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Development of autonomic dysfunction with intermittent hypoxia in a lean murine model

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Abstract

Intermittent hypoxia (IH) has been previously shown in a lean murine model to produce sustained hypertension and reverse the diurnal variation of blood glucose (BG). Concomitant glucose infusion attenuated the hypertension but exacerbated the BG fluctuations. In this study, cardiovascular variability analysis was employed to track the development of autonomic dysfunction in mice exposed to room air (IA) or IH, in combination with saline or glucose infusion. Baroreflex sensitivity was found to decrease in all animals, except in the control group. Low-frequency power of pulse interval spectrum, reflecting vagal activity, decreased more rapidly in glucose relative to saline while low-frequency power of blood pressure, reflecting sympathetic activity, decreased more slowly in IH relative to IA. Ultradian (~12 h) rhythmicity was substantially suppressed in IH groups. These findings suggest that IH acted to increase sympathetic activity while glucose infusion led to reduced parasympathetic activity. The combination of IH and hyperglycemia leads to progressively adverse effects on autonomic control independent of obesity.

1. Introduction

In sleep disordered breathing (SDB), periods of apnea or hypopnea alternate with periods of transient ventilatory overshoot that are accompanied or initiated by arousal from sleep, subjecting the patient to nocturnal intermittent hypoxia (IH). The evidence to date suggests that chronic exposure to IH, resulting from SDB, constitutes an independent causative factor for the development of systemic hypertension (Dempsey et al., 2010). Epidemiological studies in humans with SDB are supported by prospective animal studies, in which chronic exposure to nocturnal episodic hypoxia produced sustained daytime hypertension after several weeks (Brooks et al., 1997; Fletcher et al., 1992).

Although knowledge about the causal pathways linking SDB to hypertension remains incomplete, many studies suggest that abnormal autonomic control plays an important role (Carlson et al., 1993; Somers et al., 1995). Recent studies have also found an association

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between SDB and abnormal glucose metabolism (Ip et al., 2002; Punjabi et al., 2004; Punjabi et al., 2002). Taken together, the cumulative evidence suggests that chronic exposure to IH resulting from SDB can lead to both autonomic and metabolic dysfunction. Although a variety of underlying mechanisms could be responsible for these associations, abnormal autonomic nervous system control is likely to play an important role in facilitating or even initiating metabolic dysfunction. Activation of the sympatho-adrenal axis is known to reduce insulin sensitivity and increase blood glucose, which could then lead to hyperinsulinemia and further sympathetic over-activity (Chasens et al., 2003).

To examine the effects of IH on blood pressure and glucose regulation, a series of studies has been conducted in recent years using a chronically instrumented murine model (Iiyori et al., 2007; Li et al., 2005; Polotsky et al., 2003; Polotsky et al., 2006; Yokoe et al., 2008). Lean animals were used in order to circumvent the confounding influence of obesity. In this model, each animal was placed in a customized chamber and subjected to repetitive acute hypoxic exposures in which the inspired oxygen level was reduced from room air levels to 5–6% over a 30 s duration and changed back to room air over the subsequent 30 s (Polotsky et al., 2006). These cycles of IH were maintained for the 12 hours of the light, or sleeping, phase of the mouse. During the intervening dark, or active, phase, the mouse breathed room air over the entire 12-hour duration. The total length of IH exposure varied between studies from nine hours to 3 months. In the study of Yokoe et al. (2008), this protocol was repeated over a total duration of 4 consecutive days. Controls were subjected to the same protocol, except that air (IA) instead of hypoxia was introduced on an intermittent basis during sleep. Some of the mice were made mildly hyperglycemic through continuous infusion of dextrose, and the rest were kept in euglycemic conditions with continuous saline infusion. Blood pressure was monitored continuously from the femoral artery, while blood samples were withdrawn twice a day (during the sleep and active periods) for analysis of glucose, insulin and corticosteroid levels. Exposure to IH produced sustained hypertension as well as hyperglycemia in the light/sleeping phase of the mice, and reversed the diurnal variation of blood glucose. Concomitant glucose infusion attenuated the hypertension but exacerbated the blood glucose fluctuations.

In this study, we sought to examine in greater detail the time course of changes in autonomic activity in the Yokoe murine model following exposure to IH with or without concomitant hyperglycemia (Yokoe et al., 2008). Autonomic activity was monitored continuously using indices of cardiovascular variability derived from the continuous arterial blood pressure (ABP) recordings in the mice. We hypothesized that there would be a detectable and progressive increase in sympathetic activity and decrease in parasympathetic activity over the first few days of exposure to IH, and that these changes would be exacerbated by the presence of hyperglycemia.

2. Methods

2.1. Protocols

Details regarding surgery, catheterization, and post-operative maintenance are provided in the study previously published by (Yokoe et al., 2008). In brief, male mice, aged 10–12 weeks, were chronically implanted with femoral arterial and venous catheters to allow continuous arterial pressure measurements as well as infusion of saline (0.9% sodium chloride) or glucose (50% dextrose) at a constant rate of $100 \mu\text{lh}^{-1}$. Three days after the surgery, the mice were kept on a 12 hour light-dark cycle beginning at 8am for 4 days with free access to food and water where they were either exposed to IA or IH during light period (sleep period) and stable room air condition during dark period (active period). Each IH cycle consisted of 30 seconds of hypoxia (5.0–6.0% inspired oxygen) followed by 30 seconds of room air (20.9% inspired oxygen), while IA cycle only consisted of switches in

room air. Each mouse was subjected to one of four exposures: IA + saline (n = 5), IH + saline (n = 11), IA + glucose (n = 6), and IH + glucose (n = 9). Animal handling and experimentation were carried out in accordance with approved institutional animal care and use committee protocols at the University of Pittsburgh.

2.2. Data Processing

Continuous ABP data were sampled at 200 Hz. The pulse interval (PI) and beat-averaged ABP were extracted from the continuous ABP data. One PI was defined as the time interval between the point of maximum slope on the ABP waveform of the current beat and the point of maximum slope of the next beat. The beat-averaged ABP was determined by calculating the average blood pressure within the PI. For each analysis, if there was more than 25% of “excessively noisy” data within the data segment of interest, that segment would be excluded from the analysis. A data segment was deemed “excessively noisy” if the estimated signal amplitude to noise amplitude was less than 2 and/or there was an abrupt increase in amplitude in a beat that was 5 times the average amplitude of the signal.

2.3. Power spectral analyses of ABP and PI

Spectral analysis has been shown to be a useful tool in investigating the cardiovascular regulation in both humans and animals by allowing the quantification of the magnitude of sympathetic and parasympathetic activities (Just et al., 2000). Beat-to-beat ABP and PI were divided into 1-minute segments. For each segment, the beat-to-beat signals were resampled at 20 Hz and subjected to trend removal. Low- and high-frequency power (LFP: 0.15 – 1.5 Hz, HFP: 1.5 – 5 Hz respectively) of ABP and PI were calculated by integrating the power spectrum between the aforementioned frequency ranges (Just et al., 2000). The low-frequency power of ABP, denoted as LFP_{BP} , reflects sympathetic activity while the low-frequency power of PI, denoted as LFP_{PI} , in mice reflects parasympathetic activity (Janssen and Smits, 2002).

2.4. Baroreflex Sensitivity

Baroreflex sensitivity (BRS) was determined using both the sequence and spectral methods. In the sequence method, the changes in ABP and PI together in the same direction, either increasing or decreasing, for at least 3 consecutive beats were identified (Parati et al., 2000). The slope of the regression line between ABP and PI was taken to represent the estimate of the baroreflex sensitivity (BRS_{SEQ}). The change in ABP or PI from the current beat to the next beat was not required to be greater than a minimum threshold in order to qualify as an increasing or decreasing sequence (Laude et al., 2009). PI was allowed to lag ABP by 0 to 3 beats. This follows from Laude et al. (2008) whose previous study showed that the cross-spectral analysis resulted in a phase lag in PI of 0.5 sec. Therefore, we allowed the PI to lag ABP by at most 3 beats as the delay of 3 beats was approximately close to 0.5 sec. Only the sequences with the goodness of fit (R^2) of the regression line equal to or greater than 0.9 were considered in the analysis. In the spectral method, BRS was calculated as the square root of the ratio of PI and ABP power (Parati et al., 2000) in both low- and high-frequency regions and would be denoted as BRS_{LF} and BRS_{HF} , respectively.

2.5. Assessment of the Changes in Autonomic Responses over Time

2.5.1. Slopes of the linear trends—Linear trends were fitted to the time-series ABP, BRS_{SEQ} , BRS_{LF} , BRS_{HF} , LFP_{BP} and LFP_{PI} of each mouse. The slope of the linear trend, reflecting the overall change in the signal over time, was tested for any difference among the exposure groups.

2.5.2. Diurnal amplitude—The time-series ABP, BRS_{SEQ}, BRS_{LF}, BRS_{HF}, LFP_{BP} and LFP_{PI} were first smoothed out using a moving average filter with a span of 9 points (4.5 hours). The smoothed time-series of the IH groups were segmented into one-day periods. For each of these segments, the Cosinor fitting with a fixed frequency of 1 cycle/day was applied (Tong, 1976). The absolute amplitude of the fitted sinusoid, half of the peak-to-peak value, was then derived from each one-day segment. The reason why this analysis was not applied to the IA + saline and IA + glucose groups was due to the apparently pronounced ultradian rhythms (~12 hour cycle) in those groups while the diurnal rhythms of the IH + saline and IH + glucose groups were more evident.

2.5.3. Diurnal and ultradian power—After smoothing and linear trend removal, the time-series ABP, BRS_{SEQ}, BRS_{LF}, BRS_{HF}, LFP_{BP} and LFP_{PI} were subjected to power spectral analysis. The diurnal power was the average power within the frequency range of 1 ± 0.3 cycles/day (average periodicity = 24 hours) and the ultradian power was the average power within the frequency range of 2 ± 0.3 cycles/day (average periodicity = 12 hours). To determine the relative strength of ultradian to diurnal rhythmicity, the ratio of the ultradian power to the diurnal power of each of the above time-series was computed.

2.6. Statistical Analysis

2.6.1. Two-way ANOVA—To statistically assess the effect of IH and hyperglycemia, two-way ANOVA was used to compare the slopes of the fitted linear trends and the ratios of the ultradian power to the diurnal power of ABP, BRS_{SEQ}, BRS_{LF}, BRS_{HF}, LFP_{BP} and LFP_{PI}. The Holm-Sidak test was employed for post-hoc pairwise comparisons. If the normality or equal variance test failed, the data were first logarithm-transformed prior to performing the ANOVA calculations.

2.6.2. One-way Repeated Measures ANOVA—One-way repeated measures ANOVA was used to analyze the diurnal amplitude of ABP, BRS_{SEQ}, BRS_{LF}, BRS_{HF}, LFP_{BP} and LFP_{PI} of the mice in the combined IH groups (IH + saline and IH + glucose) with duration of IH exposure (in days) being the repeated factor. Holm-Sidak post-hoc comparisons versus control, with the day-1 amplitude being treated as control, were conducted. If the normality or equal variance tests failed, one-way repeated measures ANOVA on ranks with Dunnnett's post-hoc test versus control was used instead.

3. Results

3.1. Changes in Blood Pressure

As the mice were becoming acclimated to the experimental routine, the ABP showed an overall decreasing trend over four days in all exposure groups, but the decrease was slower in the IH + saline and IH + glucose groups (Figure 1). ABP in the IA + glucose group decreased the most rapidly relative to the other exposure groups, with the rate of decrease being approximately three times larger relative to the IH + saline group. The difference in the mean ABP slopes between saline and glucose infusion was significant ($p = 0.003$). Post-hoc comparisons showed that the mean ABP slope in IA + saline was significantly less steep than from IA + glucose ($p = 0.020$), and ABP in IH+saline decreased less rapidly than IH + glucose ($p = 0.035$).

3.2. Changes in Baroreflex Sensitivity

In contrast to the controls (IA + saline), the BRS_{LF} of the mice in the other exposure groups decreased over the four days of exposure (Figure 2). Although the BRS_{LF} in the control group showed a tendency to increase over time, this was not significantly different from zero

(one-sample t-test, $p = 0.23$). The differences in mean BRS_{LF} slopes between hypoxia levels (IA vs. IH) as well as between infusion levels (saline vs. glucose) were significant ($p = 0.002$ and $p = 0.010$, respectively). Pairwise comparisons showed that the BRS_{LF} decreased faster in IA + glucose ($p = 0.020$) and IH + saline ($p = 0.004$) compared to IA + saline. The findings for BRS_{SEQ} paralleled those for BRS_{LF} .

3.3 Changes in Low-Frequency Blood Pressure oscillation amplitude

Exposure to IH acted to sustain sympathetic over-activity, as reflected by the much slower decrease in LFP_{BP} in both IH groups compared to the IA groups (Figure 3). Two-way ANOVA confirmed this observation ($p < 0.001$). Pairwise comparisons showed that among the mice that were infused with saline, those that were exposed to IH had a slower decrease in LFP_{BP} ($p = 0.001$). The same conclusion could be drawn from the glucose-infusion group ($p = 0.008$).

3.4. Effect of Glucose Infusion

Hyperglycemia had a strong effect on reduction of parasympathetic activity, as reflected by LFP_{PI} (Figure 4). LFP_{PI} decreased faster over the 4 days in the IA + glucose group compared to IA + saline ($p = 0.024$). LFP_{PI} in the IH + glucose group also decreased faster than the IH + saline group ($p = 0.038$).

3.5. Changes in Amplitude of Diurnal and Ultradian rhythms

The IA + saline and IA + glucose groups displayed prominent ultradian rhythms (12-hour cycle oscillations) in the autonomic indices, whereas these oscillations were substantially less evident in the IH + saline and IH + glucose groups. In the IH groups, we focused our attention on how the diurnal amplitude changed over the experimental duration. We observed not only an overall decreasing trend in ABP over four days of exposure, but also a tendency for the diurnal amplitude to decrease over time. The LFP_{BP} , which also showed the overall decreasing trend, had a significant reduction in the diurnal amplitudes over time (Figure 5). One-way repeated measures ANOVA showed that there was a significant difference among the mean diurnal amplitude of LFP_{BP} in the combined IH group over the four days ($p < 0.001$). Post-hoc comparisons versus day-1 amplitude showed that the diurnal amplitudes of LFP_{BP} in the IH + saline and IH + glucose groups from day-2 to day-4 were significantly lower than day-1 ($p < 0.05$).

In all the autonomic parameters that were analyzed in this study except for ABP, the ratio of ultradian power to diurnal power (P_{ultr}/P_{diur}) was higher in the IA + saline and IA + glucose groups compared to the IH + saline and IH + glucose groups. Thus, IH acted to suppress ultradian rhythmicity. Figure 6 illustrates this effect on all BRS indices. Two-way ANOVA showed that P_{ultr}/P_{diur} of BRS_{LF} in IH groups was lower than that of IA groups ($p < 0.001$) and in glucose groups was lower than that of saline groups ($p = 0.036$). Pairwise comparisons showed that both IA + glucose and IH + saline had a lower ratio (P_{ultr}/P_{diur}) compared to IA + saline ($p = 0.038$ and $p = 0.001$, respectively). As was the case for BRS_{LF} , P_{ultr}/P_{diur} of BRS_{HF} in the IH groups was lower than that of IA groups ($p < 0.001$). As well, P_{ultr}/P_{diur} of BRS_{HF} in the glucose groups was lower than that of saline groups ($p = 0.041$). Pairwise comparisons for BRS_{HF} also showed similar results to BRS_{LF} : both IA + glucose and IH + saline had P_{ultr}/P_{diur} compared to IA + saline ($p = 0.015$ and $p < 0.001$, respectively). In addition, IH + glucose had significantly lower P_{ultr}/P_{diur} compared to IA + glucose ($p = 0.048$). BRS_{SEQ} , however, showed that there was a significant interaction between the hypoxia and infusion factors ($p = 0.015$). Pairwise comparisons showed that IH + saline had significantly lower P_{ultr}/P_{diur} compared to both IA + saline and IH + glucose ($p < 0.001$ and $p = 0.029$, respectively). The analysis of P_{ultr}/P_{diur} of LFP_{PI} yielded findings similar to those for P_{ultr}/P_{diur} of BRS (Figure 7). The IA + glucose and IH + saline groups

had lower $P_{\text{ultr}}/P_{\text{diur}}$ of LFP_{PI} compared to the IA + saline group ($p = 0.016$ and $p = 0.002$, respectively). The suppression of ultradian rhythmicity by IH was already apparent on the first day of IH exposure; there were no systematic changes in $P_{\text{ultr}}/P_{\text{diur}}$ over the subsequent days of exposure.

4. Discussion

4.1. Issues related to the animal preparation

As alluded to in the Results section, there was a slow downward drift in ABP in all mice over the four day study that replicates our previous study using an identical protocol but without the IH exposure (Alonso et al., 2007). This downward drift in overall ABP was likely due to the continuing adaptation of the mice to the tethering system as well as acclimation to the daily routine of the experiment which involved blood sampling twice per day requiring opening of the environmental box (controlling for light and sound), which housed the customized cages containing the tethered mice. The faster drift downwards of the glucose-infused mice was potentially related to the vasodilating properties of glucose-mediated insulin release, which was higher in the glucose infused mice than saline-infused mice. Overall, any impact on baseline blood pressure from acclimation to tethering or the sampling schedule should be comparable among all four experimental groups.

Multiple behavioral and physiologic measures demonstrate that the mice remained healthy over the course of the four day interventional component of the study. Food intake increased over each of the three recovery days from surgery and was at normal levels throughout the subsequent four day study. Body weight at the beginning of the four day experiment was 23.8 ± 0.9 gm and was unchanged at 23.9 ± 0.8 gm at the completion of the study. Blood glucose and plasma insulin levels in the IA + saline control group were all in the normal range and did not change over time. The plasma corticosterone levels during the dark/active period for days 2, 3, and 4 during the interventional study were 34 ± 11 , 14 ± 5 , and 25 ± 10 ng/ml for the IA + saline control and 55 ± 16 , 31 ± 10 , and 27 ± 9 ng/ml in the IA + glucose group. Mean heart rate also remained relatively unchanged over the 4 days. IH exposure during the light/sleep period increased plasma corticosterone to ~150–200 ng/ml, but always returned to basal levels during room air exposure in the dark/active period. By way of comparison, a study by Jacobson et al. (2006) reported basal plasma corticosterone levels in catheterized mice of ~160 ng/ml at 5–7 days after surgery that increased to ~300 ng/ml in response to hypoglycemia. Another study in chronically instrumented mice reported a basal corticosterone level of ~80 ng/ml that increased to ~180 ng/ml in response to a restraint stress (Tjurmina et al., 2002). Thus, not only do our basal plasma corticosterone levels show a complete absence of stress and no change over the course of the four day intervention, they are considerably lower than previously reported levels in healthy chronically instrumented mice.

4.2 Cardiovascular indices of autonomic function

Cardiovascular variability indices such as blood pressure variability and HRV have been valuable markers of sympathetic and parasympathetic activities in both humans and animals. Similar to humans, the ABP and its variability in mice are predominantly controlled by the sympathetic branch of the autonomic nervous system and are reflective of sympathetic tone (Janssen et al., 2000). In humans, the LFP of HRV is under the influence of both the sympathetic and parasympathetic systems (Gehrmann et al., 2000; Taskforce, 1996), but in mice, this component is largely parasympathetic (Janssen and Smits, 2002). Also, in humans, the HFP of HRV is known to be largely modulated by the parasympathetic nervous system. However, in mice, the extent to which vagal cardiac modulation contributes to HFP is more controversial; for instance, Janssen et al. (2002) suggested that this component

primarily reflects mechanical coupling of heart rate with respiration. Thus, unlike in humans (Just et al., 2000), the ratio of LFP to HFP of HRV in mice may not necessarily reflect sympathovagal balance.

We employed the sequence method as one of the ways to assess BRS. This method requires the identification of short time-segments in which beat-to-beat blood pressure and PI have to increase or decrease together. Due to the relatively low sampling rate of 200 Hz, the minimum change in the PI was automatically fixed by the sampling rate, making it more difficult to identify one sequence since the PI has to consecutively increase or decrease by 5 milliseconds at a time for at least three beats consecutively while the parallel change in blood pressure has to also occur simultaneously. This is in contrast to the suggestion by Laude et al. (2009) that the threshold for the minimum change in either the blood pressure or PI should not be imposed in order to optimize the sequence method in mice. As well, comparison of our results to other studies may be confounded by the fact that different frequency bands have been employed in the spectral analysis of both blood pressure and heart rate in mice (Baudrie et al., 2007).

4.3. Autonomic effects of intermittent hypoxia and hyperglycemia

So far, most studies have focused on the end result of the long-term exposure to IH, but few studies have looked at the early stage of development of autonomic dysfunction during chronic exposure to IH. Lin et al. (2007) showed that exposure to chronic IH resulted in a sustained impairment of baroreflex control of heart rate. As well, Dematteis et al. (2008) reported increased chemoreflex and depressed baroreflex activity as a result of exposure to chronic IH. Consistently, we found that IH suppressed the BRS and this suppression begins on the same day following the start of exposure to IH in mice. On each of the 4 days of the experiment, the mice exposed to IH had higher mean blood pressures than their corresponding counterparts exposed to IA. This is consistent with the findings in other studies on both mice (Campen et al., 2005; Kaufman et al., 2007), and humans (Cooper et al., 2005; Peppard et al., 2000) in which IH exposure, whether acute or chronic, resulted in systemic hypertension.

IH, the main characteristic of SDB, can also cause increases in sympathetic activity as reported by previous studies in both humans and animals. Direct measurements of sympathetic nervous activity from the peroneal nerve showed increased sympathetic nervous activity in SDB subjects (Narkiewicz et al., 1999) while other studies in dogs with simulated SDB (Brooks et al., 1997) and rats exposed to IH (Fletcher et al., 1999) showed elevated blood pressure but once the airway obstruction or IH was abolished, the blood pressure declined. Lai et al. (2006) investigated blood pressure variability in rats and found that LFP_{BP}, reflecting sympathetic activity, increased after the exposure to IH for 5 days. In the current study, although all mice displayed a decreasing trend in LFP_{BP}, the groups exposed to IH decreased less rapidly than those exposed to IA. We attribute this result in the IH-exposed mice to the additive effect of two opposing trends: a strong downward drift in sympathetic tone as all mice became acclimated to the daily routine of the experiment, and a countervailing upward trend in sympathetic activity produced by IH.

We found that parasympathetic activity decreased in mice exposed to hyperglycemia. While IH also seemed to cause reduction in vagal activity, this change did not achieve statistical significance. Rey et al. (2004) reported that chronic exposure to IH in cats resulted in reduction of the HF component of HRV, which reflects parasympathetic activity, similar to SDB patients. A population-based study investigating the association between multiple metabolic syndromes and HRV showed that insulin resistance, marked by increased fasting insulin, is associated with lower HF and LF components of HRV (Liao et al., 1998). Another study by Licht et al. (2010) showed similar result that decreased in parasympathetic activity,

indicated by reduction in respiratory sinus arrhythmia, and increased sympathetic activity, indicated by reduction in pre-ejection period, are associated with increased likelihood of metabolic syndrome. Besides the fact that these studies were not performed in mice, their results are consistent with our findings.

Another interesting finding in our study is the disappearance of ultradian fluctuations of BRS and the low-frequency component of heart rate in mice exposed to IH. Previous studies showed that many ultradian rhythms are related to metabolic processes such as feeding cycle (Li et al., 1999; Wollnik, 1989). The abolished high-frequency 12-hour cycle in mice exposed to IH could in part be explained by the suppressed gustatory sensory perception by hypoxia.

4.4 Other factors

There were a number of factors in the study that were not controlled, which might have contributed independently to some of the autonomic and cardiovascular differences observed between IH and IA groups. Although we did control the light dark cycle in a 12hr: 12hr format, the data have not been partitioned according to sleep state or activity as we did not have the animals instrumented for polysomnography. Body temperature was not recorded and to our knowledge there is no way to assess metabolic rate during periods of intermittent hypoxia using indirect calorimetry. However, we do know from a study in chronically instrumented rats by Hamrahi et al. (2001) that exposure to intermittent hypoxia has no effect on lowering body temperature over a period of several hours.

4.5. Implications of the findings

The findings arrived at in this study provide a useful picture of the early development of autonomic dysfunction following chronic “nocturnal” exposure to IH and how these autonomic changes are influenced also by concomitant hyperglycemia. In the murine model, the time courses for early development of insulin resistance and autonomic dysfunction appear to be quite similar. Thus, our findings do not resolve the question of whether changes in autonomic function occur first following IH exposure and that these lead to a reduction in insulin sensitivity. Extrapolation of our results to humans poses another challenge. Several prospective studies in humans have shown that the effect of IH on sympathetic over-activity is cumulative over time and that the cumulative effect occurs over a time course of several minutes. For example, Xie et al. (2000) found that exposing healthy young subjects to intermittent asphyxia over a period of 20 min led to sympathetic activation that continued even after the stimulus was removed. In another study (Cutler et al., 2004), exposure to intermittent hypoxic apnea over a 20 min duration led to prolonged sympathetic activation. The same results occurred regardless of whether these exposures occurred against a background of hypercapnia or isocapnia, thus confirming that the primary mediator for the increase in sympathetic activity was the IH. In two other studies (Leuenberger et al., 2005; Leuenberger et al., 2007), healthy young subjects exposed to repetitive hypoxic apneas for a total duration of 30 mins displayed, in the post-recovery period, a small and short-lasting increase in mean arterial blood pressure, accompanied by a more sustained and substantial increase in muscle sympathetic nerve activity. The aforementioned human studies did not include the determination of insulin sensitivity before and after IH exposure. However, it has been observed that insulin sensitivity can improve as rapidly as 48 hours after the start of CPAP treatment in patients with SDB (Harsch et al., 2004).

Taken together, the current evidence suggests that, in humans, changes in autonomic activity occur relatively rapidly with exposure to IH, whereas changes in insulin sensitivity take place at a somewhat slower pace. Thus, it is possible that some of the reduction in insulin sensitivity may have taken place as a consequence of the cumulative sympathetic over-

activity. The findings from this study are consistent with this view; however, they do not exclude the possibility that exposure to IH could also directly lead to alterations in glucose metabolism, independent of preceding changes in autonomic control.

5. Conclusion

In summary, measurements of continuous ABP from a chronically instrumented murine model were used to investigate the effects of IH, occurring under conditions of euglycemia and hyperglycemia, on the early development of autonomic nervous system dysfunction. Lean animals were used in order to eliminate the confounding influence of obesity. On the basis of the indices of cardiovascular variability derived from these measurements, we conclude that IH acted to increase sympathetic activity, whereas hyperglycemia decreased parasympathetic activity. Exposure to both IH and hyperglycemia led to the largest reductions in baroreflex sensitivity. Our findings suggest that IH, combined with hyperglycemia, exerts progressively adverse effects on autonomic control independent of obesity.

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Highlights

- A lean murine model was employed to track the development of autonomic dysfunction independent of obesity.
- Exposure to intermittent hypoxia and continuous infusion of glucose or saline over 4 days reduced baroreflex sensitivity.
- Intermittent hypoxia promoted sympathetic activity whereas hyperglycemia suppressed parasympathetic activity.
- Ultradian (12-hour) rhythmicity was substantially suppressed in mice exposed to intermittent hypoxia.
- Intermittent hypoxia combined with hyperglycemia progressively adversely affected autonomic control independent of obesity.

Figure 1. Beat-averaged blood pressure (ABP) of all exposure groups

Group median beat-averaged ABP time courses over the duration (4 days) of the experiment. (B) Each bar shows the mean of the slopes of the linear trends fitted to ABP.

The error bar represents the standard error. * indicates pairwise difference, $p < 0.05$ (Holm-Sidak method).

Figure 2. Baroreflex sensitivity at low-frequency range (BRS_{LF}) of all exposure groups
 Group median BRS_{LF} timecourses over the duration (4 days) of the experiment. (B) Each bar shows the mean of the slopes of the linear trends fitted to BRS_{LF} . The error bar represents the standard error. (C) Each bar shows the mean of the slopes of the linear trends fitted to baroreflex sensitivity computed from sequence method (BRS_{SEQ}). The error bar represents the standard error. * indicates pairwise difference, $p < 0.05$ (Holm-Sidak method).

Figure 3. Low-frequency power of blood pressure (LFP_{BP}) of all exposure groups
 (A) Group median LFP_{BP} time courses over the duration (4 days) of the experiment. (B) Each bar shows the mean of the slopes of the linear trends fitted to LFP_{BP}. The error bar represents the standard error. * indicates pairwise difference, $p < 0.05$ (Holm-Sidak method).

Figure 4. Low-frequency power of pulse interval (LFP_{PI}) of all exposure groups
 (A) Group median LFP_{PI} time courses over the duration (4 days) of the experiment. (B) Each bar shows the mean of the slopes of the linear trends fitted to LFP_{PI}. The error bar represents the standard error. * indicates pairwise difference, $p < 0.05$ (Holm-Sidak method).

Figure 5. Diurnal amplitude of low-frequency power of blood pressure (LFP_{BP}) during exposure to intermittent hypoxia

(A) Group median LFP_{BP} time courses over the duration (4 days) of the experiment. (B) Each bar shows the mean of the diurnal amplitudes of LFP_{BP}. The error bar represents the standard error. * indicates pairwise difference, $p < 0.05$ (Holm-Sidak method).

Figure 6. Ratio of ultradian power (12-hour cycle) to diurnal power (24-hour cycle) of baroreflex sensitivity (BRS)

(A) BRS by sequence method (BRS_{SEQ}). (B) BRS at low-frequency range (BRS_{LF}). (C) BRS at high-frequency range (BRS_{HF}). Each bar shows the mean of the ratios of the ultradian powers to the diurnal powers of the BRS. The error bar represents the standard error. * indicates pairwise difference, $p < 0.05$ (Holm-Sidak method).

Figure 7. Ratio of ultradian power (12-hour cycle) to diurnal power (24-hour cycle) of low-frequency power of pulse interval (LFP_{PI})

Each bar shows the mean of the ratios of the ultradian powers to the diurnal powers of the LFPPI. The error bar represents the standard error. * indicates pairwise difference, $p < 0.05$ (Holm-Sidak method).