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SLEEP DISORDERED BREATHING AND METABOLIC EFFECTS: EVIDENCE FROM ANIMAL MODELS

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INTRODUCTION

Obstructive sleep apnea (OSA) is the most common form of sleep disordered breathing (SDB) and is characterized by recurrent collapse of the upper airway during sleep leading to periods of intermittent hypoxia (IH) and sleep fragmentation (SF). OSA is prevalent in obese individuals, especially those with visceral obesity [1–3]. Metabolic syndrome, which incorporates visceral obesity, hypertension, glucose intolerance, and insulin resistance [4], is almost invariably associated with OSA [5]. Weight loss significantly alleviates OSA [6;7]. In addition, severe obesity may lead to alveolar hypoventilation during sleep, which also improves with weight loss [8;9]. However, the same degree of obesity may lead to severe SDB in some individuals, whereas others remain unaffected [1–3] suggesting that SDB is not attributable solely to amount of mechanical load on the upper airway, chest wall and diaphragm. In addition to obesity, endocrine disorders such as diabetes mellitus, hypothyroidism, and acromegaly, are also associated with SDB [10–15]. Thus, metabolic dysfunction may independently lead to OSA.

There is a growing body of epidemiological evidence that OSA is associated with metabolic abnormalities and may be implicated in causality of metabolic disorders. OSA is linked to increased risk of hypertension, insulin resistance, glucose intolerance and type 2 diabetes, dyslipidemia, atherosclerosis, and non-alcoholic fatty liver disease (NAFLD), independent of underlying obesity [1;12;16–24].

Thus, there are interactions between metabolic dysregulation and OSA which may lead to a vicious circle of cardiovascular and metabolic morbidity. The complexity of the underlying relationship between metabolism and OSA can be scrutinized most effectively with animal models, which can account for all possible confounding factors.

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ANIMAL MODELS OF SLEEP APNEA

Several strategies to simulate the physiology or the effects of OSA in animals have been attempted. They include identification of animals with spontaneous OSA, mechanical occlusion of the airway, delivery of hypoxic gases, and causing fragmentation of sleep. Some models have been used to study effects of OSA on metabolism. For the purposes of this overview, we will introduce most of the common approaches, whether or not they were in fact used to study metabolic endpoints.

Animals with Spontaneous OSA

The most natural animal model of OSA to date is the English bulldog, a breed with an enlarged soft palate and narrow oropharynx prone to snoring, hypopneas, and arousals [25;26]. Hendricks *et al* performed polysomnography (PSG) on English bulldogs and reported central and obstructive apnea with oxyhemoglobin desaturations to levels below 90% during rapid-eye-movement (REM) sleep, and hypersomnolence with shortened sleep latencies [25;26]. Thus far, the English bulldog has been used to study upper airway anatomy and physiology [27–29], and pharmacological treatment of OSA with various serotonergic medications [30;31]. OSA experienced by bulldogs is not related to obesity. In contrast, obese Yucatan mini-pigs exhibit considerably more apneas and oxyhemoglobin desaturations than their lean counterparts and have been proposed as a model of obesity-related sleep apnea [32].

Non-human primates have upper airways with structural and functional similarities to that of humans, but no primate species are known to have spontaneous OSA. Philip *et al* administered intradermal liquid collagen injections to monkeys in the uvula, tongue, and lateral pharyngeal walls every 2 weeks while performing PSG at regular intervals [33]. The injections resulted in significant hypopneas with a decrease in stage 2 and REM sleep. Animals without intrinsic OSA have been used to study the feasibility of various treatment modalities, including hypoglossal nerve stimulation in dogs [34], and MR-imaging guided radiofrequency thermal ablation to the base of the tongue in pigs [35].

In summary, spontaneous OSA is very uncommon in animals. The study of metabolic outcomes generally requires a significant sample size with well-characterized genotypic and phenotypic characteristics. Therefore, it is not feasible to conduct successful metabolic research in animals with spontaneous OSA.

Mechanical Airway Occlusion

To obviate the need for models with spontaneous apneas, researchers can simulate OSA by causing mechanical airway obstruction. While less physiologic than the apneas experienced by aforementioned models, this approach creates predictable, reliable, and modifiable events. In sedated intubated pigs, obstructive apneas were induced by repetitive clamping and unclamping of the endotracheal tubes every 30 seconds. Investigators could then carry out real-time hemodynamic measurements in the presence of vagotomy and aortic nerve sectioning [36;37]. A rat model of the upper airway occlusion, independent of sleep, has recently been developed and applied to study the effects of apnea on vascular inflammation. Tracheotomized and anesthetized rats were ventilated via a computer-controlled collapsible upper airway segment. [38–41].

More sophisticated models require monitoring of sleep-wake state in order to generate periods of sleep-induced airway obstruction terminated by animal arousal. In a canine model of sleep apnea developed in Dr. Phillipson's laboratory, electroencephalographic (EEG) and electromyography (EMG) signals were continuously monitored in tracheostomized dogs by a computer, which judged sleep-wake state [42;43]. After allowing a brief period of sleep, the

computer sent a remote-controlled signal to an occlusion valve through which the dog breathed. Upon awakening, the valve was released. This model was used to study sleep architecture prior, during, and after recovery from OSA [44] and was employed to study blood pressure responses to apnea [38;39;45]. The clear advantage of this strategy lies in its similarity to natural OSA, with occlusion of the airway closely coordinated with sleep onset. However, the need to continuously assess sleep-wake state makes this method cumbersome and technically difficult to apply to large sample sizes needed for metabolic endpoints.

Intermittent Hypoxia

Several animal models have been developed to study the effects of intermittent hypoxia (IH), a key physiological manifestation of OSA, on a variety of cardiovascular, metabolic, and neurocognitive outcomes. The most sophisticated models deliver hypoxic gases with the onset of sleep and subsequent removal of the stimulus when arousal or wakefulness occurs [46–48]. Such an approach is almost as complex as that of sleep-related airway occlusion. Consequently, the vast majority of studies examining the sequelae of OSA have utilized rodent models of IH that are not dependent on the presence of sleep. Exposing animals to IH simulates a significant aspect of OSA in a noninvasive manner allowing for control over the degree of oxyhemoglobin desaturation independent of sleep-wake state. Several systems have been designed, but all generally involve a gas control system which regulates the flow of room air, nitrogen and oxygen into customized cages [49]. Rodents are exposed to periods of hypoxia of a fixed duration (usually 30–120 sec) throughout the light phase and maintain normoxia during the dark phase. Fletcher *et al.* first attempted to use a model of chronic IH (CIH) in rats to explore relationships between OSA and hypertension [50]. CIH for 5 weeks induced hypertension in rats, both during exposure and in the period of subsequent rest, in a highly reproducible fashion [50–54]. Later studies explored whether CIH impairs sleep in a manner similar to human OSA. In rats, IH with an FiO_2 nadir of 10% caused an initial overall reduction in NREM and REM sleep in the light phase that normalized within two days [55]. In mice, Veasey *et al.* performed PSG recording after longer exposures to IH (eight weeks) with an FiO_2 nadir of 10% and found significantly altered sleep architecture [56]. Polotsky *et al.* demonstrated that IH with an FiO_2 nadir of 5% for 5 days caused arousals in mice with each hypoxic episode and led to significant disruption of sleep with a marked decrease of the EEG delta power of NREM sleep and disappearance of REM sleep without any trend to recovery by the end of exposure [57]. The validated murine model of IH was recently employed to study effects of IH on hyperlipidemia and insulin resistance [49;58–61]. Waters *et al.* explored a link between sudden infant death syndrome (SIDS) and OSA in infants, testing the hypothesis that arousal deficits can be induced by intermittent asphyxia during normal development [62]. Intermittent hypercapnic hypoxia was delivered for 4 days to young mixed-breed miniature piglets to measure frequency and latency of arousal responses. A face mask was sealed against the snout providing 6-minute intervals of 8% O_2 , 7% CO_2 alternating with air for a total of 48 minutes. The authors found that successive days of exposure resulted in fewer and progressively delayed arousals. Thus, exposure to IH produces recurrent arousals and profound changes in sleep architecture, comparable to those in humans with OSA, and can be used to study metabolic sequelae of OSA.

Sleep Fragmentation (SF)

Separately from hypoxia or cessation of airflow, OSA causes sleep fragmentation (SF), which may have independent effects on metabolism. Sleep deprivation models cannot be directly applied to SDB, because human OSA is characterized by SF, which may or may not be accompanied by partial sleep deprivation [63]. There are several animal models of SF. Brooks *et al.* developed a canine model of SF, which is similar to their model of the airway obstruction [38;64]. A dog was instrumented for PSG recording and whenever a sleep period of predetermined length was identified by the computer, it generated a signal to activate an

acoustic alarm. Polotsky *et al.* developed a mouse model of non-hypoxic SF during the light phase using an auditory/tactile stimulus and validated this model by PSG recording [57]. SF resulted in reproducible arousals over the 5-day exposure without change in total sleep time over 24 hrs. However, the effectiveness of auditory/tactile SF over longer periods of time is unknown. The major disadvantage of SF models is that none of the existing models can assure reproducible arousals during chronic exposure. In addition, as homeostatic pressure to sleep increases, animals may be capable of short episodes of micro-sleep between stimuli. Thus, an animal model of long-term SF without sleep deprivation has yet to be developed. Our subsequent discussion of animal modeling of metabolic effects of OSA will draw mainly from data obtained using IH models. This approach is the best described in the literature, and simulates the most important aspect of OSA.

INTERMITTENT HYPOXIA, INSULIN AND GLUCOSE REGULATION

A significant body of clinical evidence suggests that the hypoxic stress of OSA is associated with insulin resistance, glucose intolerance and type 2 diabetes mellitus. However, causal relationships between OSA and diabetes are not completely understood.

Effects of exposure to continuous hypoxia on glucose and insulin regulation have been studied for a number of years. Short-term continuous hypoxia (from 30 min to 2–3 days) causes acute insulin resistance in humans [65–67], whereas long-term continuous hypoxia (more than 6 weeks) reduces fasting blood glucose (FBG) levels, but does not affect insulin resistance or glucose tolerance in humans and rodents [66;68;69].

Others have examined the relationship between IH and glucose metabolism. IH was administered to mice by decreasing FiO₂ in cages from 20.9% to 5% over 30 seconds and rapidly reoxygenating to 20.9% during the subsequent 30 seconds. The exposure was carried out during the 12 hour light phase (9 p.m. to 9 a.m.) for 5 consecutive days, leaving the animals undisturbed during the 12 hr dark phase. After exposure, acute effects were studied, representative of nocturnal changes in glucose and insulin in patients with OSA. Effects 5–6 hours after exposure were representative of resultant daytime alterations in glucose and insulin. [49;59;61].

In lean C57BL/6J mice and obese *ob/ob* mice, FBG levels acutely increased during IH, whereas fasting serum insulin remained intact [59]. In lean mice of mixed C57BL/6J x 129 background, identical exposure raised both FBG and serum insulin levels [61]. These studies suggest that short-term IH leads to acute hyperglycemia. However, they do not assess degree of insulin resistance. Furthermore, they utilized anesthetized animals, with potentially altered glucose and insulin regulation [70].

The gold standard to estimate insulin resistance is a euglycemic hyperinsulinemic clamp, when a superphysiologic dose of insulin is administered, while blood glucose levels are maintained in the normal range by variable amounts of infused glucose. The rate of glucose infusion represents the whole body glucose uptake as an index of insulin sensitivity [71]. Iiyori *et al.* examined the impact of acute exposure to IH with FiO₂ nadir of 5%, 60 episodes/hr for 6 hrs, using a euglycemic hyperinsulinemic clamp in awake unrestrained mice, and found that IH significantly suppresses the whole body glucose uptake, indicating that IH acutely increases insulin resistance [72].

Short-term IH had a profound effect on levels of insulin and glucose drawn 6 hours after cessation of the exposure [49]. In lean C57BL/6J mice, IH significantly decreased FBG and glucose levels throughout the intraperitoneal glucose tolerance test (IPGTT), suggesting improved glucose tolerance, while fasting insulin levels remained unchanged. To evaluate the degree of insulin resistance, the homeostasis model assessment (HOMA) was calculated using

the following formula: $\text{HOMA} = \text{fasting serum insulin } (\mu\text{u ml}^{-1}) * \text{FBG } (\text{mmol l}^{-1})/22.5$ [73]. IH induced a greater than three-fold decrease in HOMA in lean mice. Thus, lean wildtype C57BL/6J mice exhibit a biphasic response to short-term IH: FBG levels and insulin resistance increase acutely during the exposure and improve during the subsequent period of rest, suggesting compensatory adaptation.

The effect of short-term IH was examined in leptin-deficient *ob/ob* mice 6 hours after exposure [49]. Similarly to lean mice, *ob/ob* mice showed decreases in FBG and improvement in glucose tolerance with IPGTT. In contrast to lean mice, *ob/ob* mice exhibited a marked increase in fasting and post-IPGTT serum insulin levels as well as a four-fold increase in the HOMA index after short-term IH, suggesting up-regulation of insulin secretion and progression of insulin resistance. Thus, obesity and impaired leptin significantly compromise insulin responses to IH.

Glucose and insulin regulation after exposure to chronic IH (CIH) for 35 days was assessed in anesthetized adult Sprague-Dawley rats [74]. FiO_2 in cages alternated between 5.5%–10% (70 s) and 18.9–25% (80s) from 7 a.m. to 5 p.m. and measurements were performed immediately after the exposure. CIH increased FBG levels from 195 mg/dl to 286 mg/dl, whereas serum insulin levels declined, suggesting pancreatic endocrine insufficiency. IPGTT showed a 2.5-fold decline in stimulated insulin secretion in hypoxic rats compared to controls. Furthermore, decreased serum insulin level was associated with increased insulin mRNA and protein levels in pancreatic islets suggesting that IH specifically affected insulin release.

Similar results were obtained in C57BL/6J mice after CIH exposure for 12 weeks with an FiO_2 nadir of 5% [75]. Immediately following exposure, FBG levels increased (203 mg/dl vs. 148 mg/dl in control mice, $p < 0.05$), while fasting serum insulin decreased by 30%. *Ob/ob* mice exposed to CIH for 12 weeks showed unchanged FBG levels but markedly impaired glucose tolerance in IPGTT, with steady progression of insulin resistance throughout the time course [49].

In summary, (1) in lean wildtype animals, short-term IH leads to hyperglycemia and insulin resistance, but glucose regulation remains intact during resting intervals between exposures. CIH, similar to severe OSA, leads to hyperglycemia during hypoxic exposure in association with low levels of insulin, indicating that pancreatic endocrine deficiency may have developed. It is unknown whether CIH also leads to hyperglycemia during resting intervals, similar to elevated daytime glucose levels in patients with OSA and whether CIH causes insulin resistance in the absence of obesity. (2) In obese leptin-deficient mice, short term IH exacerbates underlying hyperglycemia without changes in insulin levels during the exposure, suggesting progression of insulin resistance, which persists during resting intervals. CIH in these animals leads to relentless progression of insulin resistance and glucose intolerance.

MECHANISMS OF METABOLIC EFFECTS OF INTERMITTENT HYPOXIA

Transcriptional Regulation, Inflammatory Response, and Metabolic Effects of Intermittent Hypoxia

Metabolic effects of IH could be regulated at the transcriptional level. Most of the current studies indicate that IH induces transcription of hypoxia inducible factor 1 (HIF-1) in carotid bodies, cardiomyocytes, and liver [61;76;77], although one study in IH cell culture suggest the opposite [78]. Activation of HIF-1 during IH may occur due to the direct effect of severe hypoxia [79;80] or from excessive production of reactive oxygen species (ROS) [81]. Indeed, OSA is associated with increased generation of ROS [82;83], and experimental IH increases ROS and lipid peroxidation in the liver, myocardium, and brain [60;75;84–86]. HIF-1 is a heterodimer consisting of an O_2 -regulated HIF-1 α subunit and a constitutively expressed

HIF-1 β subunit [87;88]. HIF-1 is a master regulator of oxygen homeostasis and controls a variety of physiological responses to hypoxia, including erythropoiesis, angiogenesis, glucose metabolism, and lipid metabolism [61;80;89–92].

HIF-1 has numerous potential direct and indirect downstream influences on glucose metabolism. HIF-1 up-regulates glycolytic enzymes and GLUT-1, which is a pivotal glucose transporter in the brain and other insulin independent tissues [90]. HIF-1 also up-regulates insulin growth factors [93], which exhibit insulin-like activity, thereby increasing insulin sensitivity [94]. In addition, HIF-1 up-regulates leptin [95], which also increases insulin sensitivity [96;97]. Alternatively, HIF-1 induction during IH may increase insulin resistance. Indeed, HIF-1 up-regulates sterol regulatory element binding protein 1 (SREBP-1)[61], the main regulator of lipid biosynthesis in the liver [98–100], and up-regulation of SREBP-1 raises serum and liver fatty acid levels increasing insulin resistance [101;102]. HIF-1 also raises levels of endothelin-1 [103], which is implicated in increased insulin resistance [104;105]. Mice heterozygous for HIF-1 α do not exhibit an increase in serum insulin levels observed in their wildtype littermates during IH, suggesting that HIF-1 α activation may be responsible for IH-induced insulin resistance [61]. Thus, HIF-1 may be involved in metabolic responses to IH, but it is unclear whether HIF-1 activation alleviates or exacerbates insulin resistance and hyperglycemia.

IH increases activity of another transcription factor, nuclear factor κ B (NF- κ B) [75;78;106], which may influence glucose metabolism, inflammation, and atherosclerosis. The mechanisms of NF- κ B activation during IH are not known, but it is likely triggered by increased production of ROS [60;75;78;84–86;106;107]. NF- κ B is a major transcription factor regulating the inflammatory response [108;109]. In its inactive state, NF- κ B is located in the cytoplasm, where it is bound to inhibitory protein I κ B. After I κ B is phosphorylated by a cellular kinase complex, IKK, NF- κ B translocates to the nucleus, where it regulates expression of multiple inflammatory genes, including tumor necrosis factor α (TNF- α), interleukin 1 β (IL-1 β), and IL-6 [110–112]. In turn, TNF- α activates NF- κ B by stimulating IKK [108;110]. IKK- β induces serine phosphorylation of insulin receptor substrate 1 (IRS-1), which prevents tyrosine phosphorylation of IRS-1, disrupting the insulin signaling pathway and increasing insulin resistance [113;114]. In addition, TNF- α and IL-6 increase insulin resistance by stimulating lipolysis [115]. Obesity leads to over-expression of inflammatory cytokines [116;117]. Patients with OSA have elevated serum levels of TNF- α and IL-6, independent of body weight [2; 118;119]. Our group has shown that mice exposed to short term IH have increased levels of circulating IL-6 [61]. In addition to TNF- α , IL-1 β , and IL-6, NF- κ B up-regulates monocyte chemoattractant protein protein-1 (MCP-1), IL-8, P-selectin, E-selectin, intracellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), which are all implicated in atherosclerosis [120–128] and are elevated in patients with OSA [129–132]. The impact of experimental IH on adhesion molecules has not been studied. Thus, IH may lead to insulin resistance and atherosclerosis via transcriptional activation of inflammatory pathways, but additional animal studies are needed to characterize inflammatory effects of IH.

Intermittent Hypoxia, Sympathetic Axis and Insulin Counter-Regulatory Hormones

We will next review the evidence of how IH affects the sympathetic nervous system and insulin counter-regulatory hormones, including catecholamines, glucagon, corticosterone, and growth hormone. Acute hypoxia raises plasma epinephrine levels [67], and patients with OSA have increased circulating levels of catecholamines[133–138]. Multiple studies describe an increase in sympathetic nervous system activity in association with oxyhemoglobin desaturation in patients with OSA [134;135;139–141]. Increases in sympathetic activity accompanied each obstructive event, resolved after termination of the event, and correlated in magnitude with the degree of oxyhemoglobin desaturation [140;141]. Sympathetic activation in OSA carries over

into the daytime wakefulness and is alleviated by CPAP treatment. Human studies, however, do not isolate effects of IH from other components of OSA such as hypercapnea and sleep fragmentation (SF) as some of the following studies were able to do.

Excessive sympathetic output is implicated in pathogenesis of systemic hypertension in patients with OSA [137;142]. Sympathetic over-activity was studied in several animal models of OSA, in connection with vascular or blood pressure responses to IH or airway obstruction. Brooks *et al* found that both non-occlusive sleep fragmentation (SF) and airway obstruction during the night produced nighttime hypertension, whereas only airway obstruction caused daytime hypertension with a 16 mmHg increase in mean blood pressure, persisting for 1–3 wks after cessation of the exposure [38]. O'Donnell *et al.* showed that airway obstruction causes acute elevation of blood pressure in the absence of arousal and that pharmacological blockade of autonomic nervous system (ANS) with hexamethonium completely eliminated the blood pressure response [45]. The same group later showed that systemic hypertension in response to airway obstruction is attributable to IH and abolished by hyperoxia [39]. In a rat model of CIH, Fletcher *et al.* reported similar findings that CIH for 35 days increased mean blood pressure by 10–20 mmHg [50].

Fletcher *et al* also showed that CIH leads to daytime systemic hypertension regardless of the level of inspired CO₂ suggesting that IH is the main stimulus in OSA for hypertension and sympathetic activation [52]. Bao *et al.* showed that CIH for 5 weeks significantly increased mean plasma levels of epinephrine and norepinephrine and that an increase in epinephrine was abolished by adrenal medullectomy, whereas an increase in norepinephrine persisted. These findings indicated that IH up-regulated epinephrine release by adrenals, whereas norepinephrine was mainly produced by the sympathetic bed outside the adrenals [143]. Kumar *et al.* exposed rats to CIH (15 s of 5% O₂ followed by 5 min of 21% O₂; 9 episodes/hr; 8 hrs a day; for 3 or 10 days), continuous hypoxia (4 h of 7% O₂ followed by 20 h of 21% O₂ for 1 or 10 days), or hypercapnia (10% CO₂; either acidic, pH 6.8, or isohydric, pH 7.4) and found that noradrenaline and adrenaline effluxes from ex vivo adrenal medullae were significantly increased by CIH, whereas continuous hypoxia or hypercapnea had no effect [144]. Thus, animal data suggest that CIH of OSA may cause sympathetic activation, both acutely and throughout the day, whereas SF, hypercapnea, and sustained hypoxia have lesser effects.

OSA is associated with increased levels of circulating angiotensin II and aldosterone [145]. Rats exposed to CIH with an FiO₂ nadir of 2–3% for 35 days exhibited a fourfold increase in plasma renin activity and a 10 mm Hg increase in mean arterial blood pressure, which were abolished by renal sympathetic denervation [146]. Angiotensin II receptor blockade with losartan abolished a hypoxia-induced increase in blood pressure, whereas plasma renin activity remained elevated. These data suggest that CIH increases sympathetic output of the renal nerve, which up-regulates renin secretion ultimately leading to hypertension via the angiotensin II pathway.

Sympathetic activation has several effects on glucose metabolism. Catecholamines stimulate the mobilization of glycogen from muscle and triglycerides from adipose tissue and inhibit glucose uptake by muscle. Epinephrine stimulates secretion of glucagon, inhibits secretion of insulin, and increases gluconeogenesis in the liver. The role of the autonomic nervous system (ANS) in IH-induced insulin resistance was examined in only one study. Iiyori *et al* measured total body glucose uptake in awake unrestrained mice by hyperinsulinemia euglycemic clamp during exposure to IH with an FiO₂ nadir of 5% (60 episodes per hour) for 6 hours in the presence or absence of ANS blockade by hexamethonium [147]. The study found that IH increased insulin resistance, independent of ANS activity. However, it is conceivable that selective sympathetic blockade would have a different effect on insulin resistance during IH

and that catecholamines and the sympathetic nervous system play a role in insulin and glucose regulation during longer exposures.

IH affects not only the sympathetic system, but may lead to dysfunction of the hypothalamic-pituitary-adrenal axis (HPA) as a whole [12]. The result is increased levels of corticosteroids, predominantly cortisol in humans and corticosterone in rodents [148]. Steroid hormones disturb insulin and glucose regulation by multiple mechanisms [149]: they (a) increase lipolysis, leading to muscle insulin resistance by impairing insulin signaling pathways; (b) inhibit insulin dependent translocation of GLUT4 to the cell surface in muscle; (c) suppress glycogen synthase in muscle tissue; (d) increase hepatic glucose output; and (e) inhibit pancreatic insulin secretion. OSA changes circadian rhythm of corticosteroids, elevating plasma levels of cortisol during the night [150]. Chronic sustained hypoxia (10.8%) for 5 days nearly doubled plasma corticosterone level in rats [151]. CIH for 3 months with a FiO₂ nadir of 5% did not increase levels of circulating corticosterone in mice [75], suggesting that animals may have adapted to the stress of IH.

Another insulin counter-regulatory hormone, growth hormone (GH), is suppressed in patients with OSA. GH is secreted predominantly during slow wave sleep (SWS), and disappearance of SWS in OSA is implicated in GH suppression [152;153]. Chronic hypoxia significantly decreases GH mRNA and protein levels in pituitary gland of rats [154;155]. OSA or chronic hypoxia have not been observed to have any effect on glucagon levels [66;152]. The effects of experimental IH on GH and glucagon have not been elucidated. Thus, experimental IH increases production of catecholamines, epinephrine and norepinephrine, and induces sympathetic activation; effects of IH on corticosterone, glucagon, GH, and the role of sympathetic activation in IH-induced insulin resistance have not been sufficiently studied.

Intermittent Hypoxia, Adipokines, and Other Metabolic Hormones

IH induces changes in leptin [49], an adipocyte-derived hormone that produces satiety and increases metabolic rate [156–160]. Leptin is present in the circulation at levels proportional to the degree of obesity [157] and the severity of OSA [161–163]. However, high levels of circulating leptin in this setting do not result in low food intake and weight loss, which has led to the concept of leptin resistance [157;159]. It appears that hyperleptinemia in patients with OSA is associated with resistance to metabolic effects of the hormone.

Leptin can act centrally at the level of the hypothalamus, and peripherally at the level of β -cells of pancreatic islets and insulin-sensitive tissues, to inhibit insulin secretion and increase glucose uptake *in vivo* [96;164–167]. The presence of inappropriately low levels of leptin for a given degree of adiposity has been associated with a high level of insulin resistance [168–170]. Several clinical studies have shown that patients with OSA have significantly higher leptin levels than weight-matched subjects without OSA [161–163]. Moreover, leptin levels correlated with severity of hypoxia, and decreased with continuous positive airway pressure (CPAP) treatment [161]. Experiments in cell culture demonstrated that continuous hypoxia increases leptin gene expression *via* HIF-1 [95]. We have shown that IH causes an elevation in leptin gene expression and protein level [49], which was attenuated in mice with partial HIF-1 α deficiency [61]. Both up-regulation of leptin in wildtype mice and leptin replacement in leptin-deficient mice protected the animals against the development of glucose intolerance and insulin resistance during IH [49]. Thus, the elevation of leptin levels caused by CIH may represent an important compensatory response that acts to minimize metabolic dysfunction. However, lean mice develop insulin resistance and hyperglycemia despite increases in leptin, suggesting concurrent leptin resistance. Hence, we can conclude that IH exerts effects on insulin and glucose regulation via pathways independent of leptin stimulation.

Other adipokines involved in metabolic regulation are adiponectin and resistin. Adiponectin dramatically increases insulin sensitivity, especially of hepatocytes, by decreasing plasma lipid levels [115;171]. Resistin decreases insulin sensitivity in rodents, probably via up-regulation of lipid metabolism [172]. However, the role of resistin in humans is not clear. Clinical evidence on adiponectin in patients OSA is controversial [173;174], and resistin was not sufficiently studied [175]. In 3T3-L1 adipocyte cell culture, sustained hypoxia (1%) for 6–24 hours markedly suppressed adiponectin mRNA expression [176]. In neonatal rats, sustained hypoxia (11%) for 11 days decreased plasma adiponectin and did not affect resistin levels [177]. The effects of IH on adiponectin and resistin have not been studied in an animal model.

Ghrelin is a peptide produced predominantly by the stomach [178] which is involved in energy regulation and appetite stimulation [179]. There are only two reports studying relationships between ghrelin levels and OSA. In one study, OSA was associated with high total plasma ghrelin levels, correlating with the presence of hypertension and hypersomnolence, and extent of hypoxemia. Ghrelin levels decreased almost to control levels after 2 days of CPAP treatment [180]. The second study found no relationships between total ghrelin and OSA [181]. Exposure to sustained hypoxia had no effect on ghrelin level in neonatal and developing rats [182;183]. The effects of IH on ghrelin have not been studied.

OSA is highly prevalent in patients with hypothyroidism [184] and can be reversed in these patients with thyroid hormone replacement [185]. It was hypothesized that patients with OSA have subclinical thyroid dysfunction. This hypothesis, however, was refuted by recent clinical studies [186;187], which found no association between OSA and hypothyroidism. Sustained hypoxia decreases circulating levels of triiodothyronine (T3) and thyroxine (T4), [188] but effects of IH on thyroid function have not been elucidated.

IH and Dysregulation of Lipid Metabolism

Several lines of evidence support an independent association between OSA and dysregulation of lipid metabolism. First, OSA is associated with hypercholesterolemia independent of adiposity [189], and CPAP treatment leads to a decrease in total cholesterol and LDL cholesterol (LDL-C) [129;130;161] without any change in body weight. Second, OSA is associated with increased serum lipid peroxidation [82] and oxidized LDL [190]. Third, OSA is associated with increased carotid artery intima-media thickness [21–23] and progressive narrowing of coronary artery lumens [24]. Fourth, OSA is associated with non-alcoholic fatty liver disease [191;192], which is a risk factor for both insulin resistance and liver cirrhosis [193].

We have utilized a mouse model of IH and demonstrated that IH increases serum levels of total cholesterol, HDL cholesterol, LDL-C, triglycerides, and lipid peroxidation [58–60]. Moreover, the severity of dyslipidemia is proportional to the severity of IH [60]. IH up-regulates SREBP-1, a key transcription factor of lipid biosynthesis in the liver, and a downstream enzyme, stearoyl coenzyme A desaturase 1 (SCD-1) [59]. SCD-1 converts saturated fatty acids (SFA) 16:0 and 18:0 into monounsaturated fatty acids (MUFA) 16:1 and 18:1, which are necessary components for biosynthesis of cholesterol esters, triglycerides, and phospholipids. SCD-1 also up-regulates lipoprotein secretion [194;195]. IH increases hepatic SCD-1 mRNA and protein levels and biological activity in conjunction with increased lipoprotein secretion, suggesting that up-regulation of SCD-1 is one of the mechanisms of dyslipidemia during IH [59;60]. Partial HIF-1 α deficiency in heterozygous mice alleviated increases in serum lipids and almost completely abolished increases in active SREBP-1 and SCD-1, suggesting that IH affects lipid metabolism via HIF-1 [61]. These pathways are the likely means by which CIH induces an array of end-organ effects. CIH exposure leads to the progression of hepatic steatosis in leptin deficient *ob/ob* mice acting via the SREBP-1 pathway [58], causes liver injury in lean C57BL/6J mice by inducing oxidative stress and lipid peroxidation in liver tissue [75], and

accelerates atherosclerosis in C57BL/6J mice on a high cholesterol diet (Polotsky, unpublished observation). Thus, IH perturbs lipid metabolism, inducing both dyslipidemia and lipid peroxidation, which may lead to progression of insulin resistance, fatty liver disease and atherosclerosis.

METABOLIC EFFECTS OF SLEEP FRAGMENTATION

It is nearly impossible to dissect metabolic effects of IH from other components of OSA, such as sleep fragmentation and hypercapnea. Short term sleep deprivation (SD) in human volunteers leads to glucose intolerance, raises plasma cortisol level and suppresses insulin secretion [196], whereas chronic sleep deprivation induces insulin resistance [197]. Sleep restriction suppresses serum leptin, elevates serum ghrelin, and stimulates appetite [198], which may explain an association between short sleep duration and obesity [199;200]. However, SD is a different sleep abnormality than SF. In fact, the latter is frequently accompanied by normal or even extended total sleep time in the setting of OSA [63]. Metabolic effects of SF without SD have been studied in healthy volunteers only by Dr. Punjabi's group. In healthy volunteers, SF induced by an auditory-tactile stimulus increased sleep-wake transitions and stage 1 sleep, selectively depriving them of SWS while fragmenting stage 2 and REM sleep [201]. In this model of SF in normal subjects, investigators observed a 20% increase in insulin resistance [202].

Animal literature on metabolic and cardiovascular effects of SF is scarce. In dogs, SF increases blood pressure only during sleep, but not during daytime, unlike IH and airway obstruction [38]. In lean C57BL/6J mice, non-hypoxic SF for 5 days caused no changes in FBG, insulin, leptin, corticosterone levels, or glucose tolerance as measured by IPGTT (Polotsky, unpublished observation). Thus, the data concerning metabolic effects of SF is limited.

CAVEATS OF METABOLIC STUDIES IN ANIMAL MODELS

One potential source of concern is the protocol designed to induce IH and how closely it simulates clinical OSA. Most commonly used regimens in mice and rats use IH with an FiO_2 nadir between 5% and 10%, cycling 9 to 60 times per hour, running for 6–12 hours per day [49;52;53;58–60;85;203]. Tagaito *et al.* developed a mouse model in which hypoxia was induced during periods of sleep and was removed in response to arousal or wakefulness [46]. On average, there were over 60 events per hour of sleep throughout the 5-day protocol, and there were multiple episodes where the FiO_2 nadir was less than 10% and 5%. Tagaito *et al.* also measured arterial blood gases during wakefulness at the steady state. FiO_2 of 10% for 180–240 seconds resulted in a PaO_2 of 41 Torr, and FiO_2 of 5% for 60–90s resulted in a PaO_2 of 27 Torr. During actual IH exposure, the FiO_2 usually remains at its nadir for a much shorter interval with a higher consequential PaO_2 . Nevertheless, most of the currently employed models of IH resemble severe or very severe OSA and the findings can be applied to clinical OSA with caution. Another discrepancy in IH systems is that, without CO_2 supplementation, pure IH results in hypocapnia, as opposed to the hypercapnea of human OSA. CO_2 supplementation does not modify hypertensive effects of IH [52], but the impact of CO_2 levels on other metabolic parameters during IH and OSA are unknown.

Weight loss inevitably occurs in rodents during initial IH exposure, whereas human OSA is usually associated with obesity. However, this caveat can be easily addressed, because (1) rodents lose weight during first week of exposure to CIH, after which the animals adapt and start gaining weight [60]; and (2) experimental animals develop hypertension, insulin resistance and glucose intolerance, and dyslipidemia during IH, despite weight loss, which makes effects of IH even more striking [49;50;59;60].

The major obstacle in studying SF with animal models lies in the difficulty of causing SF without SD. Furthermore, animals can adapt to nearly all non-hypoxic stimuli due to rising homeostatic sleep drive. In spite of these limitations, animal models of IH have proven valuable for metabolic studies in OSA.

CONCLUSION AND FUTURE DIRECTIONS

Animal models of OSA explore causal links between OSA and the metabolic syndrome. Animal models provided evidence that IH leads to metabolic derangement, causing hypertension, sympathetic activation, up-regulation of renin-angiotensin and HPA systems, insulin resistance, glucose intolerance, and dyslipidemia. Molecular techniques and transgenic animals have identified transcriptional mechanisms involved in metabolic responses to IH, including activation of HIF-1, NF- κ B, and SREBP-1 transcription factors. Future research in this field should be pursued in several directions. First, efforts should be invested in developing a model of chronic non-hypoxic SF, which could be used to study sequelae of the upper airway resistance syndrome and mild apnea. Second, human translational studies should be performed to examine whether molecular pathways affected by IH in animals are relevant to human OSA. Third, methods involving RNA interference, pharmacological intervention, and use of animals with conditional gene knockouts, should be applied to identify the precise molecular mechanisms affected by IH. A combination of these novel approaches will allow us to identify future therapeutic targets for treating metabolic consequences of OSA, and the metabolic syndrome in general.

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