Dysregulation of Heart Rhythm During Sleep in Leptin-Deficient Obese Mice

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Study Objectives: Sleep deeply affects cardiac autonomic control, the impairment of which is associated with cardiovascular mortality. Obesity entails increased cardiovascular risk and derangements in sleep and cardiac autonomic control. We investigated whether cardiac autonomic control is impaired during sleep in ob/ob mice with morbid obesity caused by congenital leptin deficiency.

Design: Indexes of cardiac autonomic control based on spontaneous cardiovascular fluctuations were compared between ob/ob and lean wild-type (+/+ ) mice during wakefulness, non-rapid eye movement sleep (NREMS), and rapid eye movement sleep (REMS).

Setting: N/A

Patients or Participants: 7 ob/ob and 11 +/+ male mice.

Interventions: Instrumentation with electrodes for sleep recordings and a telemetric transducer for measuring blood pressure and heart period.

Measurements and Results: In ob/ob mice, the variability of heart period and cardiac baroreflex sensitivity (sequence technique) were significantly lower than in +/+ mice during each wake-sleep state. The vagal modulation of heart period was significantly weaker in ob/ob than in +/+ mice during NREMS and REMS. In ob/ob mice, the cross-correlation function between heart period and blood pressure suggested that the baroreflex contribution to cardiac control was lower than in +/+ mice during wakefulness and NREMS, whereas the contribution of central autonomic commands was lower than in +/+ mice during NREMS and REMS.

Conclusions: These data indicate a dysregulation of cardiac autonomic control during sleep in ob/ob mice. Ob/ob mice may represent a useful tool to understand the molecular pathways that lead to cardiac autonomic dysregulation during sleep in obesity.

Keywords: Obesity, leptin, sleep, autonomic nervous system, heart rate, blood pressure, baroreflex

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IMPAIRED IN CARDIAC AUTONOMIC CONTROLS REVEALED BY INDEXES OF HEART RATE VARIABILITY OR THE CARDIAC BAROREFLEX ARE POWERFUL negative prognostic factors in patients with cardiovascular disease.1-3 Derangements in these indexes may be a marker of other cardiovascular risk factors4 and also play a pathogenic role because vagal activation may protect against life-threatening cardiac arrhythmias.5

Sleep exerts a deep physiological impact on cardiovascular regulation.6 In particular, cardiac vagal tone and modulation7-8 and the baroreflex contribution to cardiac control9-10 are enhanced during non-rapid eye movement sleep (NREMS) with respect to wakefulness and rapid eye movement sleep (REMS). The physiological enhancement of cardiac vagal modulation in NREMS may help explain the reduced nocturnal incidence of sudden cardiac death.11 Conversely, patients with recent myocardial infarction are at high risk for sudden death and lose the capability for physiological activation of vagal activity during NREMS.12 On the other hand, the effects of sleep on cardiac autonomic control may differ between physiological and pathological conditions.9,13 Thus, assessment of pathological impairments in cardiac autonomic control may be improved by taking into account the wake-sleep behavior.

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gene, and on their male B6.V-Lep+/+/OlaHsd littermates with 2 functional leptin alleles (+/+, n = 11). The genotype of +/+ mice was assessed by PCR in the Centre for Applied Biomedical Research – CRBA, S. Orsola University Hospital, Bologna, Italy. Mice were kept on a 12:12 hour light-dark cycle with ambient temperature set at 25°C and free access to water and food (2018 diet, Harlan Italy).

Experimental Procedures

Experimental procedures for surgery, physiological recordings, and sleep scoring have been published in detail.21 Mice underwent surgery under general anesthesia for the implantation of a telemetric blood pressure (BP) transducer (TA11PAC10, DSI, Tilburg, the Netherlands), with the catheter inserted through the femoral artery into the abdominal aorta. Pairs of electrodes were positioned in contact with the dura mater and with muncal muscles to obtain electroencephalographic (EEG) and electromyographic (EMG) signals, respectively, which were transmitted by conventional cable transmission. After an 11-day period of recovery and habituation, BP (sampling rate 1024 Hz), EEG, and EMG were simultaneously and continuously measured on mice undisturbed in their own cages for 3 days. An electrical swivel and a balanced suspensor arm prevented the cable from twisting and counterbalanced its weight, allowing unhindered movements to the mice. At the end of recordings, mice were aged 15.0 ± 0.5 weeks and ob/ob mice weighted approximately twice as much as +/+ mice (48.6 ± 0.8 versus 25.5 ± 0.7 g). The states of wakefulness, NREMS, and REMS were visually scored off-line on 4-s epochs based on raw EEG and EMG signals. In 4 +/+ mice, failure of the EEG electrodes limited the analysis of wake-sleep episodes to the first 25, 47, 57, and 59 hours of recordings, respectively.

Data Analysis

The main analysis was performed on all artifact-free episodes of wakefulness, NREMS, and REMS of duration ≥ 60 s, which occurred during the 3 days of recordings. The duration requirement of ≥ 60 s allowed to compute validated indexes of autonomic control in the frequency domain.22 Ancillary analyses were performed to assess the replicability of significant findings across days as well as across the light and dark periods.

Beat-to-beat values of heart period (HP) and of systolic, diastolic, and mean BP were computed from the raw BP signal. Values of HP were computed as the time intervals between the onset of successive systolic BP upstrokes, i.e., between the minimum value of a pulse wave and the minimum value of the following one. A semi-automated procedure was performed to identify and exclude from subsequent analyses each 4-s epoch, in which errors in the automatic detection of the minima and maxima of the pulse waves produced artificial values of HP or of systolic, diastolic, and mean BP greater than twice the respective median values or lower than 10% of the respective median values within wake-sleep episode.21

The total variability of HP was quantified by the standard deviation of HP values (SDHP). The vagal modulation of HP was assessed in the time domain with the index pNN8, which corresponds to respiratory sinus arrhythmia and is based on the spectral power of HP in the frequency range 2.5–5.0 Hz.22 A validated index of the sympathetic modulation of BP (SYM) was also computed based on the spectral power of systolic BP in the frequency range 0.15–0.6 Hz.22

The cardiac baroreflex sensitivity (BRS) and the baroreflex effectiveness index (BEI) were computed within each wake-sleep episode with the sequence technique.24 In correspondence with short spontaneous sequences of increases or decreases in beat-to-beat BP values, the indexes BRS and BEI estimate the gain (i.e., the change in HP, which is associated with a unit change in systolic BP) and degree of engagement (i.e., the fraction of BP sequences, which are associated with baroreflex changes in HP) of the baroreflex, respectively. To investigate the contributions of the baroreflex and central autonomic commands to cardiac control at a longer time scale, we analyzed the cross-correlation function (CCF) between HP and systolic BP.9,10,25 The CCF yields the correlation coefficient between HP and systolic BP as a function of the time shift between these variables. Negative values of time shift indicate that changes in HP follow those in BP.

Cardiac autonomic control during the phasic hypertensive events (BP surges) of REMS was investigated with a coherent averaging procedure.15,25 The BP surges in REMS were automatically detected with a 5 mm Hg threshold for peak increase in systolic BP. After subtraction of their respective baseline values, time series of HP and systolic BP during the BP surges were synchronized at the peak increase in systolic BP and averaged.

The indexes SDHP, pNN8, BRS, and BEI were computed based on beat-to-beat values of HP and systolic BP. The other analyses were performed after re-sampling the time series of HP and systolic BP at 20 Hz with a piecewise cubic Hermite interpolation. The CCF and the BP surges in REMS were analyzed after low-pass filtering the time series of HP and BP below 0.8 Hz (3-pole Butterworth filter) to focus the analysis on fluctuations slower than the breathing rate.9,10,12,25 The CCF analysis and the indexes SDHP, pNN8, RSA, and SYM were averaged over consecutive data subsets of 60-s duration overlapped for 45 s. Data analysis was performed with custom software written in Matlab (the Mathworks, Inc., Natick, MA, USA).

Statistical Analysis

With the exception of CCF time shifts, which were not normally distributed, data were reported as mean ± SEM and analyzed with 2-way mixed model analysis of variance (ANOVA, GLM procedure) and t-tests. The CCF time shifts were reported as median and interquartile range and analyzed with the binomial single-sample sign test (cutoff value of 0; probability parameter of 0.5) to evaluate whether they significantly clustered at negative or positive values.10 Data were reported with n = 7 for ob/ob mice and n = 11 for +/+ mice. Analyses were performed with SPSS software (SPSS Inc., Chicago, IL, USA) with P < 0.05 considered statistically significant.

RESULTS

Table 1 shows the percentage of recording time spent in each wake-sleep state and the characteristics of the wake-sleep episodes, on which the analysis of cardiovascular variables was
performed. The mean values of HP and BP in these episodes are reported in Table 2.

The total variability of HP, as assessed with the index SDHP, was significantly lower in ob/ob than in +/+ mice in each wake-sleep state (P < 0.001; Figure 1, panel A).

The indexes of cardiac vagal modulation computed in the time (pNN8) and frequency (RSA) domain are reported in panels B and C of Figure 1, respectively. The indexes pNN8 and RSA were significantly lower in ob/ob than in +/+ mice in NREMS (P = 0.007 and P = 0.008, respectively) and REMS (P = 0.008 and P = 0.030, respectively), but not in wakefulness (P = 0.085 and P = 0.100, respectively).

The sympathetic modulation of BP, as assessed with the index SYM, did not differ significantly between ob/ob and +/+ mice (Figure 1, panel D).

The results of the assessment of the spontaneous cardiac baroreflex with the sequence technique are shown in Figure 2. The indexes BRS and BEI were significantly lower in ob/ob than in +/+ mice in each wake-sleep state (P < 0.002).

The average CCFs between HP and systolic BP are shown in Figure 3. The maximum (ρ\\text{MAX}) and minimum (ρ\\text{MIN}) values of the CCFs and their corresponding time shifts (τ\\text{MAX} and τ\\text{MIN}) are reported in Table 3. A CCF maximum at negative time shifts (i.e., a positive correlation between HP and the previous BP values) occurred in all wake-sleep states in +/+ mice, whereas in ob/ob mice, it was evident only in REMS. Accordingly, the values of τ\\text{MAX} in wakefulness and NREMS showed a wide dispersion in ob/ob mice. Moreover, ρ\\text{MAX} was significantly lower in ob/ob than in +/+ mice in wakefulness and NREMS (P < 0.001) but not in REMS (P = 0.276). A CCF minimum at positive time shifts (i.e., a negative correlation between HP and the following BP values) occurred in all wake-sleep states in +/+ mice, whereas in ob/ob mice, it was not evident in REMS. Accordingly, the values of τ\\text{MIN} in REMS showed a wide dispersion in ob/ob mice. In ob/ob mice, ρ\\text{MIN} was significantly lower in magnitude than in +/+ mice in NREMS (P < 0.001) and REMS (P = 0.002) but not in wakefulness (P = 0.582).

The results of the coherent averaging of BP surges during REMS are shown in Figure 4 and Table 4. With respect to +/+ mice, ob/ob mice had a similar rate of occurrence of BP surges in REMS (P = 0.536) and a slight reduction in the peak increase in BP during the surges (P = 0.050). The peak increase in BP was preceded by a decrease in HP in +/+ mice but not in ob/ob mice (P = 0.001 vs. +/+ mice). The peak increase in BP was followed by an increase in HP, the peak value of which was significantly lower in ob/ob than in +/+ mice (P = 0.026).

The significant differences, which occurred between ob/ob and +/+ mice on the whole 3-day recording period, generally showed a complete replicability across recording days as well as across the light and dark periods in ancillary analyses (indexes SDHP, BRS, BEI, ρ\\text{MAX}, ρ\\text{MIN}, and the HP decrease during BP surges in REMS; P < 0.050). Differences in pNN8 and RSA between ob/ob and +/+ mice also showed replicability across recording days as well as across the light and dark periods (P < 0.050) with the exceptions of differences in REMS in the third recording day (P = 0.067 and P = 0.158, respectively) and the dark period (P = 0.161 and P = 0.224, respectively). The reduction in the HP rise during the BP surges in REMS in ob/ob mice lost statistical significance in the ancillary analyses restricted to the second (P = 0.215) or the third (P = 0.075) recording day or to the dark period (P = 0.357).

DISCUSSION

We tested the hypothesis that cardiac autonomic control is impaired during sleep in ob/ob mice. This hypothesis was fully supported by our findings.

To our knowledge, our study was the first to investigate cardiac autonomic control during sleep in a mouse model of human disease. We assessed cardiac autonomic control based on spontaneous cardiovascular variability. This approach is inherently indirect and cannot discriminate between derangements in cardiac autonomic nerve activity and derangements in the cardiac response to such activity. On the other hand, this approach informs on real-life cardiac regulation and lends itself optimally to clinical translation, yielding indexes with prognostic significance in human patients.1,3

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**Table 1**—Characteristics of the wake-sleep episodes recorded and analyzed

| All episodes | W | NREMS | REMS |
|--------------|---|-------|------|
| % recording time | ob/ob | +/+ | ob/ob | +/+ | ob/ob | +/+ |
| Episodes > 60 s analyzed | | | | | | |
| % recording time | 40 ± 2 | 45 ± 2 | 50 ± 1 | 44 ± 1 | 7 ± 1 | 7 ± 1 |
| Number | 343 ± 23 | 383 ± 33 | 824 ± 30 | 645 ± 56 | 115 ± 12 | 101 ± 10 |
| Mean duration (s) | 210 ± 20 | 172 ± 20 | 104 ± 4 | 109 ± 4 | 100 ± 3 | 106 ± 1 |
| Total duration (h) | 19.9 ± 19.7 ± 2.3 | 23.8 ± 1.2 19.7 ± 2.0 | 3.2 ± 0.4 | 3.0 ± 0.3 |

W, wakefulness; NREMS, non-rapid eye movement sleep; REMS, rapid eye movement sleep. Data are means ± SEM in ob/ob (n = 7) and +/+ (n = 11) mice.

**Table 2**—Mean values of cardiovascular variables

| W | NREMS | REMS |
|---|-------|------|
| SBP (mm Hg) | 144 ± 3 | 138 ± 2 | 123 ± 3 | 120 ± 2 | 121 ± 3 | 122 ± 2 |
| DBP (mm Hg) | 109 ± 2 | 101 ± 1* | 93 ± 3 | 84 ± 1* | 90 ± 3 | 86 ± 1 |
| MBP (mm Hg) | 126 ± 2 | 119 ± 1* | 108 ± 3 | 101 ± 1* | 105 ± 3 | 103 ± 1 |
| HP (ms) | 98 ± 1 | 98 ± 2 | 116 ± 2 | 122 ± 3 | 112 ± 1 | 113 ± 3 |

SBP, DBP, and MBP indicate systolic, diastolic, and mean blood pressure, respectively. HP, heart period. W, wakefulness; NREMS, non-rapid eye movement sleep; REMS, rapid eye movement sleep. Data are means ± SEM in ob/ob (n = 7) and +/+ (n = 11) mice. Analysis of variance: state, P < 0.001 for all variables; strain, P = 0.397 (SBP), P = 0.010 (DBP), P = 0.049 (MBP), P = 0.465 (HP); state × strain interaction, P < 0.001 (SBP, DBP, MBP), P = 0.19 (HP), *P < 0.05 vs. ob/ob mice (t-test).
The vagal modulation of HP is specifically targeted by the indexes pNN8 and RSA in mice. However, the indexes SDHP and BRs also depend on the vagal modulation of HP in this species. In ob/ob mice, all of these indexes were lower than in +/+ mice during NREMS and REMS (Figure 1 and Figure 2), although the replicability of the difference in pNN8 and RSA during REMS was limited to the light period. On the other hand, the indexes SDHP (Figure 1) and BRs (Figure 2) were also lower in ob/ob than in +/+ mice during wakefulness. These findings indicate a dramatic impairment in the vagal modulation of HP in ob/ob mice, which is particularly prominent in NREMS and, during the light period, also in REMS. Moreover, reductions in spontaneous BRs estimates may effectively reflect reductions in the classical pharmacological estimates of BRs in mice.

To our knowledge, no validated index of cardiac sympathetic modulation is available in mice. However, a validated index of the sympathetic modulation of BP had similar values in ob/ob and +/+ mice in each wake-sleep state (Figure 1, index SYM). The mean value of HP, which reflects the balance among intrinsic HP and the effects of cardiac vagal and sympathetic tone, was also similar in ob/ob and +/+ mice in each wake-sleep state (Table 2). These data argue against any significant impairment in cardiac autonomic tone or sympathetic modulation during sleep and wakefulness in ob/ob mice.

We performed an array of analyses to test whether cardiac regulation by baroreflex and non-baroreflex mechanisms is impaired in ob/ob mice in the different wake-sleep states. Over a time scale of few (i.e., 3-4) heart beats, the values of index BEI (Figure 2) indicate that the contribution of the baroreflex to HP...
control was significantly and substantially lower in ob/ob than in +/+ mice in each wake-sleep state. On a longer time scale, a positive CCF peak at negative time shifts indicates, e.g., a pattern of cardiac slowing after an increase in BP, which is consistent with the operation of the arterial baroreflex. In +/+ mice in each wake-sleep state, such positive peak was clearly evident in the average CCFs (Figure 3) and was highly reproducible among mice, as shown by the small dispersion (interquartile range) of the corresponding time shifts (τ_{MAX} Table 3).

In ob/ob mice, the CCFs did not show any reproducible positive peak in wakefulness and NREMS, as demonstrated by the wide dispersion of the τ_{MAX} time shifts in these states. Moreover, the values of the CCF maxima (ρ_{MAX}, Table 3) were significantly lower in ob/ob than in +/+ mice during wakefulness and NREMS, whereas they did not differ between ob/ob and +/+ mice in REMS. These findings suggest that at a longer time scale than that explored by the BEI index, the baroreflex contribution to HP control is impaired in ob/ob mice in wakefulness and NREMS, but not in REMS. On the other hand, the negative CCF trough at positive time shifts indicates, e.g., a pattern of cardiac acceleration before an increase in BP, which is consistent with the operation of central autonomic commands.

In REMS, this negative CCF trough was not detectable in ob/ob mice (Figure 3 and Table 3). In ob/ob mice, moreover, the CCF minimum values (ρ_{MIN}) had significantly lower magnitude than in +/+ mice during NREMS (Table 3). These findings suggest that the contribution of central autonomic commands to HP control over a time scale of 1 minute was lower in ob/ob than in +/+ mice during the sleep states (NREMS and REMS), whereas it was maintained in wakefulness. During wakefulness and REMS, the pattern of impairment in cardiac regulation, which was evidenced by the CCF analysis in ob/ob mice, was the same as that previously reported in spontaneously hypertensive rats, which are a widely studied model of human hypertension. Notably, spontaneously hypertensive rats also show sleep related derangements in cardiac vagal modulation.

In spontaneously hypertensive rats, the impaired contribution of central commands to HP control during REMS was supported by the analysis of the phasic hypertensive events (BP surges), which are a physiological feature of this state.

Similarly, in the present study, the impaired contribution of central commands to HP control during REMS in ob/ob mice was supported by the analysis of the BP surges in REMS. A cardiac acceleration before the peak of BP surges is consistent with the central autonomic commands on HP prevailing upon the baroreflex. This cardiac acceleration was observed in +/+ mice, whereas it was absent in ob/ob mice (Figure 4 and Table 4). Conversely, the cardiac slowing after the peak of BP surges, which is consistent with baroreflex control of HP

![Figure 3](image-url) — Cross-correlation functions between low-frequency (< 0.8 Hz) fluctuations of heart period and systolic blood pressure. Data are means ± SEM during wakefulness (W), non-rapid eye movement sleep (NREMS), and rapid eye movement sleep (REMS) in ob/ob mice (red lines, n = 7) and +/+ mice (blue lines, n = 11).

| Table 3—Maximum and minimum values of the cross-correlation functions between heart period and systolic blood pressure and their corresponding time shifts |
|----------------------------------------------------------|
| Maximum and minimum CCF values | W | NREMS | REMS |
|-------------------------------|---|-------|------|
| ρ_{MAX} | ob/ob | +/+ | ob/ob | +/+ | ob/ob | +/+ |
| 0.05 ± 0.01 | 0.26 ± 0.02* | 0.18 ± 0.04 | 0.40 ± 0.03* | 0.18 ± 0.03 | 0.23 ± 0.03 |
| ρ_{MIN} | −0.19 ± 0.02 | −0.17 ± 0.02 | −0.12 ± 0.02 | −0.31 ± 0.02* | −0.04 ± 0.01 | −0.14 ± 0.02* |
| CCF time shifts | | | | | | |
| τ_{MAX} (s) | −8.7 (9.2)† | −5.6 (0.1)† | −7.5 (5.6)† | −0.6 (0.1)† | −0.4 (0.2)† | −5.6 (11.8)† |
| τ_{MIN} (s) | 0.9 (0.4)† | 0.9 (0.6)† | 0.6 (0.3)† | 0.9 (0.2)† | 0.7 (1.6)† | 1.4 (0.3)† |

Maximum and minimum values (ρ_{MAX} and ρ_{MIN}, respectively) and corresponding time shifts (τ_{MAX} and τ_{MIN}, respectively) of the cross-correlation functions (CCF) between low-frequency (< 0.8 Hz) fluctuations of heart period and systolic blood pressure. W, wakefulness; NREMS, non-rapid eye movement sleep; REMS, rapid eye movement sleep. Data are expressed as means ± SEM (ρ_{MAX}, ρ_{MIN}) or as median values and interquartile range (τ_{MAX}, τ_{MIN}) in ob/ob (n = 7) and +/+ (n = 11) mice. Analysis of variance: state and strain, P < 0.001 (ρ_{MAX}, ρ_{MIN}); state × strain interaction, P = 0.002 (ρ_{MAX}), P < 0.001 (ρ_{MIN}); *P < 0.05 vs. ob/ob mice (ρ_{MAX}, ρ_{MIN}; t-test), †P < 0.05 (τ_{MAX}, τ_{MIN}; binomial test).
controls were also observed on 24-h recordings, but the latter finding fell short of statistical significance at night. At night, however, the total variability of HP and cardiac vagal modulation in obese humans are more resistant to improvement following weight loss than during the day. The impairment in cardiac vagal modulation, which we documented during sleep states in ob/ob mice, bears a notable resemblance to the complete loss of sleep related vagal activation in human patients with a recent myocardial infarction, which are at risk of lethal arrhythmic events. This similarity emphasizes the need for clinical investigation on differences in cardiac autonomic control between obese and lean human subjects during specific sleep states.

Ob/ob mice develop massive obesity even when fed a standard diet because of congenital lack of leptin. These mice show metabolic syndrome traits, including hypertension, dyslipidemia and type 2 diabetes, as well as sleep disturbances, which resemble excessive daytime sleepiness in obese humans. A functional impairment in cardiac vagal modulation also occurs in massively obese db/db mice with dysfunctional leptin receptors. Conversely, cardiac vagal modulation is not reduced either in C57BL/6J mice with mild diet-induced obesity or in non-obese diabetic mice with overt type 1 diabetes and autonomic neuropathy. However, cardiac autonomic control was investigated in these mouse models of obesity and/or diabetes without controlling for wake-sleep states. In the present study, we found that indexes of cardiac autonomic control either differed significantly among wake-sleep states or displayed significant interaction effects between the wake-sleep state and the mouse strain (Figs. 1 and 2, Tables 2 and 3). These results demonstrate that the effects of disease or mutations on cardiac autonomic control may vary significantly as a function of the wake-sleep state in mice. Based on our findings in the different wake-sleep states, ob/ob mice may be a useful model to clarify which of the metabolic, endocrine, and inflammatory changes associated with massive obesity is involved in causing impairment in cardiac autonomic control during sleep. The study of ob/ob mice before the development of full-blown obesity, with food restriction or with leptin replacement therapy may also shed light on the role of leptin signaling on cardiac autonomic control in sleep. Accordingly, in leptin-resistant db/db mice, altered sleep regulation has been attributed to the deleterious effects of impaired leptin signaling. Finally, sleep disordered breathing may contribute to cardiovascular dysregulation in obesity. Ob/ob mice do not show signs of upper airway obstruction during sleep but have a depressed chemosensitivity to carbon dioxide, which mirrors a condition of obesity hypoventilation syndrome in humans.

In conclusion, the results of the present study provide compelling evidence of a dramatic dysregulation of heart rhythm in ob/ob mice, with a prominent impairment in cardiac vagal modulation during the sleep states of NREMS and REMS. By establishing ob/ob mice as a mouse model of cardiac autonom dysregulation in sleep, our results may open the way to a deeper understanding of the molecular pathways, which lead to cardiac autonomic impairment during sleep in obesity. Moreover, our results support the view that objective discrimination of wake-sleep states improves cardiovascular phenotyping in pre-clinical research on mice.
REFERENCES

1. Tsuji H, Larson MG, Venditti FJJ, et al. Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. Circulation 1996;94:2850-5.

2. La Rovere MT, Bigger JT, Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. Lancet 1998;351:478-84.

3. Johansson M, Gao SA, Friberg P, et al. Baroreflex effectiveness index and baroreflex sensitivity predict all-cause mortality and sudden death in hypertensive patients with chronic renal failure. J Hypertens 2007;25:163-8.

4. Lantelme P, Khettaba F, Custaud M-A, et al. Spontaneous baroreflex sensitivity: toward an ideal index of cardiovascular risk in hypertension? J Hypertens 2002;20:935-44.

5. Billman GE. A comprehensive review and analysis of 25 years of data from an in vivo canine model of sudden cardiac death: implications for future anti-arrhythmic drug development. Pharmacol Ther 2006;111:308-35.

6. Silvani A. Physiological sleep-dependent changes in arterial blood pressure: central autonomic commands and baroreflex control. Clin Exp Pharmacol Physiol 2008;35:987-94.

7. Zemaityte D, Varoneckas G. Heart rhythm control during sleep. Psychophysiology 1984;21:279-89.

8. Monti A, Medigue C, Nedelcoux H, Escourrou P. Autonomic control of the cardiovascular system during sleep in normal subjects. Eur J Appl Physiol 2002;87:174-81.

9. Bertocci T, Asti V, Ferrari V, et al. Central and baroreflex control of heart period during the wake-sleep cycle in spontaneously hypertensive rats. Am J Physiol 2007;293:R293-R298.

10. Silvani A, Grimaldi D, Vandi S, et al. Sleep-dependent changes in the coupling between heart period and blood pressure in human subjects. Am J Physiol 2008;294:R1686-R1692.

11. Portaluppi F, Hermida RC. Circadian rhythms in cardiac arrhythmias and opportunities for their chronotherapy. Adv Drug Deliv Rev 2007;59:940-51.

12. Vanoli E, Adamson PB, Ba-Lin MBH, Pinna GD, Lazzara R, Orr WC. Heart rate variability during specific sleep stages. A comparison of healthy subjects with patients after myocardial infarction. Circulation 1995;91:1918-22.

13. Bertocci T, Franzini C, Lenzi P, Zoccoli G, Silvani A. Surges of arterial pressure during rapid-eye-movement sleep in spontaneously hypertensive rats. Sleep 2008;31:111-17.

14. Ogden CL, Yanovski SZ, Carroll MD, Flegal KM. The epidemiology of obesity. Gastroenterology 2007;132:2087-102.

15. Lusis AJ, Attie AD, Reue K. Metabolic syndrome: from epidemiology to systems biology. Nat Rev Genet 2008;9:819-30.

16. Poirier P, Giles TD, Bray GA, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation 2006;113:898-918.

17. Karason K, Molgaard H, Wikstrand J, Sjöström L. Heart rate variability in obesity and the effect of weight loss. Am J Cardiol 1999;83:1242-7.

18. Nault I, Nadreau E, Paquet C, et al. Impact of bariatric surgery-induced weight loss on heart rate variability. Metabolism 2007;56:1425-30.

19. Skrpari I, Tentolouris N, Pererra D, Bakoyiannis C, Papazafiropoulou A, Katsilambros N. Baroreflex sensitivity in obesity: relationship with cardiac autonomic nervous system activity. Obesity 2007;15:1685-93.

20. da Costa Goncalves AC, Tank J, Friedlich A, et al. Diabetic hypertensive leptin receptor-deficient db/db mice develop cardiorenal autonomic dysfunction. Hypertension 2009;53:387-92.

21. Silvani A, Bastianini S, Bertocci T, et al. Sleep modulates hypertension in leptin-deficient obese mice. Hypertension 2009;53:251-5.

22. Baudrie V, Laude D, Elghozi J-L. Optimal frequency ranges for extracting information on cardiovascular autonomic control from the blood pressure and pulse interval spectrograms in mice. Am J Physiol 2007;292:R904-R912.

23. Laude D, Baudrie V, Elghozi J-L. Effects of atropine on the time and frequency domain estimates of blood pressure and heart rate variability in mice. Clin Exp Pharmacol Physiol 2008;35:454-7.

24. Laude D, Baudrie V, Elghozi J-L. Applicability of recent methods used to estimate spontaneous baroreflex sensitivity to resting mice. Am J Physiol 2008;294:R142-R150.

25. Silvani A, Asti V, Bojić T, et al. Sleep-dependent changes in the coupling between heart period and arterial pressure in newborn lambs. Pediatr Res 2005;57:108-14.

26. Swoap SJ, Li C, Wess J, Parsons AD, Williams TD, Overton JM. Vagal tone dominates autonomic control of mouse heart rate at thermoneutrality. Am J Physiol 2008;294:H1581-H1588.

27. da Costa Goncalves AC, Tank J, Plehm R, et al. Role of the multidomain protein spinophilin in blood pressure and cardiac function regulation. Hypertension 2008;52:702-7.

28. Kuo TB, Yang CC. Sleep-related changes in cardiovascular neural regulation in spontaneously hypertensive rats. Circulation 2005;112:849-54.

29. Campan MJ, Tagaito Y, Jenkins TP, Smith PL, Schwartz AR, O'Donnell CP. Phenotypic differences in the hemodynamic response during REM sleep in six strains of inbred mice. Physiol Genomics 2002;11:227-34.

30. Haluzik M, Colombo C, Gavrilova O, et al. Genetic background (C57BL/6J versus FVB/N) strongly influences the severity of diabetes and insulin resistance in ob/ob mice. Endocrinology 2004;145:3258-64.

31. Lusis AJ, Attie AD, Reue K. Metabolic syndrome: from epidemiology to systems biology. Nat Rev Genet 2008;9:819-30.

32. Lusis AJ, Attie AD, Reue K. Metabolic syndrome: from epidemiology to systems biology. Nat Rev Genet 2008;9:819-30.

33. Lusis AJ, Attie AD, Reue K. Metabolic syndrome: from epidemiology to systems biology. Nat Rev Genet 2008;9:819-30.

34. Lusis AJ, Attie AD, Reue K. Metabolic syndrome: from epidemiology to systems biology. Nat Rev Genet 2008;9:819-30.

35. Lusis AJ, Attie AD, Reue K. Metabolic syndrome: from epidemiology to systems biology. Nat Rev Genet 2008;9:819-30.

36. Lusis AJ, Attie AD, Reue K. Metabolic syndrome: from epidemiology to systems biology. Nat Rev Genet 2008;9:819-30.