Sleep Modulates Hypertension in Leptin-Deficient Obese Mice

Alessandro Silvani, Stefano Bastianini, Chiara Berteotti, Carlo Franzini, Pierluigi Lenzi, Viviana Lo Martire, Giovanna Zoccoli

Abstract—Leptin increases sympathetic activity, possibly contributing to hypertension in obese subjects. Hypertension increases cardiovascular mortality, with nighttime (sleep) blood pressure having a substantial prognostic value. We measured blood cardiovascular in male leptin-deficient obese mice (ob/ob; n = 7) and their lean wild-type littersmates (+/+; n = 11) during wakefulness, non–rapid-eye-movement sleep, and rapid-eye-movement sleep to investigate whether, in the absence of leptin, derangements of blood pressure are still associated with obesity and depend on the wake-sleep state. Mice were implanted with a telemetric pressure transducer and electrodes for discriminating wake-sleep states. Mean blood pressure was significantly higher in ob/ob than in +/+ mice during wakefulness (7.3 ± 2.6 mm Hg) and non–rapid-eye-movement sleep (6.7 ± 2.8 mm Hg) but not during rapid-eye-movement sleep (2.6 ± 2.6 mm Hg). In ob/ob and +/+ mice, mean blood pressure was substantially higher during wakefulness than during non–rapid-eye-movement sleep. On passing from non–rapid-eye-movement sleep to rapid-eye-movement sleep, mean blood pressure decreased significantly in ob/ob but not in +/+ mice. The time spent during wakefulness was lower in ob/ob than in +/+ mice during the dark (active) period, whereas the opposite occurred during the light (rest) period. Consequently, mean blood pressure was significantly higher in ob/ob than in +/+ mice during the light (8.2 ± 2.4 mm Hg) but not during the dark (3.0 ± 2.9 mm Hg) period. These data suggest that, in the absence of leptin, obesity may entail hypertensive derangements of blood pressure, which are substantially modulated by the cardiovascular effects of the wake-sleep states. (Hypertension. 2009;53:251-255.)

Key Words: arterial pressure • behavior • heart rate • hypertension • obesity • investigative techniques • mice

Obesity is a threat to health care because it is rapidly increasing in prevalence1 and is associated with hypertension and cardiovascular risk.2 The hormone leptin signals the abundance of fat stores and acts on the hypothalamus to mount adaptive adjustments of energy balance.3 Leptin also increases sympathetic activity and blood pressure (BP).4–6 Diet-induced obesity entails hyperleptinemia and resistance to the anorectic but not to the cardiovascular effects of leptin,7 which may, thus, contribute to obesity-related hypertension.8,9

Mutations that cause a lack of leptin or leptin receptors cause morbid obesity,8 allowing us to disentangle the cardiovascular correlates of obesity from those of hyperleptinemia. Although values of BP in the hypertensive range have been reported in obese subjects with congenital leptin deficiency,10 evidence of hypertension is not consistent in this rare form of obesity.11 Evidence is inconsistent also on obese mice with congenital impairment of leptin signaling, in which either hypertensive12–14 or hypertensive15–17 derangements of BP have been reported. In these mice, the occurrence15 or severity17 of hypertension vary between the light and dark periods, which entail different amounts of sleep time in mice.18 These observations are intriguing given the recent demonstration of a marked alteration of sleep structure in obese mice with congenital impairment of leptin signaling.19,20 Moreover, the observed hypertensive derangements in these mice15,17 are similar in magnitude to the physiologic-sleep-dependent changes in BP in mice.21,22 In particular, on passing from wakefulness (W) to non–rapid-eye-movement sleep (NREMS), BP substantially decreases in mice,21 similar to what occurs in human subjects.23 This decrease in BP is because of peripheral sympathetic withdrawal23,24 and cardiac vagal activation.25 On the other hand, rapid-eye-movement sleep (REMS) entails a dramatic repatterning of sympathetic activity26 and variable changes in cardiac output.27 On passing from NREMS to REMS, an increase in BP occurs in human subjects,23 whereas either increases or decreases in BP are observed in different inbred mouse strains.22

These considerations suggest that the cardiovascular effects of the wake-sleep states are a neglected yet potentially relevant source of variability in the derangements of BP, which are associated with obesity in the absence of leptin signaling. By performing simultaneous long-term recordings of BP and sleep, we directly tested the hypothesis that derangements of BP are modulated by the cardiovascular effects of wake-sleep states in leptin-deficient obese mice.

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Methods

Experiments were performed on male mice homozygous for a nonsense mutation in the leptin gene (ob/ob) and on their littermates with 2 functional leptin alleles (+/+). For a detailed description of the materials and methods of this study, please see the online data supplement (available at http://hyper.ahajournals.org).

Surgical Procedures

Mice underwent surgery under general anesthesia and sterile conditions for the implantation of a telemetric BP transducer (TA11PA-C10, DSI), with the catheter inserted through the right femoral artery into the abdominal aorta, and of electrodes to obtain electroencephalographic and electromyographic signals.

Experimental Protocol

After a 4-day period of recovery from surgery and a subsequent 7-day period of habituation to the recording apparatus, BP, electroencephalography, electromyography, and activity were simultaneously and continuously measured on mice undisturbed and freely moving in their own cages. The BP signal was transmitted via telemetry. The telemetry system also yielded the activity signal by quantifying the spatial shifts of the implanted BP transducer. The electroencephalographic and electromyographic signals were transmitted via cable. At the end of recordings, mice were aged 15.0 ± 0.5 weeks.

Data Analysis

A visual scoring of the wake-sleep states was performed on all of the consecutive 4-s epochs based on raw electroencephalographic and electromyographic recordings (Figure S1). Beat-to-beat values of systolic BP, diastolic BP, mean BP, and heart period were computed from the raw BP signal in each artifact-free 4-s epoch. For each mouse, these values were averaged within consecutive 1-hour periods, within light and dark periods, or within each wake-sleep state for the purpose of different analyses. Overall, the analysis was performed on 185.6, 245.2, and 33.8 hours of artifact-free recordings during W, NREMS, and REMS in ob/ob mice and on 283.7, 308.2, and 49.0 hours of artifact-free recordings during W, NREMS, and REMS in +/+ mice.

Statistical Analysis

Data were analyzed by ANOVA and t tests, with P < 0.05 considered to be statistically significant. Data are reported as means ± SEMs in the text and the Figure, with n = 7 for ob/ob mice and n = 11 for +/+ mice.

Results

Ob/ob mice were extremely obese, weighing approximately twice as much as +/+ mice (48.6 ± 0.8 versus 25.5 ± 0.7 g; P < 0.001), and showed severely reduced activity levels during W with respect to +/+ mice (2.2 ± 0.3 versus 6.3 ± 0.7 arbitrary units; P < 0.001).

The Table shows values of BP and heart period in ob/ob and +/+ mice as functions of the wake-sleep state. During W, ob/ob mice had higher values of mean BP (P = 0.013) and diastolic BP (P = 0.021) than +/+ mice. During NREMS, both mean BP (P = 0.030) and diastolic BP (P = 0.025) were also higher in ob/ob mice than in +/+ mice. Neither diastolic BP (P = 0.136) nor mean BP (P = 0.335) differed significantly between ob/ob and +/+ mice during REMS. Systolic BP did not differ significantly between ob/ob and +/+ mice in any wake-sleep state (P > 0.114). In both mouse strains, systolic, diastolic, and mean BPs were substantially higher in W than in NREMS (P < 0.001). On passing from NREMS to REMS, however, systolic, diastolic, and mean BPs decreased in ob/ob mice (P = 0.039, P = 0.025, and P = 0.029, respectively), whereas they did not change significantly in +/+ mice (P > 0.092). Heart period differed significantly among wake-sleep states (P < 0.001), increasing on passing from W to NREMS and decreasing on passing from NREMS to REMS (P < 0.001). Neither the state × strain interaction effect (P = 0.066) nor the main effect of the mouse strain (P = 0.452) on heart period was statistically significant.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Strain</th>
<th>W</th>
<th>NREMS</th>
<th>REMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP, mm Hg</td>
<td>ob/ob</td>
<td>125 ± 2†</td>
<td>109 ± 3*</td>
<td>106 ± 3†</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>118 ± 1†</td>
<td>102 ± 1</td>
<td>103 ± 1</td>
</tr>
<tr>
<td></td>
<td>ob/ob</td>
<td>108 ± 3†</td>
<td>94 ± 3*</td>
<td>90 ± 3†</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>100 ± 1†</td>
<td>85 ± 1</td>
<td>86 ± 1</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>ob/ob</td>
<td>142 ± 3†</td>
<td>124 ± 3</td>
<td>121 ± 3†</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>137 ± 2†</td>
<td>121 ± 2</td>
<td>122 ± 2</td>
</tr>
<tr>
<td>HS, ms</td>
<td>ob/ob</td>
<td>99 ± 1(‡)</td>
<td>116 ± 2</td>
<td>112 ± 1(‡)</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>100 ± 2(‡)</td>
<td>120 ± 3</td>
<td>114 ± 3(‡)</td>
</tr>
</tbody>
</table>

MBP, DBP, and SBP indicate mean, diastolic, and systolic BP, respectively; HP, heart period. Values are means ± SEMs, with n = 7 ob/ob mice and n = 11 +/+ mice. ANOVA: state, P < 0.001 for all variables; strain: P = 0.050 (MBP), P = 0.010 (DBP), P = 0.405 (SBP), P = 0.452 (HP); state × strain interaction: P = 0.001 (MBP, DBP, and SBP), P = 0.066 (HP).

*p < 0.05 vs W (same wake-sleep state).
†p < 0.05 vs NREMS (same mouse strain except for HP, for which brackets refer to marginal means).
The Figure shows the 24-hour profiles of mean BP and of the time spent in each wake-sleep state. During the light period, ob/ob mice had higher mean BP ($P = 0.003$) than $+/+$ mice and spent slightly more time during W ($P = 0.038$) and correspondingly less time during REMS ($P = 0.045$) with respect to $+/+$ mice. During the dark period, ob/ob mice spent less time during W ($P = 0.001$) and more time during NREMS ($P = 0.001$) and REMS ($P = 0.005$) with respect to $+/+$ mice, whereas mean BP did not differ significantly between strains ($P = 0.320$).

The 24-hour profiles of systolic and diastolic BPs were similar to that of mean BP and are shown in Figure S2, together with the 24-hour profile of the heart period. During the light period, ob/ob mice had higher diastolic BP ($P < 0.001$) but similar values of systolic BP ($P = 0.086$) and heart period ($P = 0.117$) with respect to $+/+$ mice. During the dark period, diastolic BP ($P = 0.192$), systolic BP ($P = 0.941$), and heart period ($P = 0.552$) did not differ significantly between ob/ob and $+/+$ mice.

**Discussion**

In this study, we showed that hypertensive derangements of BP occurred in leptin-deficient obese mice and were substantially modulated by the cardiovascular effects of the wake-sleep states. In ob/ob mice, we found increases in diastolic BP and mean BP with respect to $+/+$ mice (Table). Previous studies on BP in ob/ob mice yielded contrasting results, evidencing hypotension,12-13 no significant differences in BP,29,30 or hypertension limited to the light period15 with respect to lean control mice. These discrepancies bear no apparent relationship with the age, gender, and genetic background of the ob/ob mice that were studied. This is exemplified by the fact that hypertension in ob/ob mice was evidenced by the only study15 that measured BP by telemetry after prolonged postoperative recovery, as we did, regardless of differences between the mice investigated by that study15 (ie, female ob/ob mice aged 8 weeks from Jackson Laboratories) and ours (ie, male ob/ob mice aged 15 weeks from Harlan). Although telemetry is rightly considered a technique at the state of the art to measure BP in conscious freely moving mice,31 the discrepancies on BP control in obese mice lacking leptin signaling cannot be simply explained based on its adoption. In particular, obese db/db mice lacking functional leptin receptors have been reported as either hypertensive14 or hypertensive17 by using telemetry and as hypertensive by using the indirect tail-cuff method of BP measurement.16 Furthermore, although our experimental procedures were designed to minimize stress to the mice under study, we cannot exclude that ob/ob mice were more susceptible than $+/+$ mice to increases in BP induced by the surgical stress. A parsimonious explanation of the inconsistencies on the control of BP in ob/ob mice may, thus, be that, independent of leptin, obesity in these mice entails a fragility of BP control, with a proven potential for sustained hypertensive derangements. In this last respect, it should be noted that our finding of hypertension in ob/ob mice was based on the analysis of $>1100$ hours of BP recordings (see Methods section).

Combined recordings of sleep and BP have been successfully performed in few studies on mice.21,22,32 To our knowledge, our study was the first to apply this technique in a mouse model of human disease. Ob/ob mice were hypertensive with respect to $+/+$ mice during W and NREMS but not during REMS (Table). This occurred because, on passing from NREMS to REMS, BP significantly decreased in ob/ob mice, whereas BP did not differ significantly in $+/+$ mice. During REMS, central autonomic commands induce vasoconstriction in the skeletal muscles23 and vasodilatation of the mesenteric and renal vascular beds,24 the effect of these changes on BP being buffered by sino-aortic reflexes.27 The complexity of these factors generates the potential for interaction effects, whereby differences in BP on passing from NREMS to REMS reflect genetic22 and possibly also pathological derangements in cardiovascular regulation.

In agreement with previous studies,15,19 the differences in mean BP and those in the time spent in each wake-sleep state between ob/ob and $+/+$ mice depended on the light-dark period (Figure). At variance with previous studies,15,19 we simultaneously measured the 24-hour profile of BP and those of wake-sleep states in ob/ob mice (Figure) and combined this information with the analysis of the BP values in each given wake-sleep state (Table). We could, thus, demonstrate that the difference in mean BP between ob/ob and $+/+$ mice was reduced and became nonsignificant during the dark (active) period because ob/ob mice spent a lower fraction of this period awake with respect to $+/+$ mice. During the light (rest) period, conversely, the fact that ob/ob mice spent more time awake and correspondingly less time in REMS than $+/+$ mice slightly enhanced their difference in BP with respect to $+/+$ mice (Figure). The differences in BP between W and either NREMS or REMS were in fact substantial both in ob/ob and $+/+$ mice (Table) and comparable in magnitude with the average nightly decline of systolic BP in normal human subjects.33

Taken together, these observations indicate that sleep-dependent changes in BP buffered hypertension during REMS (Table) and the dark period (Figure) in ob/ob mice. Data, thus, supported our hypothesis that cardiovascular effects of the wake-sleep states are a relevant source of variability in the derangements of BP, which are associated with obesity in the absence of leptin signaling. This hypothesis fits well with recent findings that, in obese db/db mice lacking functional leptin receptors, the dark (active) period entails both an increase in the amount of NREMS time28 and a reduced severity of hypertension17 with respect to lean control mice. Nonetheless, our data remain preliminary, and additional studies to extend our findings are needed. In particular, systematic simultaneous recordings of sleep and BP are needed to determine whether sleep also modulates the hypotensive BP derangements, which may occur in ob/ob12,13 and db/db14 mice.

The increase in the amount of NREMS time during the dark (active) period, which we and others19,20 observed in obese mice with congenital impairment of leptin signaling, also occurs in mice with diet-induced obesity and functional leptin alleles.34 Intriguingly, excessive daytime sleepiness frequently occurs in obese human subjects even in the
absence of sleep apnea, which is a potential causative factor. Obesity in human subjects is clearly associated with self-reported short sleep duration but not with objective polysomnographic measurements of sleep duration, indicating that self-reported short sleep may largely be a marker of emotional stress and subjective sleep disturbances in obese human subjects. Thus, both the nature of the link between obesity and sleep and its invariance among species remain unclear. On the other hand, sleep-dependent cardiovascular changes including the decrease in BP and the increased role of the baroreflex in cardiac control during NREMS are highly conserved across species, supporting the potential for extrapolation of our results. In this respect, our finding that obesity, per se (ie, in the absence of leptin), entailed increases in mean BP, which were prominent during the light (rest) period and also occurred during NREMS, may be of particular relevance. In rats with 2-kidney, 1-clip hypertension that were subjected to different regimens of captopril treatment, the best predictor of cardiac hypertrophy was BP during the light (rest) period.41 In patients referred for ambulatory BP monitoring, nighttime (sleep) BP significantly controls not only during the day but also during the night.45 Thus, both the nature of the link between obesity and sleep and its invariance among species remain unclear.

Perspectives

The results of our study suggest that, in the absence of leptin, obesity may entail hypertensive derangements of BP. Because such derangements manifest not only during W but also during NREMS, they may have substantial relevance for cardiovascular health. Our study also demonstrates the value of teasing out W, NREMS, and REMS when investigating BP derangements, the magnitude of which is comparable to that of the physiological sleep-dependent changes in BP. This approach may be particularly useful in preclinical research when the genetic mutation under study is known or suspected to alter sleep structure.

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Disclosures

None.

References


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EXPANDED MATERIALS AND METHODS

Experiments were performed on male B6.V-Lep^{ob/ob}/OlaHsd mice (ob/ob), which are homozygous for a nonsense mutation in the leptin gene, and their male B6.V-Lep^{+/+}/OlaHsd littermate mice with two functional leptin alleles (+/+). All mice were purchased from Harlan Italy (S. Pietro al Natisone, Udine, Italy). The genotype of +/+ mice was assessed in the facilities of the Centre for Applied Biomedical Research – CRBA, S. Orsola University Hospital, Bologna, Italy. DNA was extracted from biotissue, amplified by polymerase chain reaction using oligonucleotides 5'-TGTCCAAGATGGACCAGACTC-3' and 5'-ACTGGTCTGAGGCAGGGAGCA-3', digested with DdeI restriction enzyme (Promega Italy, Milano, Italy) and resolved by gel electrophoresis. Mice were kept on a light-dark cycle of 12-hour periods with ambient temperature set at 25°C and free access to water and food (18.9 % proteins, 5.7 % fat, 57.3 % carbohydrates; 2018 diet, Harlan Italy).

The study protocol was approved by the Bologna University ethics committee on animal experimentation and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Surgical Procedures

Mice underwent surgery under general anesthesia (isoflurane, Abbott, Latina, Italy, 1-2%, N₂O 70%, and balance O₂) and sterile conditions and with the body temperature maintained at 37°C with a heating pad. A telemetric blood pressure (BP) transducer (TA11PA-C10, DSI, Tilburg, the Netherlands; 1.4 g weight) was implanted subcutaneously on the right flank, with the catheter inserted through the right femoral artery into the abdominal aorta. A pair of Teflon-coated stainless-steel electrodes (Cooner Wire, Chatsworth, CA, USA) were positioned in contact with the dura mater through burr holes in the frontal and parietal bones to obtain a differential electroencephalographic (EEG) signal. A second pair of these electrodes was inserted bilaterally in the nuchal muscles to obtain a differential electromyographic (EMG) signal. All electrodes were connected to a miniature custom-built socket, which was cemented to the skull with stainless-steel anchor screws (2.4 mm length, Plastics One, Roanoke, VA, USA), dental cement (Rely X ARC, 3M ESPE, Segrate, Milano, Italy), and dental acrylic (Respal NF, SPD, Mulazzano, Italy). Excluding the BP transmitter, the total weight of the implanted materials was 1.55 ± 0.05 g (mean ± SEM, n = 18).

Experimental protocol

During a 4 days’ period of recovery from surgery, mortality was 43 % and 29 % in ob/ob and +/+ mice, respectively. During the subsequent periods of habituation to the recording apparatus (7 days) and recordings (3 days), animals regained weight and mortality was nil in either group (ob/ob mice, n = 7; +/+ mice, n = 11). Recordings consisted of the simultaneous and continuous measurements of BP, EEG, EMG, and activity on mice undisturbed and freely-moving in their own cages. In 4 +/+ mice, recordings were limited to 25, 47, 57, and 59 hours, respectively, due to failure of the EEG electrodes. At the end of the recordings, mice were aged 15.0 ± 0.5 weeks (mean ± SEM, n = 18). The TA11PA-C10 transducer transmitted the BP signal by means of radio waves to a receiver (RPC-1, DSI) below the animal’s cage. The BP signal was then routed to a calibrated BP analog adapter (R11CPA, DSI) with compensation for barometric pressure (APR-1, DSI). The RPC-1 receiver also yielded an activity signal by quantifying the spatial shifts of the transducer due to mouse movements. The EEG and EMG signals were transmitted via cable. A rotating swivel (SL2+2C/SB, Plastics One) and a balanced suspensor arm prevented the
cable from twisting and counterbalanced its weight, thus allowing unhindered movements to
the mice.

The EEG and EMG signals were amplified and filtered (EEG: 0.3-100 Hz; EMG: 100-1000
Hz; 7P511J amplifiers, Grass, West Warwick, RI, USA). All signals were digitized at 16-bit
and 1024 Hz (PCI-6224 board, National Instruments, Austin, TX, USA). The EEG, EMG,
and locomotion signals were down-sampled at 128 Hz and stored together with the 1024 Hz
BP signal. Calibration of the telemetric BP transducers was performed against a high-
precision manometer (PCE P05, PCE Italy, Gragnano, Lucca, Italy) before implantation and
after the termination of the recordings. Data acquisition was performed by means of custom
software written in Labview (National Instruments).

**Discrimination of the wake-sleep states**

Data analysis was performed with custom software developed in Matlab and its Signal
Processing Toolbox (The MathWorks Inc., Natick, MA, USA).

A visual scoring of the wake-sleep states was performed by 2 trained investigators (C.B. and
S.B.) on all consecutive 4-s epochs. The scoring was based on EEG and EMG recordings
following criteria that were adopted in previous reports on rats by our group and that were
adjusted for application on mice. Wakefulness (W) was scored when the EMG tone was high
and the EEG was at low voltage with possible δ (0.5-4 Hz) and θ (6-9 Hz) frequency
components. Non-rapid-eye-movement sleep (NREMS) was scored when the EMG tone was
lower than in W and the EEG was at high voltage with prominent δ frequency components.
Rapid-eye-movement sleep (REMS) was scored when the EMG indicated muscle atonia with
occasional muscle twitches and the EEG was at low voltage with predominant θ frequency
components (Figure S1).

Artifacts in the EEG and EMG signals impeded the visual scoring of the wake-sleep states in
less than 0.1% of recording time in either mouse strain. The EEG and EMG signals showed
features intermediate between different wake-sleep states in 3.7 ± 0.4 % (ob/ob mice, mean ±
SEM, n = 7) and 3.7 ± 0.3 % (+/+ mice, mean ± SEM, n = 11) of the recording time. In
preliminary experiments on 4 mice, the individual differences in scoring between the 2
scorers amounted to 4.5 ± 1.3 %, 4.0 ± 0.3 % and 13.3 ± 2.9 % (mean ± SEM, n = 4) of the
consensually-scored recording time in W, NREMS, and REMS, respectively.

**Analysis of cardiovascular variables**

While performing the visual scoring of the wake-sleep states, 2 trained investigators (C.B.
and S.B.) identified the 4-s epochs, in which noise in the pulse waveforms made the BP
signal of insufficient quality to allow an accurate automatic determination of the systolic and
diastolic BP and of heart period. This identification was performed on the basis of the
standardized time series of BP in a 12-s window centered on the 4-s epoch under evaluation,
and hence with the investigators blind to the mean value of BP. In ob/ob mice, artifacts in the
BP signal represented 8.2 ± 3.4 %, 3.3 ± 2.2 %, and 1.9 ± 1.6 % (mean ± SEM, n = 7) of the
total duration of W, NREMS, and REMS, respectively. In +/+ mice, the corresponding values
were 9.1 ± 2.9 %, 0.3 ± 0.1 %, and 0.2 ± 0.1 % (mean ± SEM, n = 11). A subsequent semi-
automated procedure was performed to identify the 4-s epochs, in which errors in the
automatic detection of the minima and maxima of the pulse waves produced artifactual
values of heart period, systolic BP, diastolic BP, or mean BP greater than twice the respective
median values or lower than 10 % of the respective median values. After completion of this
procedure, the artifacts in ob/ob mice represented 8.5 ± 3.4 %, 3.6 ± 2.2 %, and 2.0 ± 1.5 %
(mean ± SEM, n = 7) of the recording time spent in W, NREMS, and REMS, respectively.
The corresponding values in +/+ mice were 9.1 ± 2.9 %, and 0.3 ± 0.1 %, and 0.2 ± 0.1 %
(mean ± SEM, n = 11).
Beat-to-beat values of systolic BP, diastolic BP, mean BP (i.e., the average BP in each cardiac cycle), and heart period (i.e., the time between the onset of successive systolic upstrokes) were computed from the raw BP signal in each artifact-free 4-s epoch. For each mouse, these values were averaged within consecutive 1-h periods, within light and dark periods, or within each wake-sleep state for the purpose of different analyses. Overall, the analysis was performed on 185.6, 245.2, and 33.8 hours of artifact-free recordings during W, NREMS, and REMS in ob/ob mice and on 283.7, 308.2, and 49.0 hours of artifact-free recordings during W, NREMS, and REMS in +/+ mice.

**Statistical analysis**
Data were analyzed by analysis of variance (ANOVA, GLM procedure with mixed-model design). The between-subject factor was the mouse strain (2 levels). In different analyses, the within-subject factor was either the wake-sleep state (3 levels) or the light-dark period (2 levels). In case of a significant state x strain interaction effect, the effect of the wake-sleep state was tested in each mouse strain with a one-way ANOVA and the simple effect of the mouse strain was tested in each wake-sleep state with t-tests. In case of a significant effect of the wake-sleep state, 2 pre-planned comparisons (NREMS vs. W and REMS vs. NREMS) were performed with paired-sample t-tests. In case of a significant light-dark period x strain interaction effect, simple effects were tested with t-tests. Statistical analyses were performed with the SPSS software (SPSS Inc., Chicago, IL, USA) with P < 0.05 considered to be statistically significant. Data are reported as mean ± SEM with n = 7 for ob/ob mice and n = 11 for +/+ mice.
REFERENCES


FIGURE S1

BP
EEG
EMG
ACT

W

BP
EEG
EMG
ACT

NREMS

BP
EEG
EMG
ACT

REMS
FIGURE S2

Time (hours)

DBP

SBP

HP

ob/ob

+/+

†††

††

††

††
FIGURE LEGENDS

Figure S1
Representative recordings obtained during wakefulness (W), non-rapid-eye-movement sleep (NREMS) and rapid-eye-movement sleep (REMS) in the same obese leptin-deficient mouse. Each recording comprises 3 epochs of 4-s duration and is shown in the graphical format, on which the visual scoring of the wake-sleep states was performed. The blood pressure (BP) signal was standardized (i.e., divided by its standard deviation after subtraction of the mean value) within the 12-s period under observation. The electroencephalographic (EEG), electromyographic (EMG), and locomotor activity (ACT) signals are shown in arbitrary units.

Figure S2
Values of diastolic blood pressure (DBP), systolic blood pressure (SBP), and heart period (HP) expressed as 24-hour profiles (left panels) or as averages in the light (L) and dark (D) periods (right panels). Data are mean ± SEM in leptin deficient obese mice (ob/ob, white bars, n = 7) and lean control mice (+/+, black bars, n = 11). ANOVA: light-dark period: P < 0.001 for all variables; mouse strain: P = 0.010 (DBP), P = 0.386 (SBP), P = 0.618 (HP); light-dark period x mouse strain interaction: P < 0.001 for all variables. *, P < 0.05 vs. ob/ob mice (same light-dark period); †, P < 0.05 vs. dark period (same mouse strain).