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Metabolic consequences of chronic sleep restriction in rats: Changes in body weight regulation and energy expenditure

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HIGHLIGHTS

- ▶ The slowly rotating drum method effectively restricted sleep time in rats.
- ▶ Short sleep increases energy expenditure but no change in food intake.
- ▶ Short sleep attenuates weight gain in rats.
- ▶ Plasma glucose, insulin and leptin levels were reduced, reflecting the nutritional status.

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ABSTRACT

Epidemiological studies have shown an association between short or disrupted sleep and an increased risk to develop obesity. In animal studies, however, sleep restriction leads to an attenuation of weight gain that cannot be explained by changes in energy intake. In the present study, we assessed whether the attenuated weight gain under conditions of restricted sleep is a consequence of an overall increase in energy expenditure. Adult male rats were subjected to a schedule of chronic sleep restriction (SR) for 8 days with a 4 h window of unrestricted rest per day. Electroencephalogram and electromyogram recordings were performed to quantify the effect of the sleep restriction schedule on sleep–wake patterns. In a separate experiment, we measured sleep restriction-induced changes in body weight, food intake, and regulatory hormones such as glucose, insulin, leptin and corticosterone. To investigate whether a change in energy expenditure underlies the attenuation of weight gain, energy expenditure was measured by the doubly labeled water method from day 5 until day 8 of the SR protocol. Results show a clear attenuation of weight gain during sleep restriction but no change in food intake. Baseline plasma glucose, insulin and leptin levels are decreased after sleep restriction which presumably reflects the nutritional status of the rats. The daily energy expenditure during SR was significantly increased compared to control rats. Together, we conclude that the attenuation of body weight gain in sleep restricted rats is explained by an overall increase in energy expenditure together with an unaltered energy intake.

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1. Introduction

A substantial number of studies have demonstrated a correlation between short sleep and increased prevalence of obesity ([1–5], for

an overview see [6]). While these studies form an important basis for the hypothesis that restricted sleep may contribute to metabolic diseases, they do not provide information on the cause and consequence in this relationship [7,8]. To determine the relationship between insufficient sleep and altered metabolic regulation, controlled studies with experimental sleep restriction are required.

The present study aimed to assess the effects of chronic sleep restriction on energy metabolism in rats. For this purpose, we used a well-established rotating drum system to keep rats awake. Previously, we reported that chronic sleep restriction induced by this rotating drum method leads to gradual and, in some cases, persistent changes in a variety of neurobiological systems (e.g., serotonergic

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signaling: [9,10]), neuroendocrine regulation (e.g., hypothalamus–pituitary–adrenal (HPA-) axis regulation: [11,12]), and physiological processes (e.g., glucose homeostasis: [13]). We now investigated the effect of sleep restriction on specific metabolic parameters, including body weight, food intake and circulating regulatory hormones, in particular insulin, leptin and corticosterone. Leptin and insulin are known adiposity signals that regulate both food intake and body weight (for reviews see: [14,15]). Corticosterone levels reflect HPA-axis activity, which may alter metabolic function and regulation of metabolic hormones, as seen during stress (for reviews see: [16,17]).

We particularly focused on changes in energy expenditure during sleep restriction. It has been shown that experimentally disturbed sleep in rats leads to an attenuation of weight gain, despite normal [13] or increased food intake [18–23]. An increase in energy expenditure might explain this, since being awake and active cost more energy than being asleep [24,25]. Spending a larger part of the day awake may therefore increase overall energy expenditure.

To assess the effects of sleep restriction on different aspects of energy balance, we exposed male rats to sleep restriction for 8 days. Body weight and food intake were measured daily and at the end of the sleep restriction protocol and after a recovery period of 5 days blood samples were taken to determine blood glucose and plasma insulin, leptin and corticosterone levels. Energy expenditure during sleep restriction was studied by the doubly labeled water method. To quantify the effect of sleep restriction on sleep–wake patterns, measurements of sleep electroencephalograms (EEG) and electromyograms (EMG) were performed.

2. Methods

2.1. Animals and housing

All experiments were performed in adult male Wistar rats (Harlan Netherlands BV, Horst, The Netherlands) weighing approximately 320 g at the start of the experiment. The rats were individually housed in Plexiglas cages in a climate-controlled room ($21\text{ }^{\circ}\text{C}\pm 1$) under a 12:12 h light–dark cycle (lights on at 10:00 am). Rats had unrestricted access to water and were maintained ad lib on a fat diet (45% fat, 30% carbohydrates, 25% proteins; Arie Blok Diervoeding B.V., Woerden, The Netherlands), which mimics the human diet and is the standard diet used in our previous studies on metabolism [13,26]. Food intake and body weights were measured daily. The bedding was carefully checked for food spillage. Experiments were approved by the Institutional Animal Care and Use Committee of the University of Groningen.

2.2. Chronic sleep restriction

Rats were subjected to chronic sleep restriction (SR) according to a previously published method [11]. The rats were allowed to sleep in their home cage for 4 h per day at the beginning of the light phase, i.e., their normal resting phase. During the remaining 20 h, they were kept awake by placing them in drums rotating at a constant speed of 0.4 m/min [9,12,13]. Rats were subjected to this schedule of sleep restriction for 8 days during which they had unlimited access to food and water inside the drums. All rats were habituated to the experimental conditions by placing them in the drums for 1–2 h on 3 consecutive days before the onset of the sleep restriction protocol. Control rats (Control) were housed in the same room but were left undisturbed in their home cage throughout the experiment. During the recovery period afterwards, all rats were left undisturbed in their home cage.

2.3. Experiment 1: sleep–wake patterns and sleep EEG

In experiment 1, we assessed the actual sleep loss during the chronic sleep restriction protocol by measuring sleep–wake patterns and sleep EEG. To be able to record EEG and EMG inside the rotating drums, we used a wireless datalogger system mounted on the head of the animals (NeuroLogger mobile system, TSE, Homburg, Germany).

The NeuroLogger head plug with electrodes for recordings of EEG and EMG was fixed to the skull under general isoflurane anesthesia (2%). Holes were drilled in the skull and 3 brass screws served as electrodes for epidural EEG (one 2.0 mm lateral of *sutura sagittalis*, 1.5 mm rostral of lambda and one 2.0 mm rostral of the measurement electrode on the right side) and a reference electrode (2.0 mm mediocaudal of lambda). For placement of EMG electrodes, the neck muscle was pierced twice with a 21-gauge needle, approximately 2 mm apart. Electrodes were then guided through these perforations and fixed into place using non-absorbable wire. Afterwards electrodes and head plug were covered with a layer of dental cement. A “dummy”, in size and weight comparable to the NeuroLogger, was attached to the head holder. After recovery from anesthesia the rat was placed back in its home cage. For postoperative care, rats received a single subcutaneous injection of finadyne (1.0 mg/kg). Rats were allowed to recover for at least 10 days before the start of the experiments.

Specialized software (CommSW, Newbehavior, Zurich, Switzerland) was used to configure and start the NeuroLogger. EEG and EMG signals were sampled at 200 Hz and directly stored on a built-in 512 mb data storage. The data was first saved in a hexadecimal format. These files were then transformed to text files using a MatLab routine and were further analyzed using SleepSign® for animals (KISSEI COMTEC, Nagano, Japan). At first, an automatic scoring took place using the wave form recognition and logic setup algorithm of the screening module of SleepSign®. Each file and epoch was then checked visually, and if necessary, corrected by an experienced observer. On the basis of this scoring, time spent in each vigilance state was calculated. In addition, the signals were subjected to spectral analysis by Fast Fourier Transformation (SleepSign®). For all NREM sleep epochs, the EEG power in the 1–4 Hz delta range was calculated as an indicator of sleep intensity. To correct for inter-individual differences in strength of the EEG signal, the delta power values were normalized by expressing them as a percentage of each rats' own average 24 hour baseline delta power. The normalized EEG delta power is referred to as slow wave activity (SWA).

In this study, EEG and EMG measurements were done for baseline and day 1 of sleep restriction as well as day 8 of sleep restriction and the first day of recovery. In this experiment, sleep restricted rats (SR: $n=4$) served as their own controls.

2.4. Experiment 2: plasma hormone levels

Experiment 2 established the effects of chronic sleep restriction on body weight, food intake and baseline circulating levels of blood glucose and plasma insulin, leptin and corticosterone. All rats in this experiment were fitted with a chronic jugular vein catheter allowing repeated and stress free blood sampling according to a previously described method [27]. Under 2% isoflurane inhalation anesthesia, a silicon heart catheter (0.95 mm OD, 0.50 mm ID) was inserted into the right jugular vein and kept in place with a ligament. The other end of the catheter was subcutaneously directed to the top of the head where it was fixed with dental cement and could be used to connect the rats to sampling tubes. Rats were allowed to recover for at least 10 days before the start of the experiment. Rats were then divided over two groups: a sleep restricted group (SR: $n=11$) and a home cage control group (Control: $n=7$). SR rats spent the first 4 h of the light phase in their regular home cages, where after they were transferred to the rotating drums. Blood samples were taken

after 8 days of SR/Control (8 d experiment) and after 5 days of recovery (5 d recovery) during the fourth hour of the light period (ZT4), at the end of the daily 4 h sleep window. In case of blood sampling, food was removed at ZT0.

Blood samples (500 μL) were collected in tubes with EDTA (20 $\mu\text{L}/\text{mL}$ blood) on ice. About 50 μL of fresh blood was immediately stored at $-20\text{ }^\circ\text{C}$ for later determination of blood glucose levels by Hoffman's ferrocyanide method. The remaining blood was centrifuged at 2600 g for 10 min and the plasma was then stored at $-20\text{ }^\circ\text{C}$ until further analysis. Plasma levels of insulin were measured by Millipore Rat Insulin Radioimmunoassay (Linco Research, St Charles, MO, USA), plasma levels of leptin were measured by Linco Research Rat leptin Radioimmunoassay (Linco Research), and plasma levels of corticosterone were measured by ImmunoChem 125I Corticosterone Radioimmunoassay (MP Biomedicals, Orangeburg, NY, USA).

2.4.1. Experiment 3: energy balance

Experiment 3 aimed to assess energy expenditure during chronic sleep restriction. Rats were divided over 2 groups: sleep restriction (SR: $n=8$) and home cage controls (Control: $n=8$). Body weight and food intake (45% fat diet: 1 g = 4.8 kcal) were measured daily. Measurement of energy expenditure during sleep restriction was achieved by the doubly labeled water method as described previously [28]. Energy expenditure was measured over a 3-day period, from day 5 until day 8 of the sleep restriction protocol, because body weights were stable during this period of time. In brief, at day 5 of the sleep restriction protocol, an intraperitoneal injection of a mixture of $^2\text{H}_2^{16}\text{O}$ (mixture enrichment of $^2\text{H}=33.32\text{ at.}\%$) and $^1\text{H}_2^{18}\text{O}$ (mixture enrichment of $^{18}\text{O}=65.62\text{ at.}\%$) was administered. The syringes containing the mixture were weighed to 0.1 mg before and after injection to obtain a dose mass. Following isotope injection rats returned to their home cage to allow isotope equilibration with the rat's water pool. Two-and-a-half hour after injection an initial blood sample was drawn from the tail [11,29]. At day 8 of the sleep restriction protocol, a second blood sample was taken at the same circadian time as the first blood sample. All samples were collected in 50 μL Vitrex pre-calibrated capillaries and were immediately flame-sealed and stored until analysis. Analysis of the blood samples was achieved by previously described methods [28].

2.5. Data analysis

In experiment 1, we measured time spent in NREM sleep, REM sleep or Wake and NREM sleep EEG SWA during a baseline day, day 1 of SR, day 8 of SR and the first day of recovery. All parameters were compared to baseline by a paired t-test. In experiment 2, body

weight and food intake were measured daily during the sleep restriction protocol and recovery period afterwards. At day 8 of SR, regulatory metabolic hormones were measured. To test the effect of 8 days of sleep restriction and the effect of 5 days of recovery thereafter on body weight, data were subjected to analysis of variance (ANOVA) with repeated measures. To test for effects of sleep restriction on food intake and glucose, insulin, leptin and corticosterone levels, data were subjected to One Way ANOVA. In experiment 3, body weight and food intake were measured daily. Energy expenditure was measured by doubly labeled water during day 5 until day 8 of the experimental protocol. Energy balance was calculated by subtracting energy expenditure from energy intake. To test for effects of sleep restriction on delta body weight, data were subjected to Repeated Measures ANOVA. To test for the effects of sleep restriction on food intake, energy expenditure and energy balance, data were subjected to One Way ANOVA. For all the three experiments, data in text and figures are expressed as averages \pm SEM and $P<0.05$ was considered statistically significant.

3. Results

3.1. Experiment 1: sleep–wake patterns and sleep EEG

The average sleep–wake pattern is shown in Fig. 1 for baseline, day 1 and day 8 of sleep restriction and the first day of recovery. The sleep restriction protocol reduced sleep time and increased overall waking time compared to baseline, both on the first and eighth days of the experiment (paired t-test: total sleep time day 1 SR: $t=16.27$, $P<0.01$; total sleep time day 8 SR: $t=18.11$, $P<0.01$; total wake time day 1 SR: $t=-26.97$, $P<0.001$; total wake time day 8 SR: $t=-72.59$, $P<0.001$; see Fig. 2 and Table 1). NREM and REM sleep times were significantly decreased during the first and eighth days of sleep restriction (total NREM sleep day 1 SR: $t=25.69$, $P<0.001$; total NREM sleep day 8 SR: $t=34.42$, $P<0.01$; total REM sleep day 1 SR: $t=15.94$, $P<0.01$; total REM sleep day 8 SR: $t=11.85$, $P<0.01$; see Table 1).

Although the sleep restriction procedure reduced NREM and REM sleep times, rats did have occasional micro sleeps in the rotating drum ($<20\text{ s}$), which added up to approximately 1 h of sleep during the daily 20 h sleep deprivation phase, both on the first and eighth days of the experimental protocol (Table 1). Most of the sleep in the rotating drum consisted of NREM sleep, although sporadic REM sleep epochs occurred as well. In fact, the amount of REM sleep in the rotating drum significantly increased from day 1 to day 8 of the sleep restriction protocol ($t=-7.65$, $P<0.05$).

During the daily 4 hour sleep window, REM sleep time was significantly increased as compared to the same period under baseline

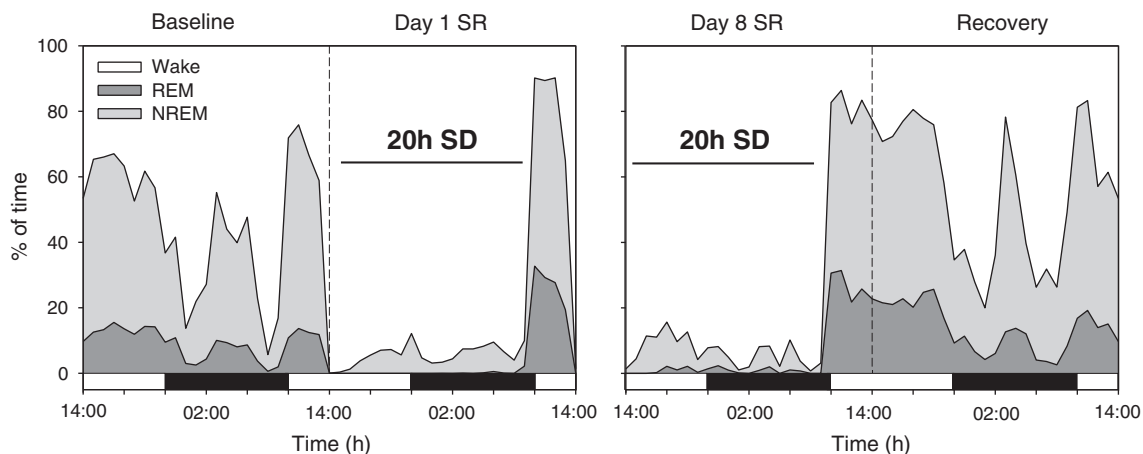


Fig. 1. Average sleep–wake patterns under baseline conditions, during day 1 and day 8 of the sleep restriction protocol and on the first recovery day. Rats ($n=4$) were subjected to sleep deprivation (SD) for 20 h/day. The horizontal black-and-white bar at the bottom of the panels represents the light–dark cycle.

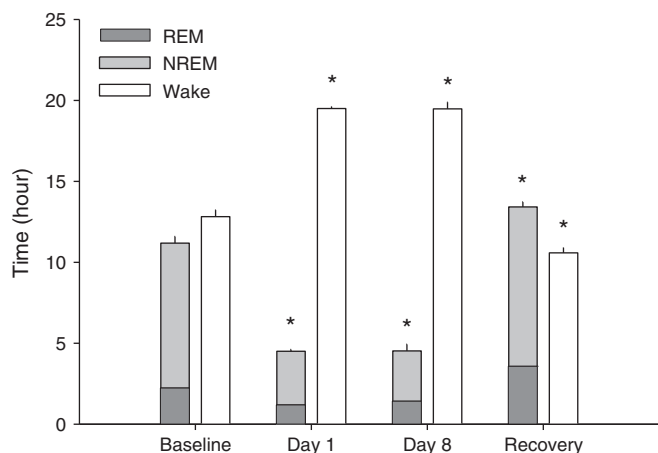


Fig. 2. The total time spent asleep or awake during the baseline day, during day 1 and day 8 of SR protocol, and on the first recovery day. Time spent asleep is divided into NREM and REM sleep. Data are average values \pm SEM ($n=4$). Statistics is done on total sleep time and total wake time. Asterisks indicate a significant difference in comparison to the baseline day (* $P<0.05$).

conditions (day 1 SR: $t=-20.96$, $P<0.001$; day 8 SR: $t=-10.73$, $P<0.01$). NREM sleep time during this 4 hour window was not significantly changed compared to baseline conditions. However, the average NREM sleep EEG SWA during this 4 h window was higher on sleep restriction days than during baseline. Due to the small sample size and variation, this did not reach statistical significance (SWA baseline: $116.7 \pm 1.1\%$, SWA day 1 SR: $132.5 \pm 5.1\%$, SWA day 8 SR: $129.0 \pm 10.2\%$; paired t -test: baseline vs. day 1 SR: $t=-3.06$, $P=0.055$, baseline vs. day 8 SR: $t=-1.17$, $P=0.36$).

During the first day of recovery total sleep time was significantly increased ($t=-4.99$, $P<0.05$) and total wake time significantly decreased when compared to baseline ($t=5.00$, $P<0.05$). Total REM sleep time was significantly increased ($t=-29.13$, $P<0.01$), but NREM sleep time was only increased during 20 h ($t=-12.32$, $P<0.05$) and not during total 24 h of recovery.

3.2. Experiment 2: plasma hormone levels

Sleep restriction by forced locomotion significantly suppressed the weight gain that was seen in home cage control rats (Repeated Measures ANOVA: time \times treatment interaction: $F(18,306)=17.38$, $P<0.001$; see Fig. 3A). Upon termination of the sleep restriction protocol, weight gain seemed to normalize, but the overall increase in body weight

Table 1

Time (minutes) spent in NREM, REM or wake.

	Baseline	Day 1	Day 8	Recovery
NREM (min)				
20 h SR	411.8 \pm 4.7	65.8 \pm 9.9*	65.1 \pm 13.6*	473.0 \pm 9.2*
4 h rest	132.8 \pm 12.1	142.1 \pm 4.1	131.5 \pm 3.7	130.8 \pm 11.3
24 h total	544.7 \pm 16.7	207.9 \pm 9.0*	196.6 \pm 17.3*	603.8 \pm 11.3
REM (min)				
20 h SR	99.5 \pm 6.9	2.1 \pm 1.0*	9.2 \pm 1.6**	162.3 \pm 12.0*
4 h rest	26.7 \pm 3.8	60.2 \pm 3.1*	65.7 \pm 6.8*	39.1 \pm 2.2
24 h total	126.2 \pm 10.2	62.4 \pm 3.4*	74.9 \pm 8.2*	201.4 \pm 12.0*
Wake (min)				
20 h SR	688.6 \pm 10.3	1132.1 \pm 9.9*	1125.7 \pm 15.1*	564.7 \pm 18.6*
4 h rest	80.5 \pm 15.5	37.6 \pm 6.7*	42.8 \pm 10.4	70.1 \pm 1.1
24 h total	769.1 \pm 25.3	1169.8 \pm 7.4*	1168.8 \pm 19.3*	634.8 \pm 19.3*

Time spent in different sleep stages during the total 24 h, 20 h of sleep restriction and 4 h window of sleep allowance (rest) during days 1 and 8 of SR. The same time points were used for the baseline and the first recovery day. Data are average \pm SEM.

* $P<0.05$ compared to baseline.

** $P<0.05$ compared to day 1.

remained significantly lower compared to controls even after 5 days of recovery. There were no significant differences in total food intake (Fig. 3B).

The changes in blood glucose and plasma insulin, leptin and corticosterone levels after 8 days of sleep restriction and after a subsequent 5 day recovery period are shown in Fig. 4. Blood glucose levels were significantly decreased after 8 days of SR (One Way ANOVA: $F(1,14)=8.16$, $P<0.05$) but levels had returned to control levels after 5 days of recovery (One Way ANOVA $F(1,10)=0.88$, $P>0.5$).

Insulin levels were decreased after 8 days of SR compared to controls (One Way ANOVA: $F(1,14)=17.42$, $P<0.01$) and after 5 days of recovery they were no longer significantly different compared to controls (One Way ANOVA: $F(1,9)=1.30$, $P>0.1$). Plasma levels of leptin showed the same pattern. After 8 days of SR, leptin levels were significantly decreased compared to control rats (One Way ANOVA: $F(1,12)=11.63$, $P<0.01$) and after 5 days of recovery they were no longer significantly different compared to controls (One Way ANOVA: $F(1,12)=0.71$, $P>0.1$).

Corticosterone levels after 8 days of SR and 5 days of recovery did not differ between groups.

3.3. Experiment 3: energy balance

Energy expenditure in SR rats, assessed by the doubly labeled water method from day 5 until day 8 of the sleep restriction protocol, was significantly higher compared to energy expenditure in home cage control rats ($F(1,14)=118.0$, $P<0.0001$; see Fig. 5). During the same 3-day period, food intake was not different between the groups, leading to a significant difference in energy balance (energy intake minus energy expenditure: Control: 18.8 ± 2.5 kcal/day; SR: 0.1 ± 2.2 kcal/day; One Way ANOVA: $F(1,14)=30.7$, $P<0.001$). In agreement with this, weight gain during this 3-day period was significantly lower in sleep restricted rats than control animals ($F(1,14)=58.1$, $P<0.0001$; see Fig. 5). Body weight over the 3-day period was significantly increased over time for control rats (Repeated Measures ANOVA: $F(1,7)=63.57$, $P<0.001$) and significantly decreased over time for SR rats ($F(1,7)=13.32$, $P<0.01$).

4. Discussion

The slowly rotating drum method effectively restricted sleep time in rats. The reduction of total sleep time led to an attenuation of weight gain. During the sleep restriction period, energy intake in terms of food consumption was not affected while daily energy expenditure was significantly increased. This implies that the attenuation in weight gain during sleep restriction is caused by an increase in energy expenditure. Plasma levels of glucose, insulin and leptin were reduced, reflecting the nutritional status and attenuation of weight gain.

In the present study, chronic sleep restriction, achieved by the slowly rotating drum method, significantly reduced total sleep time. Total sleep decreased from 11 h on the baseline day to about 4.5 h on sleep restriction days. Most of this sleep occurred in the daily 4 h sleep window, but during the daily 20 h forced wakefulness periods, inside the rotating drums, rats had occasional micro sleeps as well. The latter only added up to approximately 1 h of sleep. It cannot be excluded that sleep restricted rats experienced some additional sleep like processes, perhaps even locally in specific brain regions but this was not accounted for in our global analysis of vigilance states. A recent study in rats showed that under conditions of sleep deprivation, local clusters of cortical neurons may go offline while the rest of the brain stays awake [30]. However, it is unlikely that such local processes are sufficient to compensate for the substantial deficit of sleep that our rats experienced.

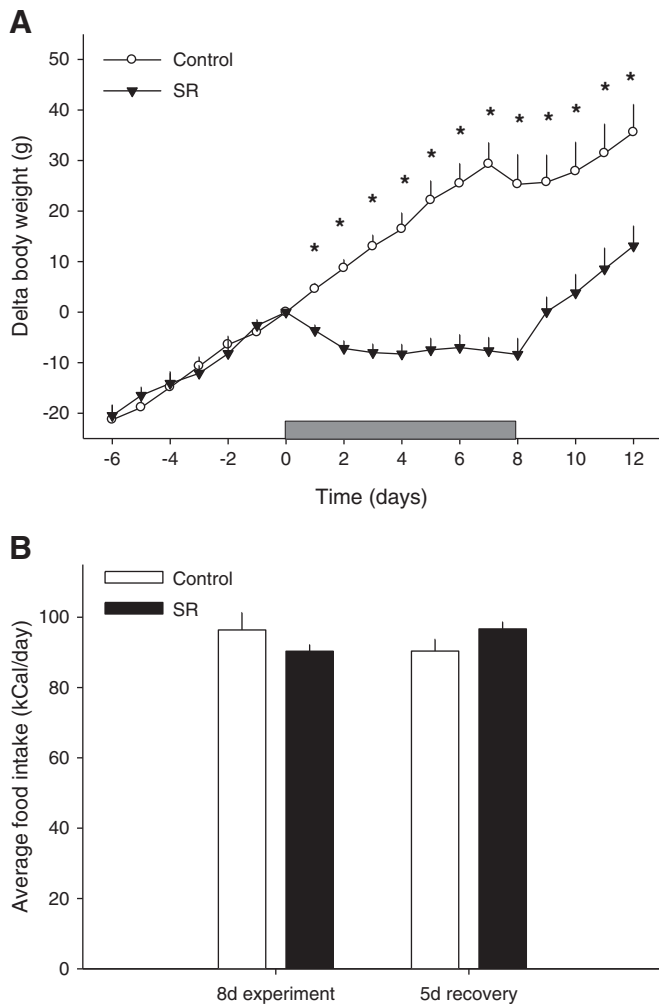


Fig. 3. Daily body weight (A) and food intake (B) during the baseline, experimental and recovery phase of the experiment for sleep restricted rats (SR, $n = 11$) and control rats (Control, $n = 7$). The horizontal gray bar at the bottom of panel A represents the 8 day sleep restriction period. Data are average values \pm SEM. Asterisks indicate a significant difference between SR and control rats ($*P < 0.01$).

Weight gain was attenuated as a consequence of chronic sleep restriction. This attenuation appears to be a direct result of increased energy expenditure. In control rats, energy intake was higher than energy expenditure, resulting in weight gain. In sleep restricted rats, energy expenditure was increased compared to controls, resulting in an attenuation of weight gain compared to controls. One has to keep in mind that energy expenditure measurement by means of the doubly labeled water method is a relative underestimation [31]. This method does not take heat production and anaerobic phosphorylation into account. Furthermore, the ingested nutrients have a certain efficiency by which they finally end up in the respiration chain. Incomplete nutrient absorption by the intestinal lumen probably causes the largest decrement in this efficiency. Counting in such a loss explains why sleep restricted animals lose weight while “energetically” being in balance. Absorption efficiency of a HF diet in rodents is estimated to be between 90 and 95% [32], and this would still relate to ~10–15 kcal/day by which the amount of absorbed nutrients exceeds energy expenditure in the control rats and thus would cause weight gain in these rats. Absorption efficiency of 90–95% in the sleep restricted rats would also explain the somewhat lower body weight loss by absolute numbers, than the body weight gain in the controls. This may explain why SR rats lost some weight during the 3-day period when energy expenditure was measured,

even though energy intake and energy expenditure seem to be in balance.

There may be multiple explanations for the increase in energy expenditure in sleep restricted rats. First, being awake costs more energy than being asleep [24,25,33]. During wakefulness and sleep deprivation, the activity of the sympathetic autonomic nervous system is higher than during sleep (for review, see [34]). As a consequence, body temperature [35–38] and heart rate are increased [39,40]. As a result, it is not surprising to see an increase in energy expenditure during prolonged wakefulness.

A second explanation for the increase in energy expenditure during sleep restriction may be that the procedure causes stress in rats. Various studies have shown that uncontrollable stress leads to increased energy expenditure [41] together with an attenuation of weight gain [42]. However, in our experiment, plasma levels of the stress hormone corticosterone were not changed after 8 days of SR, suggesting that forced locomotion as a sleep restriction method is not a major stressor.

A third explanation for the increase in energy expenditure might be that our sleep deprivation procedure involves a certain degree of (forced) locomotion. However, our lab previously found that rats on both a forced or voluntary exercise protocol did not show an attenuation of body weight gain, even though both groups of rats walked approximately 5500 m/day [43]. The latter is ten times as much as the distance walked by SR rats in the present study. Therefore, it is unlikely that the daily forced locomotion during sleep restriction is the only factor involved in the increase in energy expenditure and in turn the attenuation of weight gain. In fact, the increase in energy expenditure may be due to a combination of all previous factors mentioned.

The physiological mechanism underlying the increase in energy expenditure, and in turn the attenuation of weight gain, could be an increase in the gene expression of uncoupling protein-1 (UCP-1) in the brown adipose tissue. The brown adipose tissue is known for its regulatory non-shivering thermogenesis in rodents and heat production is mediated by UCP-1 [44]. It has been demonstrated that during sleep deprivation UCP-1 is increased over time, together with an increase in O_2 consumption [45]. In addition, Cirelli and Tononi demonstrated that UCP-2 is upregulated in muscles after sleep deprivation [46]. This elevated muscular UCP-2 expression is likely to affect energy expenditure and may therefore contribute to the increase in energy expenditure during SR.

It is intriguing that rats in our sleep restriction model do not increase their food intake, despite an increase in energy expenditure. Studies by other investigators have shown hyperphagia in sleep deprived rats, which may indicate an attempt to compensate for the increased energy use [18,21,22,47]. However, even in those studies, sleep deprived rats lost weight compared to controls. It is not clear what the explanation might be for this variation in effects of sleep disturbance on food intake. Several interacting factors may be involved, including the exact nature and duration of sleep deprivation protocol (total sleep deprivation versus REM sleep deprivation, continuous sleep deprivation versus intermittent sleep restriction). Also the method of sleep deprivation may play a role. The latter mentioned studies all used sleep deprivation methods in which rats touch or fall into the water as soon as they fall asleep, which may have immediate and non-specific effects on metabolism and eating behavior.

An explanation for the decrease in glucose levels during sleep restriction may be an increase in utilization of nutrients due to the increase in energy expenditure, which has been published before [48,49]. We furthermore showed that plasma insulin and leptin levels were decreased after 8 days of SR. These hormonal changes are likely a reflection of the nutritional state of the rats, as seen in literature [50,51]. At the end of the 5 day recovery phase, body weight of SR rats remained significantly lower compared to controls, which still

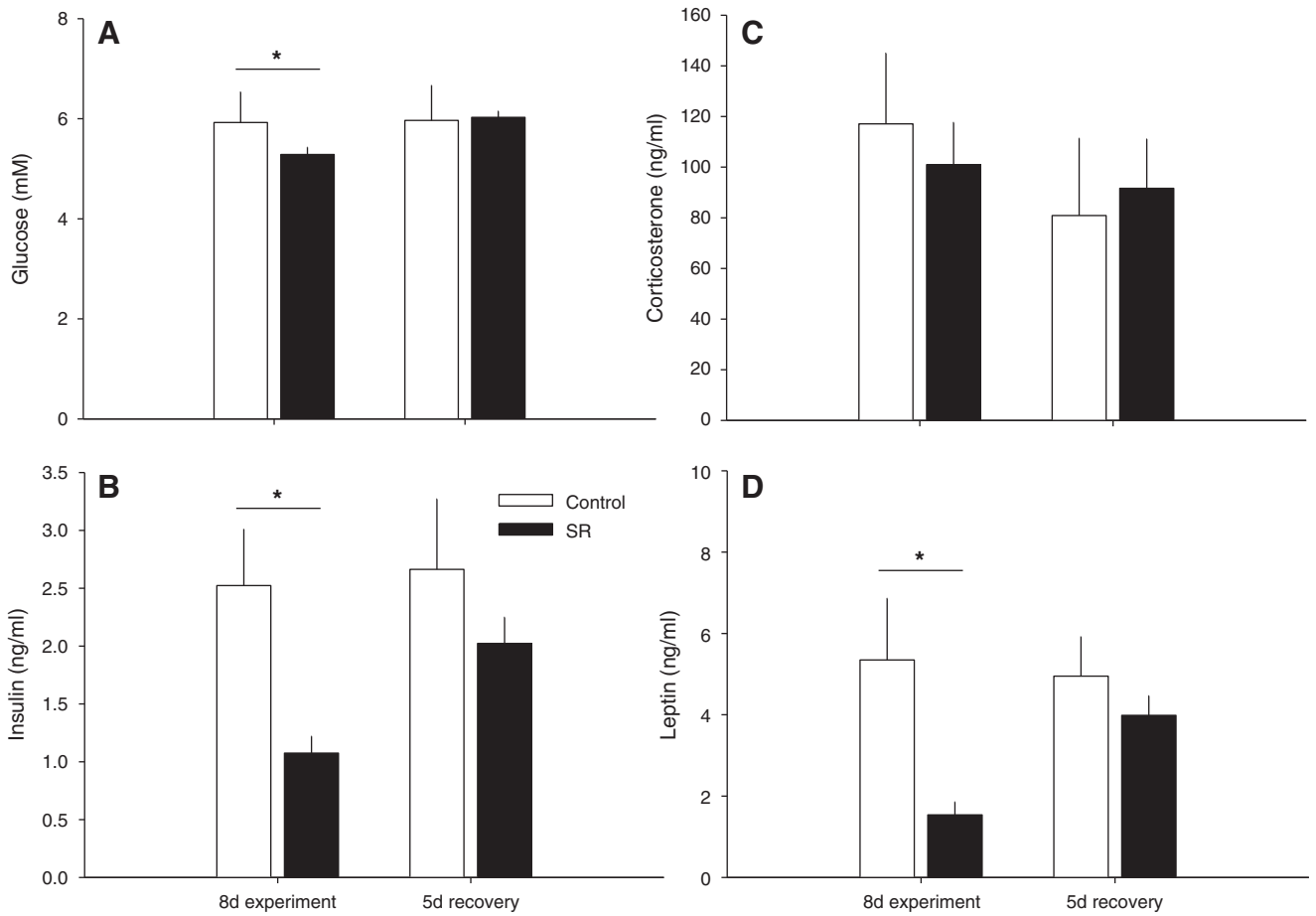


Fig. 4. Plasma levels of glucose, insulin, corticosterone and leptin at the end of the 8 d experiment and after 5 d of recovery in sleep restricted rats (SR, n = 11) and control rats (Control, n = 7). Data are average values \pm SEM. Asterisks indicate a significant difference ($*P < 0.05$).

seemed to be reflected in leptin and insulin levels, even though these levels were no longer significantly different from controls. Interestingly, since leptin is a satiety signal [14,15], one might expect that a decrease in the levels of this hormone would lead to an increase in food intake. However, in the present study SR rats refrained from increasing their food intake, despite a decrease in leptin and insulin levels.

Since the decreased levels of leptin and insulin did not lead to increased food intake, it may be that these neuroendocrine signals are processed differently at the central level. Some sleep deprivation studies in literature show hyperphagia together with increased neuropeptide Y mRNA levels and orexin/hypocretins mRNA levels in the hypothalamus [21,52,53]. Thus it may be that the increases in these neuropeptides, together with decreased levels of leptin and

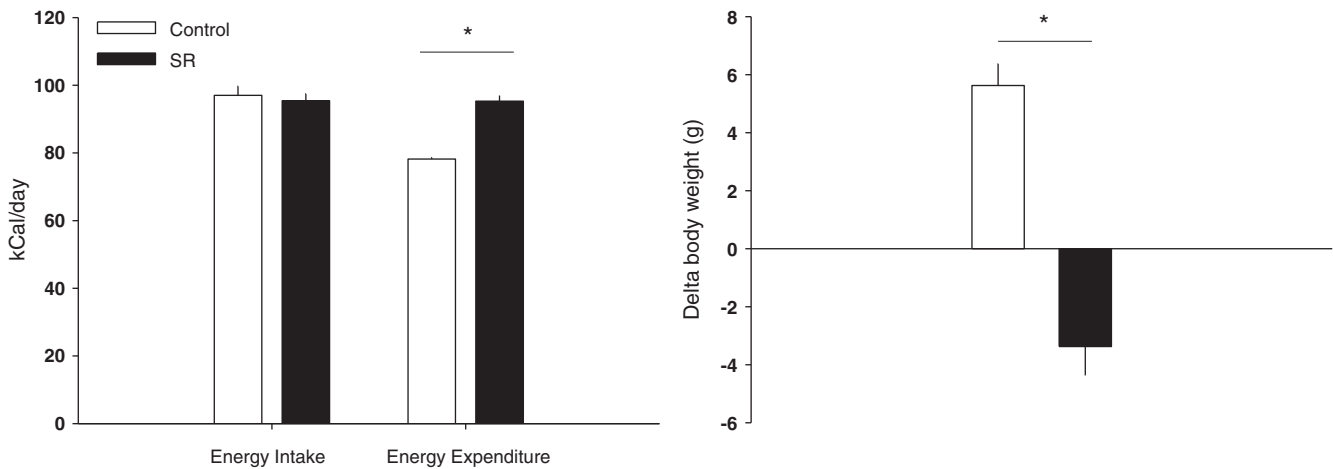


Fig. 5. Daily energy expenditure and energy intake measured during the last 3 days of the 8-day sleep restriction protocol (left panel) and the change in body weight during the same 3-d period (right panel). Measurement of energy expenditure was performed by the doubly labeled water method and energy intake was calculated on the basis of food intake and the caloric value of the diet. n = 8 for both groups. Data are average values \pm SEM. Asterisks indicate a significant difference ($*P < 0.05$).

insulin, are necessary to induce hyperphagia. Future experiments should be performed to verify this.

In conclusion, eight days of sleep restriction leads to an attenuation of weight gain which is largely explained by an increase in energy expenditure. During sleep restriction, food intake is not changed despite a decrease in the regulatory hormonal factors insulin and leptin. An explanation may be that sleep restriction disturbs the regulation of food intake at a more central level such that the decrease in plasma leptin and insulin is not sufficient to induce hyperphagia.

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