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## Metabolic consequences of chronic sleep restriction in rats

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*Published in:*  
Physiology & Behavior

*DOI:*  
[10.1016/j.physbeh.2012.09.005](https://doi.org/10.1016/j.physbeh.2012.09.005)

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*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2012

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Barf, R. P., Van Dijk, G., Scheurink, A. J. W., Hoffmann, K., Novati, A., Hulshof, H. J., Fuchs, E., Meerlo, P., & Desprez, T. (2012). Metabolic consequences of chronic sleep restriction in rats: Changes in body weight regulation and energy expenditure. *Physiology & Behavior*, *107*, 322-328.  
<https://doi.org/10.1016/j.physbeh.2012.09.005>

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## Increased food intake and changes in metabolic hormones in response to chronic sleep restriction alternated with short periods of sleep allowance

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Submitted 22 June 2011; accepted in final form 14 October 2011

**Barf RP, Desprez T, Meerlo P, Scheurink AJ.** Increased food intake and changes in metabolic hormones in response to chronic sleep restriction alternated with short periods of sleep allowance. *Am J Physiol Regul Integr Comp Physiol* 302: R112–R117, 2012. First published October 19, 2011; doi:10.1152/ajpregu.00326.2011.—Rodent models for sleep restriction have good face validity when examining food intake and related regulatory metabolic hormones. However, in contrast to epidemiological studies in which sleep restriction is associated with body weight gain, sleep-restricted rats show a decrease in body weight. This difference with the human situation might be caused by the alternation between periods of sleep restriction and sleep allowance that often occur in real life. Therefore, we assessed the metabolic consequences of a chronic sleep restriction protocol that modeled working weeks with restricted sleep time alternated with weekends with sleep allowance. We hypothesized that this protocol could lead to body weight gain. Male Wistar rats were divided into three groups: sleep restriction (SR), forced activity control (FA), and home cage control (HC). SR rats were subjected to chronic sleep restriction by keeping them awake for 20 h per day in slowly rotating drums. To model the human condition, rats were subjected to a 4-wk protocol, with each week consisting of a 5-day period of sleep restriction followed by a 2-day period of sleep allowance. During the first experimental week, SR caused a clear attenuation of growth. In subsequent weeks, two important processes occurred: 1) a remarkable increase in food intake during SR days, 2) an increase in weight gain during the weekends of sleep allowance, even though food intake during those days was comparable to controls. In conclusion, our data revealed that the alternation between periods of sleep restriction and sleep allowance leads to complex changes in food intake and body weight, that prevent the weight loss normally seen in continuous sleep-restricted rats. Therefore, this “week-weekend” protocol may be a better model to study the metabolic consequences of restricted sleep.

sleep deprivation; obesity; body weight; metabolism; energy expenditure

SLEEP LOSS IS A COMMON PROBLEM in our modern society. Both epidemiological and clinical data suggest that disturbed sleep may contribute to the development of various diseases, e.g., obesity and type 2 diabetes (4, 6, 7, 12, 36, 37). Restricted sleep also leads to alterations in food intake and its regulatory hormones, particularly an increased appetite and preference for fat, together with increased levels of ghrelin and decreased levels of leptin (38, 39).

There are several rodent models for sleep deprivation; for example, the disk-over-water method (8, 28), the inverted flowerpot (or platform) paradigm (13, 24), and the slowly rotating drum paradigm (1, 26, 30). In general, the changes in

blood hormone levels and food intake in these models are similar to the findings in humans. Sleep deprivation decreased plasma insulin (13) and leptin levels (8), and increased food intake was observed in some (8, 13, 16, 17, 28, 29) but not all studies (1). However, the most consistent finding among these studies is that sleep-deprived rats lose weight (1, 8, 13, 16, 17, 28, 29), which is in contrast to the human finding where a lack of sleep generally is associated with weight gain (6, 14).

The reason for this difference between rats and humans is unknown. There is some indirect evidence that the differences in weight gain vs. weight loss might be related to the nature of the sleep restriction protocol. Sleep loss in humans is, in general, an alternation between sleep restriction during the week and recuperation from sleep loss in the weekend (40). In contrast, experimental studies in rats often consist of a continuous period of sleep restriction without periods of recovery. Indirect evidence comes from a shift work study by Salgado-Delgado et al. (33, 34), who subjected rats to a protocol consisting of alternating 5-day periods of shift work and 2-day period of undisturbed sleep-wake rhythms, and indeed they found a clear increase in body weight.

On the basis of these studies, we hypothesized that an alternation between periods of sleep restriction and periods of sleep allowance is critical for the induction of body weight gain in a sleep restriction paradigm. Therefore, we evaluated the behavioral and metabolic consequences of a sleep restriction protocol consisting of 5 days of sleep restriction alternated by 2 days in which the rats were allowed to recover. This protocol was continued for 4 wk. We hypothesize that the 2 days of sleep allowance per week will prevent the weight loss normally seen in chronically sleep-restricted rats.

### METHODS

**Animals and housing.** Male Wistar rats (weight  $302.1 \pm 1.2$  g at start of the experiment, derived from Harlan Netherlands BV, Horst, The Netherlands) were individually housed in Plexiglas cages in a climate-controlled room ( $21^\circ\text{C} \pm 1$ ) under a 12:12-h light-dark cycle (lights on at 1:00 PM). Animals were maintained ad libitum on medium fat food (45% fat: Arie Blok Diervoeding B.V., Woerden, The Netherlands), which mimics the human diet and is the standard diet in our previous studies on metabolism (1). Water was available ad libitum throughout the study. Food intake and body weights were measured daily. Experiments were approved by the Ethical Committee of Animal Experiments of the University of Groningen.

**Chronic sleep restriction and forced activity.** The rats were assigned to one of three groups. The first group was subjected to chronic sleep restriction (SR;  $n = 12$ ). SR was achieved by placing the rats in slowly rotating drums, according to previously described methods (23). Briefly, rats were allowed to sleep in their home cage for only 4 h per day at the beginning of the light phase. During the remaining 20 h, the rats were kept awake by placing them in slowly rotating drums (diameter, 40 cm), rotating at a constant speed of 0.4 m/min (1, 26, 30).

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To model the human condition with alternating working weeks and weekends of rest, rats were subjected to a 4-wk schedule with each week consisting of a 5-day "working week" [5 consecutive days with SR or forced activity (FA)] followed by a 2-day "weekend" [2 days of uninterrupted sleep allowance in the home cage (HC)]. The second group was a forced activity control group (FA;  $n = 7$ ). The FA rats served as controls for the amount of exercise and walked the same distance as the SR rats in 2 h at the end of the dark phase (wheels by TSE, Bad Homburg, Germany). Thus, the FA group walked the same distance but was not sleep restricted. Both the SR and FA groups were forced to walk 480 m/day during the "working week", which is ~10–20% of the distance rats cover voluntarily (35). The rats of the SR and FA groups had unlimited access to food and water inside the drums. The third group of rats consisted of HC controls ( $n = 5$ ), which remained in their home cage throughout the experiment.

**Blood samples and chemical analysis.** To assess the effects of sleep restriction on metabolic hormones, blood samples were taken in *week 1* and *week 4* of the experiment for analysis of glucose, insulin, leptin, and corticosterone. Samples were taken immediately after the 5-day working week (working week: *day 5* and *day 26*) at the beginning of the lights phase (ZT0) and after 4 h of rest (ZT4). Another blood sample was taken after 2 days of rest (weekend: *day 7* and *day 28*) during the 4th h of the light phase (ZT4). Blood samples of ~0.5 ml were drawn from the tail (10, 23) and collected in pre-cooled cups containing EDTA. Afterwards, the samples were centrifuged at 4°C for 10 min at 2,600 g, and the plasma was stored at -20°C until further analysis. Blood glucose was measured by Hoffman's ferrocyanide method, and plasma levels of insulin, leptin, and corticosterone were measured by Millipore Rat Radioimmunoassays (Linco Research, St. Charles, MO).

**Indirect calorimetry.** At the first day of the final weekend of sleep allowance (*day 26*), immediately after the last SR/FA period, rats were transferred to respirometric chambers (45×25×30 cm) to determine oxygen consumption ( $\dot{V}O_2$ , l/h) and carbon dioxide production ( $\dot{V}CO_2$ , l/h). Oxygen and carbon dioxide concentration of dried inlet and outlet air (drier: molecular sieve 3 Å, Merck) from each chamber was measured with a paramagnetic oxygen analyzer (Servomex Xentra 4100) and carbon dioxide by an infrared gas analyzer (Servomex 1440). The system recorded the differentials in oxygen and carbon dioxide between dried reference air and dried air from the metabolic chambers. Flow rate of inlet air (60 l/h) was measured with a mass-flow controller (type 5850 Brooks). Samples were collected every 10 min (allowing optimal air mixing) for each animal and automatically stored on a computer. Behavioral activity of the animals was recorded with calibrated passive infrared detectors [PIR: Optex Wonderex FX-35; OPTEX (Europe), Berkshire, UK]. Animals were measured at an ambient temperature of 21°C, and food and water were provided ad libitum over the whole period. Energy expenditure (kJ) was calculated using the following equation of Ferrannini (9):  $EE = (16.18 \times \dot{V}O_2 \times 0.001) + (5.02 \times \dot{V}CO_2 \times 0.001)$ .

During the 24 h in the indirect calorimetry, fecal pellets were collected. The rotating drum system to sleep-restrict rats could not be combined with the respirometry system, and therefore, it was not possible to measure oxygen consumption during the working weeks of SR/FA.

**Statistical analysis.** The data in the figures and text are expressed as averages  $\pm$  SE. The effects of the sleep restriction and forced activity protocols on food intake, body weight, and blood hormone levels were tested by repeated-measures ANOVA with between-subjects factor of "treatment" (SR, FA, or HC) and within-subjects factor of "time" (day of the experimental protocol). Indirect calorimetry data, body weight gain, average daily food intake, and feces weight were tested by one-way ANOVA with factor "treatment" (SR, FA, or HC). When appropriate, a post hoc Tukey test was applied to establish differences between the three groups (controls, SR, and FA).  $P < 0.05$  was considered statistically significant.

## RESULTS

Daily body weight and food intake are shown in Fig. 1. Both body weight and food intake differed significantly over time [repeated-measures ANOVA effect of time for body weight:  $F(32,672) = 411.73$ ,  $P < 0.001$ ; food intake:  $F(30,600) = 7.57$ ,  $P < 0.001$ ]. The increase in body weight was significantly attenuated in SR rats compared with home cage controls [repeated-measures ANOVA treatment  $\times$  time interaction:  $F(64,672) = 3.26$ ,  $P < 0.001$ ; post hoc Tukey test: SR vs. HC:  $P < 0.05$ ]. Food intake of SR rats increased after the first recovery weekend compared with both FA and HC rats [repeated-measures ANOVA treatment  $\times$  time interaction:  $F(60,600) = 6.48$ ,  $P < 0.001$ ; post hoc Tukey test: experimental weeks 2–4: SR vs. FA and SR vs. HC:  $P < 0.05$ ].

In Fig. 2, data are averaged for weeks 2–4, when the effects of sleep restriction and patterns of body weight and daily food intake had stabilized [average body weight gain or food intake divided by the number of days (7, 5, or 2 days)]. SR rats had a slight reduction in weight gain during the 5-day working weeks [ $F(2,21) = 3.48$ ,  $P < 0.05$ , post hoc Tukey test: SR vs. HC:  $P < 0.05$ ]. Both SR and FA rats showed an increase in

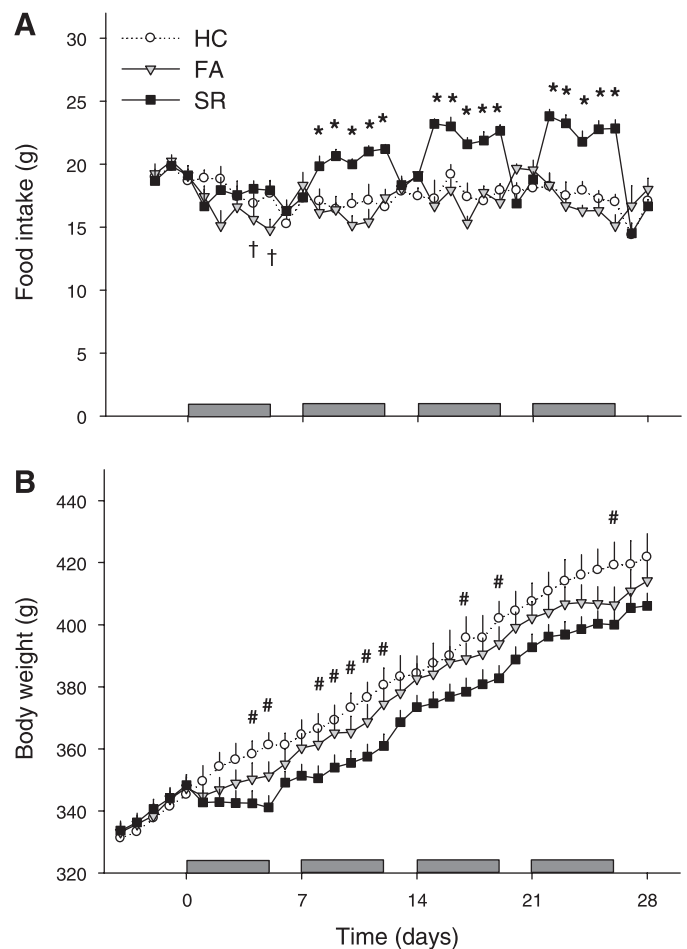


Fig. 1. Daily food intake (A) and body weight (B) during baseline, 4 experimental weeks, and 4 weekends of sleep allowance for sleep restriction (SR;  $n = 12$ ), forced activity (FA;  $n = 7$ ), and home cage (HC;  $n = 5$ ) rats. The gray bars represent the 5-day wk of SR or FA. Data are average values  $\pm$  SE. Significant differences ( $P < 0.05$ ): \*SR vs. both control groups; #SR vs. HC; †FA vs. HC.

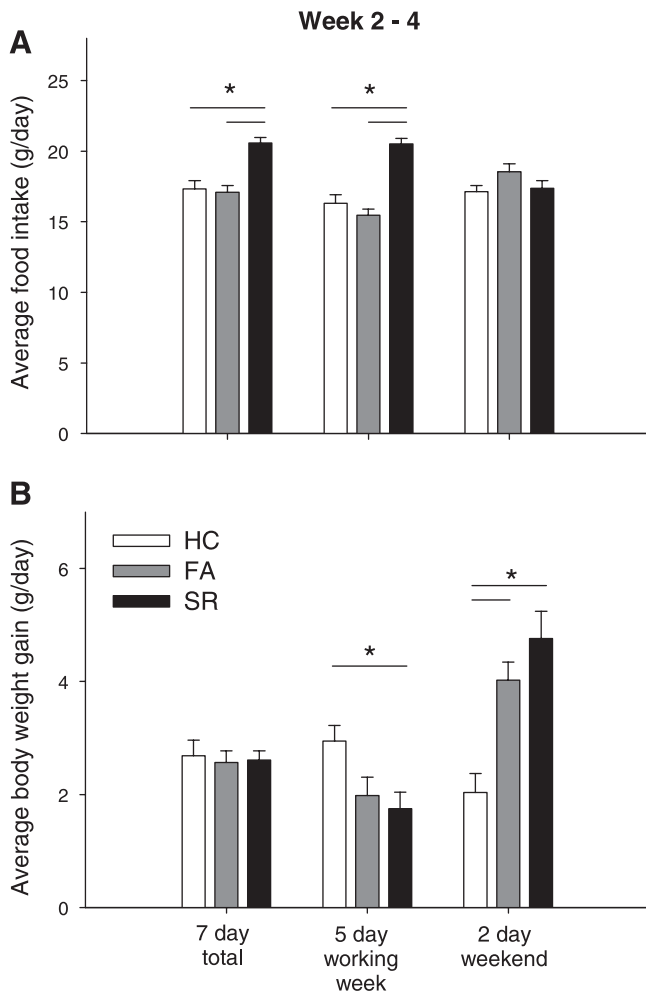


Fig. 2. Average food intake and body weight gain per day during experimental weeks 2–4 for SR ( $n = 12$ ), FA ( $n = 7$ ), and HC ( $n = 5$ ) rats. Averages are shown per total 7 days, per experimental week (5 days) and per weekend of sleep allowance (2 days). Data are average values  $\pm$  SE. \* $P < 0.05$ .

their body weight gain during the weekends of rest [ $F(2,21) = 8.38$ ,  $P < 0.01$ , post hoc Tukey test: SR vs. HC and FA vs. HC:  $P < 0.05$ ]. The total body weight gain was not different between groups.

During weeks 2–4, average daily food intake was significantly increased during the 5-day sleep restriction periods [one-way ANOVA:  $F(2,21) = 43.26$ ,  $P < 0.001$ , post hoc Tukey test: SR vs. FA and SR vs. HC:  $P < 0.001$ ] but returned to control levels during the 2-day weekends of rest [ $F(2,21) = 1.65$ ,  $P > 0.1$ ]. For the week totals, including both the working week and recovery weekend, average daily food intake remained significantly increased for SR rats [ $F(2,21) = 23.38$ ,  $P < 0.001$ , post hoc Tukey test: SR vs. HC and SR vs. FA:  $P < 0.001$ ].

Glucose, insulin, leptin, and corticosterone levels (Table 1) were measured at the end of the first and fourth working week, immediately after the rats had returned to their home cage at the beginning of the light phase (ZT0), after 4 h of rest (ZT4), and after 2 days rest (weekend: ZT4). No changes over time or between groups were found in glucose levels. For insulin levels, repeated-measures ANOVA revealed a significant time effect [ $F(5,105) = 2.60$ ,  $P < 0.05$ ] and a significant treatment  $\times$

time interaction [ $F(10,105) = 2.26$ ,  $P < 0.05$ ]. Post hoc Tukey revealed decreased insulin levels in SR and FA rats compared with HC rats at the end of working weeks 1 and 4. Insulin levels returned to control values after 2 days of rest.

Leptin levels showed a similar pattern. Repeated-measures ANOVA revealed a significant time effect [ $F(5,105) = 6.48$ ,  $P < 0.001$ ] and a nearly significant treatment  $\times$  time interaction [ $F(10,105) = 1.74$ ,  $P = 0.08$ ]. A post hoc Tukey test revealed decreased leptin levels after both 1 and 4 wk of SR, but not FA. Leptin levels in SR were back to control levels after 2 days of rest.

Corticosterone levels showed a significant time effect [ $F(5,105) = 26.00$ ,  $P < 0.001$ ] and a significant treatment  $\times$  time interaction [ $F(10,105) = 10.77$ ,  $P < 0.001$ ]. At the end of the first 5-day working week, immediately after the last 2 h forced locomotion session, FA rats had significantly increased corticosterone levels (post hoc Tukey test, FA vs. HC and FA vs. SR:  $P < 0.05$ ), which had returned to control levels after 4 h of rest. The same pattern was visible after 4 wk of FA (post hoc Tukey test, FA vs. HC and FA vs. SR:  $P < 0.05$ ), although the increase in corticosterone levels after FA was less pronounced compared with week 1 (paired  $t$ -test:  $t = 4.11$ ,  $P < 0.01$ ).

Data derived from indirect calorimetry on the first recovery day after the fourth working week is shown in Fig. 3. Daily energy expenditure did not differ between groups during the light phase, dark phase, and total 24 h. The respiratory quotient (RQ:  $\text{CO}_2$  production/ $\text{O}_2$  consumption) during the 24-h respirometry measurement was significantly lower in SR rats compared with control rats [SR:  $0.92 \pm 0.01$ ; FA:  $0.95 \pm 0.01$ ; HC:  $0.95 \pm 0.01$ ;  $F(2,21) = 3.85$ ,  $P < 0.05$ ; post hoc Tukey test: SR vs. FA and SR vs. HC:  $P < 0.05$ ]. Levels of activity were significantly decreased for SR rats during the light phase [ $F(2,15) = 7.47$ ,  $P < 0.01$ , post hoc Tukey test, SR vs. FA:  $P < 0.05$ ]. Total 24-h activity levels tended to be lower, but this did not reach statistical significance [ $F(2,15) = 3.11$ ,  $P = 0.07$ ]. Furthermore, feces were weighed for all rats. SR rats had significantly less feces during the first day of sleep allowance in the indirect calorimetry [SR:  $3.8 \pm 0.2$  g; FA:  $4.8 \pm 0.3$  g; HC:  $4.7 \pm 0.3$  g; one-way ANOVA:  $F(2,20) = 4.79$ ,  $P < 0.05$ , post hoc Tukey test, SR vs. FA:  $P < 0.05$ ].

## DISCUSSION

The most striking result of the present study is the significant increase in food intake, which appeared after the first weekend of sleep allowance. The first period of sleep restriction was similar to our previously published data, in which 8 days of sleep restriction led to unchanged food intake, but a clear weight loss (1). After the first weekend of sleep allowance, rats become hyperphagic, preventing further weight loss. A second interesting finding is that the rats have normal food intake during the weekends of sleep allowance, but significant weight gain in this period. Together, these data support the hypothesis that an alternation between periods of sleep restriction and sleep allowance can prevent the weight loss normally seen in sleep-restricted rats. Therefore, this “week-weekend” protocol has increased face validity compared with our earlier sleep restriction protocol that did not include the intervening periods of sleep allowance.

Table 1. Effect of chronic sleep restriction on plasma levels of glucose, insulin, leptin, and corticosterone

	Working Week 1		Weekend 1	Working Week 4		Weekend 4
	ZT0	ZT4	ZT4	ZT0	ZT4	ZT4
Glucose, mM						
HC	5.9 ± 0.2	6.1 ± 0.1	6.2 ± 0.2	5.9 ± 0.1	6.1 ± 0.1	6.2 ± 0.3
FA	6.0 ± 0.1	5.8 ± 0.1	6.1 ± 0.1	6.0 ± 0.2	5.8 ± 0.2	6.0 ± 0.1
SR	5.9 ± 0.1	5.7 ± 0.1	6.0 ± 0.1	6.1 ± 0.2	5.9 ± 0.1	6.1 ± 0.1
Insulin, ng/ml						
HC	4.8 ± 1.1	4.5 ± 0.5	4.4 ± 0.9	4.9 ± 0.9	5.2 ± 0.7	3.7 ± 0.5
FA	2.3 ± 0.2*	3.4 ± 0.5	3.8 ± 0.5	2.6 ± 0.6*	3.1 ± 0.4*	3.4 ± 0.4
SR	2.7 ± 0.3*	2.6 ± 0.3*	3.7 ± 0.4	2.9 ± 0.2*	3.9 ± 0.4	3.3 ± 0.3
Leptin, ng/ml						
HC	16.3 ± 3.7	11.9 ± 1.1	11.6 ± 1.7	17.6 ± 2.7	15.3 ± 2.8	13.5 ± 2.4
FA	10.7 ± 3.1	8.3 ± 2.0	10.8 ± 2.3	10.7 ± 2.7	10.0 ± 3.2	12.3 ± 4.5
SR	8.3 ± 0.9*	6.3 ± 0.6*	8.7 ± 0.8	10.2 ± 0.7*	9.3 ± 0.9	10.3 ± 1.3
CORT, ng/ml						
HC	19.2 ± 2.1	16.2 ± 5.0	31.7 ± 9.7	11.3 ± 2.5	7.7 ± 2.3	26.8 ± 14.1
FA	376.6 ± 71.9*	11.7 ± 1.3	18.0 ± 2.2	158.9 ± 27.5*	4.9 ± 1.3	14.4 ± 1.7
SR	132.4 ± 38.7†	11.2 ± 0.7	21.3 ± 2.3	77.8 ± 20.5†	10.3 ± 1.4	27.8 ± 5.3

Blood samples were taken during the 1st and 4th week of the experiment. For both weeks, samples were taken immediately after five experimental days (working week) at the beginning of the light phase [zeitgeber (ZT0)] and after 4 h of rest (ZT4); another sample was taken after 2 days of rest (weekend) during the 4th hour of the light phase (ZT4). Data are average values ± SE [sleep restriction (SR):  $n = 12$ , forced activity (FA)  $n = 7$ , and home cage (HC)  $n = 5$ ]. Significant differences ( $P < 0.05$ ): \*compared to HC, †compared to FA.

It is important to note that our experimental rats had increased body weight gain only during the weekends of sleep allowance compared with the home cage controls, but not during the working weeks. Overall, even at the end of the 4-wk protocol, the SR rats were still slightly lighter than controls. Even though our current protocol of sleep restriction alternated with weekends of sleep allowance attenuates the weight loss seen with continuous sleep restriction in literature, rats still do not become overweight or obese. This suggests that sleep restriction per se is not sufficient to produce obesity in rats.

The present experiment was indirectly based on a study by Salgado-Delgado et al. (33, 34), who showed that subjecting rats to a shift work protocol with forced locomotion during the normal rest phase led to a significant increase in body weight compared with controls. In their study, shift working weeks were alternated with weekends of undisturbed rest, which may have been a crucial factor in the reported body weight increase. The reason why their shift work protocol resulted in a net increase in body weight above control levels, while our current protocol only attenuated the body weight loss seen in previous studies, may lie in the methodological differences. While both models interfered with sleep by subjecting rats to forced activity, the Salgado-Delgado shift work protocol specifically disrupted circadian organization, whereas our model only aimed to shorten sleep. It may thus be that disrupting circadian organization has additional effects beyond sleep disruption that contribute to the body weight increase.

In our present study, SR rats lost only weight during the first week of the protocol, similar to what was reported before (1). This decrease in weight may be a result of increased energy expenditure associated with prolonged wakefulness and increased activity. Indeed several studies have shown increased energy expenditure during sleep deprivation (2, 5, 13, 18), and one explanation for this change in energy expenditure, and, in turn, the attenuation of body weight gain, could be an increase in the gene expression of uncoupling protein-1 (UCP-1) in the brown adipose tissue (BAT). BAT is known for its regulatory nonshivering thermogenesis in rodents and heat production is

mediated by UCP-1 (3). Indeed, Koban and Swinson (18) have demonstrated that during sleep deprivation, UCP-1 is increased over time, together with an increase in  $O_2$  consumption. Thus, in our study, it might be that the 5 days of sleep restriction leads to increased energy expenditure and increased UCP-1, whereas during the weekends of sleep allowance, both return to baseline.

During the second week of the protocol, rats started compensating for the presumed increased energy expenditure associated with sleep restriction by increasing their food intake. These changes in food intake may be related to changes in hypothalamic neuropeptides, such as orexin and neuropeptide Y. Orexin is involved in the regulation of both the sleep/wake cycle and food intake regulation (31, 32). Some studies have demonstrated that rapid eye movement (REM) sleep deprivation increases orexin levels in the cerebrospinal fluid and orexin immunoreactivity in the lateral hypothalamic area (11, 27). Furthermore, orexin neurons project densely to the arcuate nucleus, which is known for its involvement in food intake regulation (25). Indeed, REM sleep deprivation leads to significant increases in neuropeptide Y mRNA levels (15, 16). Recently, it has also been demonstrated that sleep deprivation increases orexin mRNA levels, which, in turn, activate the arcuate neuropeptide Y neurons that could lead to hyperphagia (22). Thus, it might be that in our experiment, sleep restriction after the first weekend of sleep allowance leads to increased orexin and neuropeptide Y levels in the brain, causing the rats to increase their food intake only during periods of sleep restriction. During the weekends rats are allowed to sleep, which might lead to a decrease in central orexin levels, and, in turn, cause food intake to return to baseline values. Future experiments should be performed to verify this.

The data in Fig. 2 reveal that the sleep-restricted rats are only hyperphagic during the periods of sleep restriction, whereas the weight gain only occurs during the periods of sleep allowance when the rats are not hyperphagic. Why the rats do not gain weight when they are hyperphagic during the periods of sleep restriction may be due to a number of factors. One may

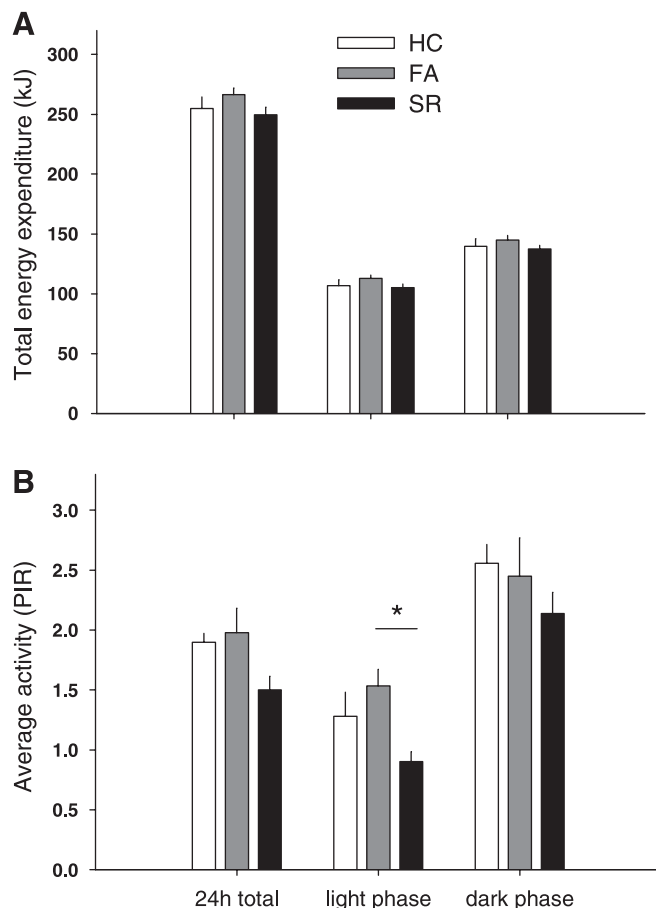


Fig. 3. Total energy expenditure and average cage activity (PIR) measured with indirect calorimetry during the first day of sleep allowance after *week 4* for SR ( $n = 12$ ), FA ( $n = 7$ ), and HC ( $n = 5$ ) rats. Data are average values  $\pm$  SE. \* $P < 0.05$ .

argue that the exercise protocol of 480 m/day may have increased the energy expenditure of the rats. However, the FA control rats walked the same distance per day, did not increase their food intake during these periods of forced locomotion and did not lose body weight. Nevertheless, it might be that the sleep restriction protocol itself has effects on the energy expenditure beyond the increase in locomotor activity. Because of methodological limitations, we were not able to measure energy expenditure during the 5-day periods of SR; therefore, we can only speculate that energy expenditure most probably is increased during SR, similar to what has been published before (2, 5, 13, 18).

The finding that SR rats gain weight when they are not hyperphagic during the periods of sleep allowance can be interpreted in relationship to the effects of sleep restriction on sleep time and intensity. It is tenable to assume that recovery from sleep restriction is associated with increased sleep time and sleep intensity, as others have demonstrated that rats do sleep longer and deeper after sleep acute sleep deprivation and chronic sleep restriction (20, 21). However, the data demonstrate that energy expenditure is not different between groups during these periods of sleep allowance, even though total activity is decreased. The question remains about how SR rats grow faster during the fourth weekend of sleep allowance, despite similar food intake and overall energy expenditure in

the different groups. One explanation might be increased food efficiency. One may argue that increased energy absorption in the intestinal tract can be used for recovery, storage, and thus weight gain. This is indirectly supported by the fact that the feces weight of SR rats is lower during weekends of sleep allowance compared with the control groups. This decrease indicates that, even though energy intake and energy expenditure are similar compared with controls, the food efficiency might be higher in SR rats during a period of sleep allowance, leading to increased body weight gain.

Although SR rats increase their food intake from *week 2* onward, leptin and insulin levels were still lower at the end of the fourth working week, which is in agreement with the fact that these rats still had a slightly lower weight than control rats. These hormones reflect the nutritional status of the rat. However, both leptin and insulin are also satiety hormones, which could also be another explanation for the increase in food intake during the periods of sleep restriction. The corticosterone levels of FA rats, immediately after the experimental period, were strongly increased. For SR rats, this did not reach significance. This increase in corticosterone for FA rats is in agreement with the notion that corticosterone may, in part, reflect and support behavioral activity (19). For instance, Koolhaas et al. (19) have demonstrated that stressful events but also pleasurable events, such as a sexual experience, can lead to similar increases in corticosterone. Therefore, an increase in corticosterone is associated with behavior, and in our case, forced locomotion. The fact that corticosterone levels rapidly returned to baseline during 4 h of sleep allowance suggests that our sleep restriction protocol is not a chronic stressor.

### Perspectives and Significance

Our data revealed that the alternation between periods of sleep restriction and periods of sleep allowance leads to complex changes in food intake and body weight that prevented the negative energy balance normally seen during continuous sleep restriction in rat studies. Although the discrepancy between epidemiological studies and rat studies remains, the alternation between periods of sleep loss and periods of sleep allowance seems to be a crucial factor and an important addition to the sleep deprivation literature.

### ACKNOWLEDGMENTS

The authors thank Jan Bruggink and Gerard Overkamp for technical assistance. We also thank Henriëtte Hulshof, Arianna Novati, Siebrigje Hooijsma, and Lisa Brouwer for their assistance with the experiments. Furthermore, we thank Kim Kinzig for carefully reading the manuscript.

### GRANTS

This work was supported by the Netherlands Organization for Scientific Research (VIDI Grant 864.04.002 to P. Meerlo).

### DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

### AUTHOR CONTRIBUTIONS

Author contributions: R.P.B., P.M., and A.J.S. conception and design of research; R.P.B. and T.D. performed experiments; R.P.B. and T.D. analyzed data; R.P.B., T.D., P.M., and A.J.S. interpreted results of experiments; R.P.B., P.M., and A.J.S. prepared figures; R.P.B., P.M., and A.J.S. drafted manuscript; R.P.B., P.M., and A.J.S. edited and revised manuscript; R.P.B., T.D., P.M., and A.J.S. approved final version of manuscript.

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