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# The effect of adrenal medullectomy on metabolic responses to chronic intermittent hypoxia

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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.


## 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, 2004; Punjabi and Beamer, 2009; Pamidi et al., 2010; Pamidi and 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 and Beamer, 2009; Stamatakis et al., 2008; Drager et al., 2010). Exposure of healthy human volunteers to IH leads to insulin resistance and impaired insulin secretion (Louis and 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. (2008) reported that IH induces replication of pancreatic β-cells, whereas Xu et al. (2009) reported increases both in β-cell apoptosis and proliferation. 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. (2006) showed that exposure to chronic IH increased norepinephrine and epinephrine efflux from the adrenal medulla *ex vivo*.

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;

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Dungan et al., 2009) and stimulation of adipose tissue lipolysis (Jaworski et al., 2007; Lafontan and 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 and 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 h 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., 2011, 2012, 2013a,b). 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 $O_2$ decreased from ~21% to ~6–7% over a 30 s period, followed by a rapid return to ~21% over the subsequent 30 s period. We have previously shown that this regimen of IH induces oxyhemoglobin desaturations from 99% to ~65%, 60 times/h (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 12 pm and 1 pm. 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-h fast by injecting 1 g/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 min after glucose injection. The IPGTT areas under the curve were calculated after subtracting the fasting glucose baseline. ITT was performed after a 2-h 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 min 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 ± 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