Published in final edited form as:

Respir Physiol Neurobiol. 2014 November 1; 0: 60–67. doi:10.1016/j.resp.2014.08.018.

THE EFFECT OF ADRENAL MEDULLECTOMY ON METABOLIC RESPONSES TO CHRONIC INTERMITTENT HYPOXIA

Mi-Kyung Shin, PhD, Woobum Han, Shannon Bevans-Fonti, BS, Jonathan C. Jun, MD, Naresh M. Punjabi, MD, PhD, and Vsevolod Y. Polotsky, MD, PhD

Division of Pulmonary and Critical Care Medicine, Department of Medicine, Johns Hopkins University School of Medicine

Abstract

Obstructive sleep apnea causes intermittent hypoxia (IH) and is associated with insulin resistance and type 2 diabetes. IH increases plasma catecholamine levels, which may increase insulin resistance and suppress insulin secretion. The objective of this study was to determine if adrenal medullectomy (MED) prevents metabolic dysfunction in IH. MED or sham surgery was performed in 60 male C57BL/6J mice, which were then exposed to IH or control conditions (intermittent air) for 6 weeks. IH increased plasma epinephrine and norepinephrine levels, increased fasting blood glucose and lowered basal and glucose-stimulated insulin secretion. MED decreased baseline epinephrine and prevented the IH induced increase in epinephrine, whereas the norepinephrine response remained intact. MED improved glucose tolerance in mice exposed to IH, attenuated the impairment in basal and glucose-stimulated insulin secretion, but did not prevent IH-induced fasting hyperglycemia or insulin resistance. We conclude that the epinephrine release from the adrenal medulla during IH suppresses insulin secretion causing hyperglycemia.

Keywords

sleep apnea; type 2 diabetes; epinephrine; insulin secretion

1. Introduction

Obstructive sleep apnea (OSA) has been associated with insulin resistance, glucose intolerance, decreased insulin secretion and type 2 diabetes (Punjabi *et al.*, 2002;Punjabi *et al.*, 2004;Punjabi & Beamer, 2009;Sharma *et al.*, 2011;Pamidi *et al.*, 2010;Pamidi & Tasali, 2012;Foster *et al.*, 2009). OSA leads to chronic intermittent hypoxia (IH) during sleep (Gastaut *et al.*, 1966). Insulin resistance and decreased insulin secretion in OSA patients have been attributed to IH (Punjabi & Beamer, 2009;Stamatakis *et al.*, 2008;Drager *et al.*,

© 2014 Elsevier B.V. All rights reserved.

Corresponding Author: Vsevolod (Seva) Polotsky, M.D., Ph.D., Associate Professor of Medicine, Division of Pulmonary and Critical Care Medicine, Johns Hopkins University School of Medicine, Johns Hopkins Asthma and Allergy Center, Rm. 4B 65, 5501 Hopkins Bayview Circle, Baltimore, MD 21224, (410) 550-6386 (Phone), (410) 550-2612 (Fax), vpolots1@jhmi.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

2010). Exposure of healthy human volunteers to IH leads to insulin resistance and impaired insulin secretion (Louis & Punjabi, 2009). We have previously developed a mouse model of chronic IH, which mimics the oxyhemoglobin saturation profile in patients with OSA (Jun *et al.*, 2010; Polotsky *et al.*, 2006; Reinke *et al.*, 2011; Tagaito *et al.*, 2001). Acute and chronic IH induces insulin resistance in mice (Polotsky *et al.*, 2003; Iiyori *et al.*, 2007; Drager *et al.*, 2011; Polak *et al.*, 2013; Lee *et al.*, 2013) and impairs glucose stimulated insulin secretion in mice and rats (Polak *et al.*, 2013; Lee *et al.*, 2013; Fenik *et al.*, 2012). Yokoe *et al.* reported that IH induces replication of pancreatic β -cells (Yokoe *et al.*, 2008), whereas Xu *et al.* reported increases both in β -cell apoptosis and proliferation (Xu *et al.*, 2009). Mechanisms of dysregulation of glucose metabolism in IH remain unclear.

Human OSA has been firmly linked to the SNS activation detected by measurements of muscle sympathetic nerve activity (Narkiewicz *et al.*, 1998; Somers *et al.*, 1995; Carlson *et al.*, 1993; Hedner *et al.*, 1988; Leuenberger *et al.*, 1995) and urine and plasma catecholamines (Baruzzi *et al.*, 1991; Marrone *et al.*, 1993; Carlson *et al.*, 1993). Treatment of OSA with continuous positive airway pressure (CPAP) abolished increases in muscle sympathetic nerve activity (Narkiewicz *et al.*, 1999; Imadojemu *et al.*, 2007; Somers *et al.*, 1995) and decreased plasma (Heitmann *et al.*, 2004) and urine (Pinto *et al.*, 2013) catecholamine levels. In rodents, IH increased cervical (Greenberg *et al.*, 1999), renal (Huang *et al.*, 2009), splanchnic (Dick *et al.*, 2007), thoracic (Zoccal *et al.*, 2008) and lumbar (Marcus *et al.*, 2010) sympathetic nerve activity (reviewed in (Prabhakar *et al.*, 2012)), enhanced norepinephrine turnover in sympathetic endings (Gonzalez-Martin *et al.*, 2011), and raised plasma epinephrine and norepinephrine levels (Bao *et al.*, 1997a; Kumar *et al.*, 2006). Kumar *et al.* showed that exposure to chronic IH increased norepinephrine and epinephrine efflux from the adrenal medulla *ex vivo* (Kumar *et al.*, 2006).

Norepinephrine and epinephrine may induce insulin resistance via multiple mechanisms including up-regulation of glycogenolysis and gluconeogenesis (Barth *et al.*, 2007; Ziegler *et al.*, 2012; Dungan *et al.*, 2009) and stimulation of adipose tissue lipolysis (Jaworski *et al.*, 2007; Lafontan & Langin, 2009; Zechner *et al.*, 2009; Bickel *et al.*, 2009). However, the autonomic nervous system blockade with hexamethonium did not reverse insulin resistance induced by acute IH in mice (Iiyori *et al.*, 2007) questioning the role of the sympathetic nervous system in insulin resistance during IH. Norepinephrine and epinephrine can also directly suppress insulin secretion by pancreatic islets (Szollosi *et al.*, 2010; Skoglund *et al.*, 1986; Ahren & Lundquist, 1985; Liggett, 2009; Ruohonen *et al.*, 2012). The roles of sympathetic nervous system and catecholamines secreted by adrenal medulla in pancreatic β cell dysfunction during IH are unknown. We hypothesize that perturbations of glucose metabolism by IH can be abolished by adrenal medullectomy. To test this hypothesis we performed adrenal medullectomy or sham surgery in C57BL/6J mice, which were subsequently exposed to chronic IH or intermittent air (IA) followed by fasting blood glucose and plasma insulin measurements, the intraperitoneal glucose tolerance test (IPGTT) and insulin tolerance test (ITT).

2. Materials and Methods

2.1 Experimental Animals

Sixty adult male C57BL/6J mice, 6–8 weeks of age (Jackson Laboratory, Bar Harbor, MA) underwent adrenal medullectomy (MED, n = 30) or sham surgery (n=30). After a 2-week recovery animals were exposed to chronic intermittent hypoxia (IH, n = 15 in both MED and sham groups) or intermittent air (IA, n = 15 in both MED and sham groups) for 6 weeks while fed a regular chow diet. Forty mice (IH-MED, IA-MED, IH-sham, IA-sham, n = 10 per group) were used for intraperitoneal glucose tolerance test (IPGTT), insulin tolerance test (ITT), and all other metabolic measurements as described further. Twenty mice (n = 5 per group) were used for catecholamine measurements in adrenal tissue. Mice were housed in the humidity controlled standard laboratory environment at 22°C in the 12 hr light/dark cycle (9 am – 9 pm lights on/ 9 pm – 9 am lights off). The study was approved by the Johns Hopkins University Animal Use and Care Committee (Protocol # MO12M309) and complied with the American Physiological Society Guidelines for Animal Studies.

2.2 Adrenal Medullectomy

Mice were anesthetized with 1–2% isoflurane, the lumbar area was shaved and prepped with chlorhexidine and alcohol. A 1 cm dorsal midline incision was performed between the 1st and 3rd lumbar vertebra. The muscle wall was entered with mosquito forceps 1.5 cm lateral to the spine on each side. The left adrenal gland was located lateral and cranial to the spleen and the right adrenal gland was located cranial to the right kidney. The adrenal glands were exteriorized. Small incisions were made on the adrenal capsule bilaterally and medulla was gently squeezed out. The adrenal capsule and attached fat pads were returned to the abdominal cavity and the skin incision was closed. Burpenorphine (0.01 mg/kg) was administered subcutaneously at the end of surgery to minimize discomfort. Sham surgery was performed in an identical fashion, except that adrenal medulla was not removed.

2.3 Intermittent Hypoxia

IH was performed as previously described (Drager *et al.*, 2012; Drager *et al.*, 2011; Drager *et al.*, 2013). Briefly, a gas control delivery system was designed employing programmable solenoids and flow regulators, which controlled the flow of air, nitrogen, and oxygen into cages. During each cycle of intermittent hypoxia, % of O₂ decreased from ~21% to ~6–7% over a 30 seconds period, followed by a rapid return to ~21% over the subsequent 30 seconds period. We have previously shown that this regimen of IH induces oxyhemoglobin desaturations from 99% to ~65%, 60 times/hr (Jun *et al.*, 2010). A control group was exposed to an identical regimen of intermittent air (IA) delivered at the same flow rate as IH. All animals had free access to water. The IH animals had free access to food. The IA group was weight matched to the IH group by varying food intake as previously described (Jun *et al.*, 2010). IH and IA were administered during the light phase (9 am – 9 pm) for 6 weeks. Upon completion of the exposure, mice were bled by retro-orbital puncture and sacrificed under 1–2% isoflurane anesthesia after a 5 h fast. The liver and epididymal fat pads were dissected and weighed. All terminal blood draws and sacrifices were performed between 12pm and 1pm. Plasma samples were immediately frozen in liquid nitrogen and stored at –80°C.

2.4 Intraperitoneal Glucose Tolerance Test (IPGTT), Insulin Tolerance Test (ITT), and Glucose Stimulated Insulin Secretion

IPGTT and ITT were performed between 12 pm and 3 pm during weeks 3 and 4 of IH or IA exposures in unanesthetized animals. IH and IA exposures were continued during the tests. IPGTT was performed after a 5-hour fast by injecting 1g/kg glucose intraperitoneally (i.p.). Glucose levels were measured by tail-snip technique using a hand-held glucometer (Accu-Check Aviva, Roche, Indianapolis, IN) at baseline and at 10, 20, 30, 60, 90 and 120 minutes after glucose injection. The IPGTT areas under the curve were calculated after subtracting the fasting glucose baseline. ITT was performed after a 2-hour fast by injecting 0.5 IU/kg insulin (Humulin R, Eli Lilly, Indianapolis, IN, USA) i.p. Glucose levels were measured at baseline, and at 10, 20, 30, 40, 50 and 60 minutes post-injection. The ITT blood glucose values were presented as percent of the fasting glucose baseline. To assess glucose stimulated insulin secretion, plasma insulin levels were measured at fasting (5 h) conditions and 30 min after glucose injection in the IPGTT.

2.5 Assays

For adrenal tissue catecholamine measurements, adrenal glands were homogenized in 0.01 N hydrochloric acid, 1 mM ethylenediaminetetraacetic acid (EDTA) and 4 mM sodium metabisulfate; epinephrine and norepinephrine were extracted by using a cis-diol-specific affinity gel, acylated, converted enzymatically and measured with the competitive ELISA kit from Labor Diagnostika Nord GmbH & Co (Nordhorn, Germany). Plasma insulin was measured with an ELISA kit from Millipore (Billerica, MA). Plasma epinephrine and norepinephrine were measured with ELISA from Rocky Mountain Diagnostics (Colorado Springs, CO). Fasting plasma total cholesterol, free fatty acids (FFA) and triglycerides were measured with enzymatic colorimetric kits from Wako Diagnostics, Inc. (Richmond, VA). Corticosterone and glucagon were determined by ELISA kits from R&D Systems, Inc. (Minneapolis, MN). All plasma samples were tested simultaneously in a respected assay.

2.6 Statistical Analysis

All values are reported as means $\pm$ SEM. All the data in the study were checked for normality with a chi-square goodness of fit test. All values were normally distributed, except insulin, glucose and epinephrine levels. Statistical significance for all comparisons between normally distributed values was determined by two-way analysis of variance test with Bonferroni post-hoc correction for multiple comparisons. Statistical significance for all comparisons between non-normally distributed values was determined by Friedman and Mann-Whitney tests. For the assessment of glucose-stimulated insulin secretion, we performed a one- sample Wilcoxon signed rank test. A p-value of <0.05 was considered significant. Statistical analysis was performed using Minitab 16 (State College, PA).

3. Results

Adrenal medullectomy resulted in a greater than 5-fold decrease in adrenal epinephrine (0.18 ± 0.07 $\mu\text{g}/\text{mg}$ compared to 1.17 ± 0.13 $\mu\text{g}/\text{mg}$ in sham-operated mice exposed to IA, $n = 5$ per group, $p < 0.001$) and a greater than 10-fold reduction in adrenal norepinephrine

(0.10 ± 0.03 $\mu\text{g}/\text{mg}$ compared to 1.10 ± 0.13 $\mu\text{g}/\text{mg}$ in sham-operated mice exposed to IA, $p < 0.001$) (Figure 1).

All groups of mice were identically weight matched on Day 0 (Table 1). By Day 42, mice exposed to IH were lighter than normoxic mice in both sham-operated and medullectomized groups (IH-sham, 20.5 ± 0.3 g; IA-sham, 21.8 ± 0.4 g; IH-MED, 21.2 ± 0.3 g; IA-MED; 21.9 ± 0.4 g, $p = 0.012$ for the effect of IH). Among mice exposed to IH, medullectomized mice appeared to be slightly heavier than sham-operated animals ($p = 0.045$). Both medullectomized and sham-operated mice exhibited an 8–9% increase in liver weight and loss of epididymal fat during IH (Table 1). Neither surgery nor hypoxic exposure affected plasma levels of corticosterone, glucagon, and free fatty acids. Fasting plasma total cholesterol and triglyceride levels were increased by IH (Table 1).

IH did not affect adrenal epinephrine and norepinephrine content (Figure 1). In sham-operated mice, chronic IH increased plasma epinephrine (2.05 ± 0.54 ng/ml vs 0.93 ± 0.19 ng/ml in the IA group, $n = 8$ per group, $p = 0.0019$) and norepinephrine (19.1 ± 1.4 ng/ml vs 12.1 ± 1.1 ng/ml in the IA group, $n = 8$ per group, $p < 0.001$ levels (Figure 2). Adrenal medullectomy resulted in a 4-fold decrease in plasma epinephrine at baseline compared to sham (0.24 ± 0.03 ng/ml, $n = 7$, $p = 0.0028$) and abolished a surge in epinephrine in response to IH (0.35 ± 0.04 , $n = 8$, Figure 2A). In contrast, adrenal medullectomy did not affect plasma norepinephrine levels (Figure 2B).

IH increased fasting blood glucose levels in sham-operated mice (153 ± 4 mg/dl vs. 88 ± 2 mg/dl in the IA group, $n = 10$ per group, $p < 0.001$, Figure 3A) and medullectomized mice (160 ± 6 mg/dl vs. 94 ± 4 mg/dl in the IA group, $n = 10$ per group, $p < 0.001$). Fasting serum insulin levels were not affected by IH in sham-operated mice (0.79 ± 0.07 ng/ml vs. 0.71 ± 0.02 ng/ml in the IA group), but were markedly increased by IH in medullectomized mice (1.17 ± 0.13 ng/ml vs. 0.61 ± 0.02 ng/ml, $n = 10$ per group, $p < 0.01$, Figure 3B). IH did not affect glucose tolerance in sham-operated mice in the IPGTT (Figure 4). In the IA-sham group ($n = 10$), blood glucose levels increased from 88 ± 2 mg/dl at baseline to 222 ± 13 mg/dl after the glucose bolus demonstrating an increase of 134 ± 14 mg/dl. In the IH-sham group ($n = 10$), blood glucose levels increased from 153 ± 4 mg/dl at baseline to 285 ± 14 mg/dl exhibiting an increase of 132 ± 15 mg/dl, which was identical to the IA-sham group (Figure 4A). The IPGTT areas under the curve were similar between IA-sham and IH-sham groups (Figure 4C). In contrast, medullectomized mice showed improved glucose tolerance during IH. In the IA-MED group ($n = 10$) blood glucose levels increased from 94 ± 4 mg/dl at baseline to 225 ± 7 mg/dl demonstrating an increase of 119 ± 8 mg/dl, which was similar to sham mice. In the IH-MED group ($n = 10$), blood glucose levels increased from 160 ± 6 mg/dl at baseline to 201 ± 11 mg/dl exhibiting an increase of only 41 ± 15 mg/dl (Figure 4B). IH-MED mice showed a striking decrease in the IPGTT area under the curve above the baseline compared to all other groups ($p < 0.001$, Figure 4C).

IH decreased the magnitude of glucose response to insulin in the ITT in both sham-operated and medullectomized mice suggesting that insulin resistance may have developed. In sham-operated mice, blood glucose levels declined to $50 \pm 2\%$ of baseline in the IH group ($n = 6$) vs. $36.5 \pm 2.8\%$ of baseline in the IA group ($n = 9$, $p = 0.002$, Figure 5). In medullectomized

mice, blood glucose levels declined to $48.7 \pm 2.4\%$ of baseline in the IH group ($n = 6$) vs. $34.7 \pm 0.9\%$ of baseline in the IA group ($n = 9$, $p = 0.002$, Figure 5). Medullectomy did not affect IH-induced insulin resistance (Figure 5).

Glucose stimulated insulin levels were assessed at the peak of glucose levels in the IPGTT, 30 min after the glucose injection (Figure 6). In sham operated mice exposed to IA, glucose injection led to a 1.55 fold increase in plasma insulin, from 0.79 ± 0.07 to 1.32 ± 0.25 ng/ml ($n = 9$, $p = 0.012$, Fig. 6A). IH inhibited a glucose-stimulated insulin increase in mice with intact adrenal medulla: fasting plasma insulin level remained unchanged from 0.71 ± 0.02 ng/ml, despite a 1.8-fold increase in blood glucose levels after glucose injection ($n = 9$, Figs. 4 and 6B). Adrenal medullectomy did not significantly affect glucose stimulated insulin levels in normoxic mice (Fig. 6C). Adrenal medullectomy restored the insulin response to glucose during IH with plasma insulin levels increasing from 1.17 ± 0.15 to 1.71 ± 0.27 ng/ml after the glucose bolus ($n = 10$, $p = 0.035$, Fig. 6D).

3. Discussion

The main finding of our study was that IH suppressed basal and glucose stimulated insulin secretion in mice and that adrenal medullectomy reversed IH-mediated inhibition of insulin secretion. Adrenal medullectomy also improved glucose tolerance, but did not affect insulin resistance in mice exposed to IH.

4.1. IH, catecholamines and insulin resistance

IH increased plasma epinephrine and norepinephrine levels without a change in catecholamine content in the adrenal glands suggesting that IH may increase catecholamine efflux from the adrenal medulla (Kumar *et al.*, 2006). IH has been previously reported to increase tyrosine hydroxylase activity and catecholamine biosynthesis via oxidative stress mechanisms (Raghuraman *et al.*, 2012; Kumar *et al.*, 2006). As expected adrenal medullectomy abolished the IH-induced increase in plasma epinephrine. In contrast, adrenal medullectomy did not affect plasma norepinephrine levels. Our results were consistent with a previous report in rats, which has also shown that IH increased plasma epinephrine and norepinephrine levels and that adrenal medullectomy prevented an increase in epinephrine, but not in norepinephrine (Bao *et al.*, 1997a). The lack of an impact of adrenal medullectomy on plasma norepinephrine suggests that plasma norepinephrine represents mostly spill over from the sympathetic endings unaffected by adrenal medullectomy (Patel *et al.*, 2000; Esler *et al.*, 1990).

Adrenal medullectomy did not attenuate fasting hypoglycemia during IH. Medullectomized mice exposed to IH showed increased fasting plasma insulin levels in response to elevated glucose suggesting both intact insulin secretion and increased hepatic glucose output. IH stimulates the splanchnic sympathetic nerve (Dick *et al.*, 2007), which branches to the liver. The liver has abundant sympathetic innervation (Nobin *et al.*, 1978; Metz & Forssmann, 1980; Hartmann *et al.*, 1982; Henriksen *et al.*, 1998; Oben *et al.*, 2004). IH increases liver sympathetic innervation and this effect was prevented by carotid body denervation (Shin *et al.*, 2014). IH also increases baseline hepatic output and this increase was prevented by the autonomic nervous system blockade and carotid body denervation (Iiyori *et al.*, 2007; Shin *et*

et al., 2014). We have previously shown that hypoxia-induced activation of the sympathetic nervous system leads to adipose tissue lipolysis, which may cause insulin resistance during IH (Jun *et al.*, 2010; Jun *et al.*, 2011; Jun *et al.*, 2012). Adrenal medullectomy did not affect the epididymal fat pad size, liver weight or plasma lipid levels suggesting no effect on adipose tissue lipolysis. The lack of the effect of adrenal medullectomy on fasting hepatic glucose output and insulin resistance during IH may be related to preserved sympathetic efferent innervation of insulin sensitive tissues, i.e. liver, skeletal muscle and adipose tissue. This type of sympathetic activation would not be affected by medullectomy, and is reflected by the increased “spillover” of plasma norepinephrine during IH in both medullectomized and sham-operated mice (Kopin *et al.*, 1978). Overall, our data suggest that circulating epinephrine secreted by adrenal medulla does not play a role in IH-induced insulin resistance.

4.2. IH, insulin secretion and catecholamines

In mice, IH suppressed glucose-stimulated insulin secretion suggesting pancreatic β -cell dysfunction (Lee *et al.*, 2013). The pathogenesis of β cell dysfunction during IH has been attributed to oxidative stress, apoptosis of β cells and up-regulation of the renin-angiotensin system (Wang *et al.*, 2013; Xu *et al.*, 2009; Yokoe *et al.*, 2008; Fenik *et al.*, 2012). Our data suggest that the impairment of insulin secretion in IH is caused by the surge in circulating epinephrine.

Sham-operated mice showed no increase in fasting and glucose-stimulated plasma insulin levels during IH, despite increased glucose levels. This phenomenon could be related either to suppressed insulin secretion or increased insulin clearance during IH. However, we have previously shown that IH does not affect insulin clearance (Polak *et al.*, 2013). Thus, our data are consistent with previous reports that IH suppressed both basal and glucose-stimulated insulin secretion (Lee *et al.*, 2013; Yokoe *et al.*, 2008). In contrast, medullectomized mice exposed to IH exhibited increases in basal and glucose-stimulated insulin levels, which were similar to IA control conditions and previous observations in unexposed C57BL/6J mice (Andrikopoulos *et al.*, 2008). In parallel, these animals showed a striking improvement in glucose tolerance in the IPGTT suggesting that adrenal medullectomy increases glucose tolerance in hypoxic mice by augmenting insulin secretion. Medullectomized mice exposed to IH exhibited better glucose tolerance than normoxic animals, which may be related to unmasked in the absence of epinephrine beneficial effects of hypoxia on insulin secretion, e.g. hypoxia inducible factor 1 alpha (Cheng *et al.*, 2010). Overall, given that IH increased plasma epinephrine level and adrenal medullectomy abolished this increase, our data implicate adrenal epinephrine in the pancreatic endocrine defect during IH.

An inhibitory effect of epinephrine on insulin secretion, especially in response to glucose, has been described several decades ago (Lerner & Porte, Jr., 1971). Our findings are in line with the known control of pancreatic insulin secretion by α_{2A} -adrenoreceptors (Ahren & Lundquist, 1985; Liggett, 2009; Ruohonen *et al.*, 2012). Mice lacking α_{2A} -adrenoreceptors show hyperinsulinemia, reduced blood glucose levels and improved glucose tolerance (Ruohonen *et al.*, 2012). Increased expression of α_{2A} -adrenoreceptors depresses insulin

release, which is a pathogenic mechanism of type 2 diabetes (Rosengren *et al.*, 2010). Our data suggest that IH of OSA may contribute to the development or progression of type 2 diabetes via excessive efflux of epinephrine, which can overstimulate α_2A -adrenoreceptors on pancreatic β -cells.

4.3. Limitations

Our study had several limitations. First, our IH mouse model induces hypocapnic hypoxia (Savransky *et al.*, 2007; Lee *et al.*, 2009) rather than hypercapnic IH noted in OSA patients. However, previous publications in human volunteers and rodents implicated IH in activation of the sympathetic nervous system and dysregulation of glucose metabolism, regardless of CO₂ levels (Bao *et al.*, 1997a; Bao *et al.*, 1997b; Drager *et al.*, 2011; Lee *et al.*, 2013; Louis & Punjabi, 2009). Second, differences in fasting blood glucose between hypoxic and normoxic mice prevent us from a definitive statement on the effect of IH on insulin resistance in the ITT (Ayala *et al.*, 2010). Nonetheless, fasting blood glucose levels were identical in medullectomized and sham operated mice suggesting that adrenal medullectomy did not affect insulin resistance. Third, we estimate glucose stimulated insulin secretion based on a single measurement 30 min after i.p. glucose injection. This is a valid estimate given that IH does not affect insulin clearance (Polak *et al.*, 2013). The recently developed murine intravenous glucose tolerance test (IVGTT, (Alonso *et al.*, 2012) would be a more sensitive technique to measure time course of insulin secretion. However, the peak of glucose levels in IPGTT occurs 20–30 min after the glucose injection (Drager *et al.*, 2011; Polak *et al.*, 2013) and the lack of appropriate insulin secretion at this time is a highly specific (although not as sensitive as the IVGTT) marker of β -cell failure. Finally, we did not quantify sympathetic activation at the tissue level and were thus unable to characterize non-adrenal versus adrenal stimulation of the sympathetic nervous system by IH.

4.4. Conclusions

Adrenal medullectomy abolished IH-induced hyperglycemia by increasing basal and glucose stimulated insulin secretion. The IH-stimulated epinephrine efflux from adrenal medulla may be implicated in the development of type 2 diabetes in OSA.

Acknowledgments

This work has been supported by NIH grants HL080105 (VYP) and HL109475 (JCJ), and the American Heart Association Grants 10GRNT3360001(VYP) and 12POST118200001 (M-KS).

References

- Ahren B, Lundquist I. Effects of alpha-adrenoceptor blockade by phentolamine on basal and stimulated insulin secretion in the mouse. *Acta Physiol Scand.* 1985; 125:211–217. [PubMed: 2866660]
- Alonso LC, Watanabe Y, Stefanovski D, Lee EJ, Singamsetty S, Romano LC, Zou B, Garcia-Ocana A, Bergman RN, O'Donnell CP. Simultaneous measurement of insulin sensitivity, insulin secretion, and the disposition index in conscious unhandled mice. *Obesity (Silver Spring).* 2012; 20:1403–1412. [PubMed: 22331130]
- Andrikopoulos S, Blair AR, Deluca N, Fam BC, Proietto J. Evaluating the glucose tolerance test in mice. *Am J Physiol Endocrinol Metab.* 2008; 295:E1323–E1332. [PubMed: 18812462]

- Ayala JE, Samuel VT, Morton GJ, Obici S, Croniger CM, Shulman GI, Wasserman DH, McGuinness OP. Standard operating procedures for describing and performing metabolic tests of glucose homeostasis in mice. *Dis Model Mech*. 2010; 3:525–534. [PubMed: 20713647]
- Bao G, Metreveli N, Li R, Taylor A, Fletcher EC. Blood pressure response to chronic episodic hypoxia: role of the sympathetic nervous system. *J Appl Physiol*. 1997a; 83:95–101. [PubMed: 9216950]
- Bao G, Randhawa PM, Fletcher EC. Acute blood pressure elevation during repetitive hypocapnic and eucapnic hypoxia in rats. *J Appl Physiol*. 1997b; 82:1071–1078. [PubMed: 9104841]
- Barth E, Albuszies G, Baumgart K, Matejovic M, Wachter U, Vogt J, Radermacher P, Calzia E. Glucose metabolism and catecholamines. *Crit Care Med*. 2007; 35:S508–S518. [PubMed: 17713401]
- Baruzzi A, Riva R, Cirignotta F, Zucconi M, Cappelli M, Lugaresi E. Atrial natriuretic peptide and catecholamines in obstructive sleep apnea syndrome. *Sleep*. 1991; 14:83–86. [PubMed: 1839810]
- Bickel PE, Tansey JT, Welte MA. PAT proteins, an ancient family of lipid droplet proteins that regulate cellular lipid stores. *Biochim Biophys Acta*. 2009; 1791:419–440. [PubMed: 19375517]
- Carlson JT, Hedner J, Elam M, Ejnell H, Sellgren J, Wallin BG. Augmented resting sympathetic activity in awake patients with obstructive sleep apnea. *Chest*. 1993; 103:1763–1768. [PubMed: 8404098]
- Cheng K, Ho K, Stokes R, Scott C, Lau SM, Hawthorne WJ, O'Connell PJ, Loudovaris T, Kay TW, Kulkarni RN, Okada T, Wang XL, Yim SH, Shah Y, Grey ST, Biankin AV, Kench JG, Laybutt DR, Gonzalez FJ, Kahn CR, Gunton JE. Hypoxia-inducible factor-1 α regulates beta cell function in mouse and human islets. *J Clin Invest*. 2010; 120:2171–2183. [PubMed: 20440072]
- Dick TE, Hsieh YH, Wang N, Prabhakar N. Acute intermittent hypoxia increases both phrenic and sympathetic nerve activities in the rat. *Exp Physiol*. 2007; 92:87–97. [PubMed: 17138622]
- Drager LF, Jun JC, Polotsky VY. Metabolic consequences of intermittent hypoxia: relevance to obstructive sleep apnea. *Best Pract Res Clin Endocrinol Metab*. 2010; 24:843–851. [PubMed: 21112030]
- Drager LF, Li J, Reinke C, Bevans-Fonti S, Jun JC, Polotsky VY. Intermittent hypoxia exacerbates metabolic effects of diet-induced obesity. *Obesity (Silver Spring)*. 2011; 19:2167–2174. [PubMed: 21799478]
- Drager LF, Li J, Shin MK, Reinke C, Aggarwal NR, Jun JC, Bevans-Fonti S, Sztalryd C, O'Byrne SM, Kroupa O, Olivecrona G, Blaner WS, Polotsky VY. Intermittent hypoxia inhibits clearance of triglyceride-rich lipoproteins and inactivates adipose lipoprotein lipase in a mouse model of sleep apnoea. *Eur Heart J*. 2012; 33:783–790. [PubMed: 21478490]
- Drager LF, Togeiro SM, Polotsky VY, Lorenzi-Filho G. Obstructive sleep apnea: a cardiometabolic risk in obesity and the metabolic syndrome. *J Am Coll Cardiol*. 2013a; 62:569–576. [PubMed: 23770180]
- Drager LF, Yao Q, Hernandez KL, Shin MK, Bevans-Fonti S, Gay J, Sussan TE, Jun JC, Myers AC, Olivecrona G, Schwartz AR, Halberg N, Scherer PE, Semenza GL, Powell DR, Polotsky VY. Chronic Intermittent Hypoxia Induces Atherosclerosis via Activation of Adipose Angiopoietin-like 4. *Am J Respir Crit Care Med*. 2013b; 188:240–248. [PubMed: 23328524]
- Dungan KM, Braithwaite SS, Preiser JC. Stress hyperglycaemia. *Lancet*. 2009; 373:1798–1807. [PubMed: 19465235]
- Esler M, Jennings G, Lambert G, Meredith I, Horne M, Eisenhofer G. Overflow of catecholamine neurotransmitters to the circulation: source, fate, and functions. *Physiol Rev*. 1990; 70:963–985. [PubMed: 1977182]
- Fenik VB, Singletary T, Branconi JL, Davies RO, Kubin L. Glucoregulatory consequences and cardiorespiratory parameters in rats exposed to chronic-intermittent hypoxia: effects of the duration of exposure and losartan. *Front Neurol*. 2012; 3:51. [PubMed: 22509173]
- Foster GD, Sanders MH, Millman R, Zammit G, Borradaile KE, Newman AB, Wadden TA, Kelley D, Wing RR, Sunyer FX, Darcey V, Kuna ST. Obstructive sleep apnea among obese patients with type 2 diabetes. *Diabetes Care*. 2009; 32:1017–1019. [PubMed: 19279303]

- Gastaut H, Tassinari CA, Duron B. Polygraphic study of the episodic diurnal and nocturnal (hypnic and respiratory) manifestations of the Pickwick syndrome. *Brain Res.* 1966; 1:167–186. [PubMed: 5923125]
- Gonzalez-Martin MC, Vega-Agapito MV, Conde SV, Castaneda J, Bustamante R, Olea E, Perez-Vizcaino F, Gonzalez C, Obeso A. Carotid body function and ventilatory responses in intermittent hypoxia. Evidence for anomalous brainstem integration of arterial chemoreceptor input. *J Cell Physiol.* 2011; 226:1961–1969. [PubMed: 21520047]
- Greenberg HE, Sica A, Batson D, Scharf SM. Chronic intermittent hypoxia increases sympathetic responsiveness to hypoxia and hypercapnia. *J Appl Physiol.* 1999; 86:298–305. [PubMed: 9887143]
- Hartmann H, Beckh K, Jungermann K. Direct control of glycogen metabolism in the perfused rat liver by the sympathetic innervation. *Eur J Biochem.* 1982; 123:521–526. [PubMed: 6281012]
- Hedner J, Ejnell H, Sellgren J, Hedner T, Wallin G. Is high and fluctuating muscle nerve sympathetic activity in the sleep apnoea syndrome of pathogenetic importance for the development of hypertension? *J Hypertens Suppl.* 1988; 6:S529–S531. [PubMed: 3241251]
- Heitmann J, Ehlenz K, Penzel T, Becker HF, Grote L, Voigt KH, Peter JH, Vogelmeier C. Sympathetic activity is reduced by nCPAP in hypertensive obstructive sleep apnoea patients. *Eur Respir J.* 2004; 23:255–262. [PubMed: 14979500]
- Henriksen JH, Moller S, Ring-Larsen H, Christensen NJ. The sympathetic nervous system in liver disease. *J Hepatol.* 1998; 29:328–341. [PubMed: 9722218]
- Huang J, Lusina S, Xie T, Ji E, Xiang S, Liu Y, Weiss JW. Sympathetic response to chemostimulation in conscious rats exposed to chronic intermittent hypoxia. *Respir Physiol Neurobiol.* 2009; 166:102–106. [PubMed: 19429526]
- Iiyori N, Alonso LC, Li J, Sanders MH, Garcia-Ocana A, O'doherty RM, Polotsky VY, O'Donnell CP. Intermittent hypoxia causes insulin resistance in lean mice independent of autonomic activity. *Am J Respir Crit Care Med.* 2007a; 175:851–857. [PubMed: 17272786]
- Imadojemu VA, Mawji Z, Kunselman A, Gray KS, Hogeman CS, Leuenberger UA. Sympathetic chemoreflex responses in obstructive sleep apnea and effects of continuous positive airway pressure therapy. *Chest.* 2007; 131:1406–1413. [PubMed: 17494791]
- Jaworski K, Sarkadi-Nagy E, Duncan RE, Ahmadian M, Sul HS. Regulation of triglyceride metabolism. IV. Hormonal regulation of lipolysis in adipose tissue. *Am J Physiol Gastrointest Liver Physiol.* 2007; 293:G1–G4. [PubMed: 17218471]
- Jun J, Reinke C, Bedja D, Berkowitz D, Bevans-Fonti S, Li J, Barouch LA, Gabrielson K, Polotsky VY. Effect of intermittent hypoxia on atherosclerosis in apolipoprotein E-deficient mice. *Atherosclerosis.* 2010; 209:381–386. [PubMed: 19897196]
- Jun J, Savransky V, Nanayakkara A, Bevans S, Li J, Smith PL, Polotsky VY. Intermittent hypoxia has organ-specific effects on oxidative stress. *Am J Physiol Regul Integr Comp Physiol.* 2008; 295:R1274–R1281. [PubMed: 18703411]
- Jun JC, Drager LF, Najjar SS, Gottlieb SS, Brown CD, Smith PL, Schwartz AR, Polotsky VY. Effects of sleep apnea on nocturnal free fatty acids in subjects with heart failure. *Sleep.* 2011; 34:1207–1213. [PubMed: 21886358]
- Jun JC, Shin MK, Yao Q, Bevans-Fonti S, Poole J, Drager LF, Polotsky VY. Acute hypoxia induces hypertriglyceridemia by decreasing plasma triglyceride clearance in mice. *Am J Physiol Endocrinol Metab.* 2012; 303:E377–E388. [PubMed: 22621867]
- Kopin IJ, Lake RC, Ziegler M. Plasma levels of norepinephrine. *Ann Intern Med.* 1978; 88:671–680. [PubMed: 646261]
- Kumar GK, Rai V, Sharma SD, Ramakrishnan DP, Peng YJ, Souvannakitti D, Prabhakar NR. Chronic intermittent hypoxia induces hypoxia-evoked catecholamine efflux in adult rat adrenal medulla via oxidative stress. *J Physiol.* 2006; 575:229–239. [PubMed: 16777938]
- Lafontan M, Langin D. Lipolysis and lipid mobilization in human adipose tissue. *Prog Lipid Res.* 2009; 48:275–297. [PubMed: 19464318]
- Lee EJ, Alonso LC, Stefanovski D, Strollo HC, Romano LC, Zou B, Singamsetty S, Yester KA, McGaffin KR, Garcia-Ocana A, O'Donnell CP. Time-dependent changes in glucose and insulin

- regulation during intermittent hypoxia and continuous hypoxia. *Eur J Appl Physiol.* 2013; 113:467–478. [PubMed: 22801715]
- Lee EJ, Woodske ME, Zou B, O'Donnell CP. Dynamic arterial blood gas analysis in conscious, unrestrained C57BL/6J mice during exposure to intermittent hypoxia. *J Appl Physiol.* 2009; 107:290–294. [PubMed: 19056995]
- Lerner RL, Porte D Jr. Epinephrine: selective inhibition of the acute insulin response to glucose. *J Clin Invest.* 1971; 50:2453–2457. [PubMed: 4938132]
- Leuenberger U, Jacob E, Sweer L, Waravdekar N, Zwillich C, Sinoway L. Surges of muscle sympathetic nerve activity during obstructive apnea are linked to hypoxemia. *J Appl Physiol* (1985). 1995; 79:581–588. [PubMed: 7592221]
- Li J, Savransky V, Nanayakkara A, Smith PL, O'Donnell CP, Polotsky VY. Hyperlipidemia and lipid peroxidation are dependent on the severity of chronic intermittent hypoxia. *J Appl Physiol.* 2007; 102:557–563. [PubMed: 17082365]
- Li J, Thorne LN, Punjabi NM, Sun CK, Schwartz AR, Smith PL, Marino RL, Rodriguez A, Hubbard WC, O'Donnell CP, Polotsky VY. Intermittent hypoxia induces hyperlipidemia in lean mice. *Circ Res.* 2005; 97:698–706. [PubMed: 16123334]
- Liggett SB. α 2A-adrenergic receptors in the genetics, pathogenesis, and treatment of type 2 diabetes. *Sci Transl Med.* 2009; 1:12ps15.
- Louis M, Punjabi NM. Effects of acute intermittent hypoxia on glucose metabolism in awake healthy volunteers. *J Appl Physiol.* 2009; 106:1538–1544. [PubMed: 19265062]
- Marcus NJ, Li YL, Bird CE, Schultz HD, Morgan BJ. Chronic intermittent hypoxia augments chemoreflex control of sympathetic activity: role of the angiotensin II type 1 receptor. *Respir Physiol Neurobiol.* 2010; 171:36–45. [PubMed: 20153844]
- Marrone O, Riccobono L, Salvaggio A, Mirabella A, Bonanno A, Bonsignore MR. Catecholamines and blood pressure in obstructive sleep apnea syndrome. *Chest.* 1993; 103:722–727. [PubMed: 8449058]
- Metz W, Forssmann WG. Innervation of the liver in guinea pig and rat. *Anat Embryol (Berl).* 1980; 160:239–252. [PubMed: 7457919]
- Narkiewicz K, Kato M, Phillips BG, Pesek CA, Davison DE, Somers VK. Nocturnal continuous positive airway pressure decreases daytime sympathetic traffic in obstructive sleep apnea. *Circulation.* 1999; 100:2332–2335. [PubMed: 10587337]
- Narkiewicz K, van de Borne PJ, Cooley RL, Dyken ME, Somers VK. Sympathetic activity in obese subjects with and without obstructive sleep apnea. *Circulation.* 1998; 98:772–776. [PubMed: 9727547]
- Nobin A, Baumgarten HG, Falck B, Ingemansson S, Moghimzadeh E, Rosengren E. Organization of the sympathetic innervation in liver tissue from monkey and man. *Cell Tissue Res.* 1978; 195:371–380. [PubMed: 103622]
- Oben JA, Roskams T, Yang S, Lin H, Sinelli N, Torbenson M, Smedh U, Moran TH, Li Z, Huang J, Thomas SA, Diehl AM. Hepatic fibrogenesis requires sympathetic neurotransmitters. *Gut.* 2004; 53:438–445. [PubMed: 14960531]
- Pamidi S, Aronsohn RS, Tasali E. Obstructive sleep apnea: role in the risk and severity of diabetes. *Best Pract Res Clin Endocrinol Metab.* 2010; 24:703–715. [PubMed: 21112020]
- Pamidi S, Tasali E. Obstructive sleep apnea and type 2 diabetes: is there a link? *Front Neurol.* 2012; 3:126. [PubMed: 23015803]
- Patel KP, Zhang K, Carmines PK. Norepinephrine turnover in peripheral tissues of rats with heart failure. *Am J Physiol Regul Integr Comp Physiol.* 2000; 278:R556–R562. [PubMed: 10712272]
- Pinto P, Barbara C, Montserrat JM, Patarrao RS, Guarino MP, Carmo MM, Macedo MP, Martinho C, Dias R, Gomes MJ. Effects of CPAP on nitrate and norepinephrine levels in severe and mild-moderate sleep apnea. *BMC Pulm Med.* 2013; 13:13. [PubMed: 23497046]
- Polak J, Shimoda LA, Drager LF, Undem C, McHugh H, Polotsky VY, Punjabi NM. Intermittent Hypoxia Impairs Glucose Homeostasis in C57BL6/J Mice: Partial Improvement with Cessation of the Exposure. *Sleep.* 2013; 36:1483–1490. [PubMed: 24082307]

- Polotsky VY, Li J, Punjabi NM, Rubin AE, Smith PL, Schwartz AR, O'Donnell CP. Intermittent hypoxia increases insulin resistance in genetically obese mice. *J Physiol*. 2003; 552:253–264. [PubMed: 12878760]
- Polotsky VY, Rubin AE, Balbir A, Dean T, Smith PL, Schwartz AR, O'Donnell CP. Intermittent hypoxia causes REM sleep deficits and decreases EEG delta power in NREM sleep in the C57BL/6J mouse. *Sleep Med*. 2006; 7:7–16. [PubMed: 16309961]
- Prabhakar NR, Kumar GK, Peng YJ. Sympatho-adrenal activation by chronic intermittent hypoxia. *J Appl Physiol*. 2012; 113:1304–1310. [PubMed: 22723632]
- Punjabi NM, Beamer BA. Alterations in Glucose Disposal in Sleep-disordered Breathing. *Am J Respir Crit Care Med*. 2009; 179:235–240. [PubMed: 19011148]
- Punjabi NM, Shahar E, Redline S, Gottlieb DJ, Givelber R, Resnick HE. Sleep-disordered breathing, glucose intolerance, and insulin resistance: the Sleep Heart Health Study. *Am J Epidemiol*. 2004; 160:521–530. [PubMed: 15353412]
- Punjabi NM, Sorkin JD, Katzel LI, Goldberg AP, Schwartz AR, Smith PL. Sleep-disordered breathing and insulin resistance in middle-aged and overweight men. *Am J Respir Crit Care Med*. 2002; 165:677–682. [PubMed: 11874813]
- Raghuraman G, Prabhakar NR, Kumar GK. Differential regulation of tyrosine hydroxylase by continuous and intermittent hypoxia. *Adv Exp Med Biol*. 2012; 758:381–385. [PubMed: 23080186]
- Reinke C, Bevens-Fonti S, Drager LF, Shin MK, Polotsky VY. Effects of different acute hypoxic regimens on tissue oxygen profiles and metabolic outcomes. *J Appl Physiol*. 2011; 111:881–890. [PubMed: 21737828]
- Rosengren AH, Jokubka R, Tojjar D, Granhall C, Hansson O, Li DQ, Nagaraj V, Reinbothe TM, Tuncel J, Eliasson L, Groop L, Rorsman P, Salehi A, Lyssenko V, Luthman H, Renstrom E. Overexpression of alpha2A-adrenergic receptors contributes to type 2 diabetes. *Science*. 2010; 327:217–220. [PubMed: 19965390]
- Ruohonen ST, Ruohonen S, Gillsbach R, Savontaus E, Scheinin M, Hein L. Involvement of alpha2-adrenoceptor subtypes A and C in glucose homeostasis and adrenaline-induced hyperglycaemia. *Neuroendocrinology*. 2012; 96:51–59. [PubMed: 22327786]
- Savransky V, Bevens S, Nanayakkara A, Li J, Smith PL, Torbenson MS, Polotsky VY. Chronic intermittent hypoxia causes hepatitis in a mouse model of diet-induced fatty liver. *Am J Physiol Gastrointest Liver Physiol*. 2007; 293:G871–G877. [PubMed: 17690174]
- Skoglund G, Lundquist I, Ahren B. Effects of alpha 1- and alpha 2-adrenoceptor stimulation and blockade on plasma insulin levels in the mouse. *Pancreas*. 1986; 1:415–420. [PubMed: 2882501]
- Shin MK, Yao Q, Jun JC, Bevens-Fonti S, Yoo DY, Han W, Mesarwi O, Richardson RA, Fu YY, Pasricha PJ, Schwartz AR, Shirahata M, Polotsky VY. Carotid body denervation prevents fasting hyperglycemia during chronic intermittent hypoxia. *J Appl Physiol*. 2014 Aug.; 7. pii: jap. 01133.2013. [Epub ahead of print].
- Somers VK, Dyken ME, Clary MP, Abboud FM. Sympathetic neural mechanisms in obstructive sleep apnea. *J Clin Invest*. 1995; 96:1897–1904. [PubMed: 7560081]
- Stamatakis K, Sanders MH, Caffo B, Resnick HE, Gottlieb DJ, Mehra R, Punjabi NM. Fasting glycemia in sleep disordered breathing: lowering the threshold on oxyhemoglobin desaturation. *Sleep*. 2008; 31:1018–1024. [PubMed: 18652097]
- Szollasi A, Nenquin M, Henquin JC. Pharmacological stimulation and inhibition of insulin secretion in mouse islets lacking ATP-sensitive K⁺ channels. *Br J Pharmacol*. 2010; 159:669–677. [PubMed: 20128805]
- Tagaito Y, Polotsky VY, Campen MJ, Wilson JA, Balbir A, Smith PL, Schwartz AR, O'Donnell CP. A model of sleep-disordered breathing in the C57BL/6J mouse. *J Appl Physiol*. 2001; 91:2758–2766. [PubMed: 11717244]
- Wang N, Khan SA, Prabhakar NR, Nanduri J. Impairment of Pancreatic Beta Cell Function by Chronic Intermittent Hypoxia. *Exp Physiol*. 2013; 98:1376–85. [PubMed: 23709585]
- Xu J, Long YS, Gozal D, Epstein PN. Beta-cell death and proliferation after intermittent hypoxia: role of oxidative stress. *Free Radic Biol Med*. 2009; 46:783–790. [PubMed: 19133326]

- Yokoe T, Alonso LC, Romano LC, Rosa TC, O'doherty RM, Garcia-Ocana A, Minoguchi K, O'Donnell CP. Intermittent hypoxia reverses the diurnal glucose rhythm and causes pancreatic beta-cell replication in mice. *J Physiol.* 2008; 586:899–911. [PubMed: 18033815]
- Zechner R, Kienesberger PC, Haemmerle G, Zimmermann R, Lass A. Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores. *J Lipid Res.* 2009; 50:3–21. [PubMed: 18952573]
- Ziegler MG, Elayan H, Milic M, Sun P, Gharaibeh M. Epinephrine and the metabolic syndrome. *Curr Hypertens Rep.* 2012; 14:1–7. [PubMed: 22124970]
- Zoccal DB, Simms AE, Bonagamba LG, Braga VA, Pickering AE, Paton JF, Machado BH. Increased sympathetic outflow in juvenile rats submitted to chronic intermittent hypoxia correlates with enhanced expiratory activity. *J Physiol.* 2008; 586:3253–3265. [PubMed: 18450774]

Highlights

- Intermittent hypoxia (IH) increased plasma epinephrine and norepinephrine levels.
- IH increased fasting blood glucose and decreased insulin levels.
- Adrenal medullectomy improved glucose tolerance during IH.
- Adrenal medullectomy prevented the decrease in insulin secretion during the IH.
- Adrenal medullectomy did not affect IH-induced insulin resistance.

Figure 1.

Adrenal tissue epinephrine (**A**) and norepinephrine (**B**) levels in C57BL/6J mice subjected to adrenal medullectomy or sham surgery and then exposed to chronic intermittent hypoxia or intermittent air for 6 weeks. $p < 0.001$ for the effect of medullectomy for both epinephrine ($F = 63.54$) and norepinephrine ($F = 69.14$). The degree of freedom = 19.

Figure 2.

Plasma epinephrine (**A**) and norepinephrine (**B**) levels in C57BL/6J mice subjected to adrenal medullectomy or sham surgery and then exposed to chronic intermittent hypoxia or intermittent air for 6 weeks. * and †, $p = 0.0019$ and $p < 0.001$ ($F = 40.58$, the degree of freedom = 37), respectively, for the difference with intermittent air.

□ Intermittent Air
 ■ Chronic Intermittent Hypoxia

Figure 3.

Fasting (5 h) blood glucose (**A**) and fasting plasma insulin levels (**B**) in C57BL/6J mice subjected to adrenal medullectomy or sham surgery and then exposed to chronic intermittent hypoxia or intermittent air for 6 weeks. *, $p < 0.001$ for the difference between intermittent air and intermittent hypoxia in both sham and medullectomized animals; †, $p < 0.05$ for the effect of medullectomy and $p < 0.01$ for the difference with the intermittent air – medullectomy group; ‡, $p < 0.05$ for the difference with intermittent air – sham.

Figure 4. Intraperitoneal glucose tolerance test (1g/kg) in C57BL/6J mice subjected to sham surgery (A) or adrenal medullectomy (B) and then exposed to chronic intermittent hypoxia or intermittent air for 6 weeks. The area under the curve above the baseline is depicted on panel C. *, $p < 0.001$ for the difference with all other groups.

Figure 5.

Intraperitoneal insulin tolerance test (0.5U/kg) in C57BL/6J mice subjected to sham surgery (A) or adrenal medullectomy (B) and then exposed to chronic intermittent hypoxia or intermittent air for 6 weeks. The area under the curve is depicted on panel C. *, $p = 0.048$ for the difference with intermittent air.

A. Sham Surgery – Intermittent Air **B. Sham Surgery – Intermittent Hypoxia** **C. Adrenal Medullectomy – Intermittent Air** **D. Adrenal Medullectomy – Intermittent Hypoxia** **Figure 6.**

Changes in plasma insulin levels 30 min after i.p. glucose administration (1 g/kg) from fasting baseline. The lines represent mean levels. *, †, and ‡, $p = 0.012$, 0.0062 , and 0.035 , respectively, for the change of the mean from the baseline level.

Basic characteristics of C57BL/6J mice exposed to chronic intermittent hypoxia (IH) or intermittent air (IA) after adrenal medullectomy (MED) or sham surgery

Table 1

	Sham		Adrenal Medullectomy		Effect of IH p (F-value)		Effect of medullectomy p (F-value)	
	IA	IH	IA	IH	IA	IH	IA	IH
N	10	10	10	10				
Age	6	6	6	6				
(weeks)	8	8	8	8				
Body	20.7 ± 0.4	21.5 ± 0.5	20.9 ± 0.3	21.2 ± 0.4	0.51 (0.46)	0.51 (0.46)	0.51 (0.46)	0.51 (0.46)
weight (g)	21.8 ± 0.4	20.5 ± 0.3	21.9 ± 0.4	21.2 ± 0.3	0.012 (13.89)	0.012 (13.89)	0.045 (9.84)	0.045 (9.84)
Liver weight (g)	0.71 ± 0.01	0.77 ± 0.01	0.73 ± 0.02	0.80 ± 0.02	0.002 (16.3)	0.002 (16.3)	0.213 (1.61)	0.213 (1.61)
Epididymal fat (g)	0.41 ± 0.02	0.33 ± 0.02	0.41 ± 0.02	0.36 ± 0.02	0.004 (9.85)	0.004 (9.85)	0.444 (0.60)	0.444 (0.60)
Fasting plasma corticosterone (ng/ml)	158.2 ± 11.6	157.8 ± 19.1	140.4 ± 12.1	165.9 ± 20.9	0.140 (2.30)	0.140 (2.30)	0.314 (1.05)	0.314 (1.05)
Fasting plasma glucagon (pg/ml)	92.9 ± 12.5	89.0 ± 7.7	85.0 ± 6.4	77.7 ± 5.2	0.547 (0.37)	0.547 (0.37)	0.306 (1.09)	0.306 (1.09)
Fasting plasma triglycerides (mg/dl)	22.5 ± 2.1	31.27 ± 3.3	21.7 ± 1.7	31.3 ± 2.6	0.001 (15.86)	0.001 (15.86)	0.295 (1.13)	0.295 (1.13)
Fasting plasma cholesterol (mg/dl)	76.7 ± 1.9	88.2 ± 2.3	80.3 ± 2.8	84.9 ± 3.5	0.025 (5.50)	0.025 (5.50)	0.56 (0.35)	0.56 (0.35)
Fasting plasma free fatty acids (mmol/l)	0.60 ± 0.02	0.68 ± 0.19	0.52 ± 0.03	0.44 ± 0.07	0.989 (0.00)	0.989 (0.00)	0.144 (2.23)	0.144 (2.23)

Note: the degree of freedom for body, liver and epididymal fat weights was 39, for the change in body weight it was 9 per group and for all other measurements it was 37.