Biological plausibility linking sleep apnoea and metabolic dysfunction

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Abstract | Obstructive sleep apnoea (OSA) is a very common disorder that affects 10–25% of the general population. In the past two decades, OSA has emerged as a cardiometabolic risk factor in both paediatric and adult populations. OSA-induced metabolic perturbations include dyslipidaemia, atherogenesis, liver dysfunction and abnormal glucose metabolism. The mainstay of treatment for OSA is adenotonsillectomy in children and continuous positive airway pressure therapy in adults. Although these therapies are effective at resolving the sleep-disordered breathing component of OSA, they do not always produce beneficial effects on metabolic function. Thus, a deeper understanding of the underlying mechanisms by which OSA influences metabolic dysfunction might yield improved therapeutic approaches and outcomes. In this Review, we summarize the evidence obtained from animal models and studies of patients with OSA of potential mechanistic pathways linking the hallmarks of OSA (intermittent hypoxia and sleep fragmentation) with metabolic dysfunction. Special emphasis is given to adipose tissue dysfunction induced by sleep apnoea, which bears a striking resemblance to adipose dysfunction resulting from obesity. In addition, important gaps in current knowledge and promising lines of future investigation are identified.

Obstructive sleep apnoea (OSA), the most severe entity in the spectrum of sleep-disordered breathing, is characterized by repetitive upper airway narrowing or collapse during sleep that leads to oxyhaemoglobin desaturation events (intermittent hypoxia), and recurrent arousal from sleep (sleep fragmentation). OSA of at least mild severity, defined as 5–15 obstructive apnoea events per hour of sleep, occurs in 10–25% of the general population worldwide[1,2]. Even mild-severity OSA has been identified as an independent risk factor for a variety of adverse cardiovascular outcomes, such as arterial hypertension, ischaemic heart disease, arrhythmias and ischaemic stroke[3]. In addition, OSA has been linked to excessive daytime sleepiness and an increased incidence of motor vehicle accidents[4], poor overall quality of life[5] and increased mortality[6].

In the past decade, a number of both population and interventional studies have provided substantial evidence supporting a bidirectional relationship between OSA and metabolic dysfunction[7–10]. However, the mechanisms underlying these associations have not been thoroughly explored. We review salient studies in cohorts of patients with OSA, discuss in detail the evidence from animal and cellular models of OSA, and propose future research avenues and opportunities.

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Animal models of OSA

To better understand the implications of OSA for morbidity, a substantial search for adequate animal models that reliably mimic the human disease was necessary. Although naturally occurring OSA models exist, for example the English bulldog[11] and the obese Zucker rat[12], these models only partially recapitulate the human disease, and their use has been restricted to specific pharmacological or ventilatory control studies. Thus, even though OSA does not occur in mice or rats, the unique advantages afforded by use of these species in research, more specifically the high throughput and relative ease of biological and genetic manipulations, has led to the development of experimental models consisting of the imposition of intermittent hypoxia and sleep fragmentation of varying severities and durations[13,14]. In this Review, we focus on the findings derived primarily from studies in rodents in which chronic exposures to intermittent hypoxia or sleep fragmentation were employed. Chronic exposures mimic human disease more closely than acute exposures, and the physiological responses to acute intermittent hypoxia or sleep fragmentation can differ markedly from the responses to chronic exposures[15]. It should be emphasized that, as a result of technical difficulties, no animal model to date has been able to encompass

Key points

- Obstructive sleep apnoea (OSA) is a very common disorder in the general population, and 50–65% of adult and pediatric patients with OSA are obese
- Intermittent hypoxaemia during sleep and fragmentation of sleep architecture are the two major constitutive perturbations that characterize OSA
- In epidemiological studies, OSA is independently associated with metabolic comorbidities, such as the metabolic syndrome, fatty liver disease, adipose tissue dysfunction, insulin resistance and atherosclerosis, particularly when obesity is concurrently present
- Despite divergent phenotypic effects on adipose tissue, both intermittent hypoxaemia during sleep and sleep fragmentation have been mechanistically linked to altered metabolic phenotypes in preclinical studies performed in rodent models
- Systemic and organ-specific inflammation, oxidative stress and autonomic nervous system imbalance probably contribute to OSA-associated metabolic dysfunction; other mechanisms, including gut microbiota dysbiosis and endoplasmic reticulum stress, are under investigation
- Interventional trials in which patients with OSA were effectively treated reveal variable subsequent improvements in metabolic morbidity, which suggests complex interactions between alterations in sleep and oxygenation and obesity

all the major components of OSA in humans, namely intermittent hypoxia, fragmented sleep, swinging shifts in intrathoracic pressure and episodic hypercapnia. Thus, the pathophysiological mechanisms deduced from the results of animal studies should be translated into clinical practice with caution.

Chronic intermittent hypoxia

Because night-time haemoglobin desaturations are one of the main characteristics of OSA, models of intermittent hypoxia during sleep are probably the most widely used animal models of OSA. High-throughput studies in rats and mice, facilitated by the development of automated gas-exchange systems[16], have increased our understanding of the pathophysiological mechanisms that can be induced by varying the degree, frequency and time course of intermittent hypoxia exposure. Most models induce hypoxia using environmental oxygen concentrations of 5–7% and aim to elicit nadir arterial oxyhaemoglobin levels of 75–80%, which correspond to the oxyhaemoglobin levels seen in moderate-to-severe OSA in humans. Models of chronic intermittent hypoxia (CIH) in mice and rats, defined as intermittent hypoxia exposure for 2 weeks or longer, produce phenotypic manifestations that are strikingly similar to the clinical features of human OSA. Mouse and rat models of CIH show increased oxidative stress, autonomic nervous system dysregulation and activation of inflammatory pathways[17], which results in cardiovascular derangements including hypertension[18] and increased atherogenesis[19], along with metabolic perturbations that include insulin resistance and dyslipidaemia[20,21]. These findings have been replicated in a very small number of experimental studies involving humans, whereby relatively short 2–14 day exposures to intermittent hypoxia[22,23] or sleep fragmentation[24] in young healthy volunteers have resulted in measurable alterations in systemic blood pressure, glucose disposition and calculated sensitivity of peripheral tissues to insulin.

OSA and metabolic health

Circulating lipids in OSA and CIH

The potential adverse effects of OSA on the levels of circulating lipids are still poorly characterized in humans. In adult individuals, large cross-sectional studies have shown independent associations between fasting levels of total serum cholesterol, HDL cholesterol and triglycerides and the severity of OSA, particularly the frequency of intermittent hypoxic events[25–27]. Increased plasma levels of free fatty acids (FFAs)[28,29] were also reported in patients with OSA. These levels were reduced with supplemental oxygen treatment[28]. A randomized trial of continuous positive airway pressure (CPAP) treatment for 1 month in patients with OSA found that plasma levels of total cholesterol decreased by 0.28 mmol/l in the treatment group, whereas triglyceride levels remained unaffected[30]. In another randomized trial in which the overall cardiovascular and metabolic effects of CPAP treatment were examined, with or without weight reduction induced by a dietary intervention, serum levels of triglycerides were reduced only when CPAP treatment was accompanied by weight loss[31]. In a long-term study in a small cohort of 39 patients with OSA treated with auto-adjusting positive airway pressure (APAP), a technique that allows for continuous adjustment of the positive pressure applied to the patient during sleep, no evidence was found for favourable outcomes in serum lipid levels despite improvements in OSA after 8 years of APAP treatment[32]. By contrast, in another randomized controlled trial, marked reductions were observed in the 24-hour triglyceride profile, particularly postprandially, in patients with OSA treated with CPAP[33]. However, in a subsequent smaller study, no evidence was found for favourable changes to markers of oxidative lipid damage in postprandial circulating lipids in patients with OSA treated with CPAP[33,34]. Taken together, divergent findings have emerged across different studies. Indeed, a 2014 meta-analysis found that OSA seemed to be associated with increased LDL cholesterol and reduced HDL cholesterol levels, but not with serum triglyceride levels[35]. The inclusion in the meta-analysis of a study that was later retracted casts some doubts on these findings, particularly since a systematic review of the literature concluded that no effects of OSA treatment on the lipid profile of patients were discernible[36].

In adult individuals, the adverse effects of OSA on metabolic parameters might be masked by the concurrent presence of long-term obesity, which also negatively effects metabolic health. Long-term obesity could also inhibit the ability of OSA treatments to reverse any OSA-mediated morbid effects, on the basis that obesity results in dysfunction in related organs. We and other researchers therefore investigated this particular issue in children with OSA, who are less likely to have organ dysfunction related to obesity, although the average severity of paediatric OSA is markedly lower than that of adult OSA. Overall, the same conflicting findings seen in studies of adult patients with OSA persist in studies of younger patients. Although the results from several studies suggest that children with OSA have altered serum lipid levels, and that treatment of the underlying sleep-disordered breathing results in improvements in both serum cholesterol and

triglyceride levels, particularly among obese children[37,38], other studies have failed to identify any specific improvements with standard treatment of OSA in children (that is adenotonsillectomy)[39,40].

Glucose homeostasis in patients with OSA

Multiple studies have provided compelling evidence that in adult individuals OSA impairs insulin sensitivity when assessed by various methods, such as insulin resistance as defined by HOMA, insulin-stimulation test, glucose-tolerance test or euglycaemic clamp. In adult individuals, OSA has been associated with insulin resistance in those with obesity[41] and in lean individuals[42], increased risk of the metabolic syndrome[43,44] and type 2 diabetes mellitus (T2DM)[45,46], and with poorer glycemic control in those with existing T2DM[47–49] independently of obesity. The converse relationship is also seen; the prevalence of OSA (apnoea hypopnoea index (AHI) ≥5 events per hour of sleep) among adult individuals with known T2DM ranges from 58% to 86%[50,51], substantially higher than a 2013 estimate from a community-based cohort of 17% in women and 34% in men[1]. Treatment of OSA in adult individuals with CPAP improves insulin sensitivity in most but not all studies[31,52,53], although data regarding the effect of OSA therapy upon lowering blood glucose and glycosylated hemoglobin levels has been more unpredictable[54,55]. Accordingly, randomized clinical trials have primarily shown a beneficial effect of CPAP treatment on OSA-associated insulin resistance, but the results were mostly related to short-term trials lasting <6 months. Consequently, the long-term benefits of OSA treatment remain unexplored[52,55–61].

In children, the association of OSA with insulin resistance is less obvious than in adult individuals; in children with mild-to-moderate OSA without accompanying obesity, insulin sensitivity seems to be preserved, but the presence of obesity induces insulin resistance, particularly when OSA is concurrently present, such that OSA emerges as an independent risk factor for insulin resistance[40,62,63,64]. Results published in 2015 demonstrated that treatment with adenotonsillectomy in children with and without obesity who had sleep-disordered breathing improves insulin resistance, and the residual metabolic dysfunction after treatment is associated with the degree of adiposity, rather than the remaining sleep-disordered breathing as indicated by polysomnography[38]. These results underscore the importance of a multidisciplinary approach to treatment that concurrently targets other determinants of metabolic dysfunction, such as obesity, when addressing the metabolic health of a patient with OSA.

CIH in animal models

Initial studies in mice that investigated the consequences of intermittent hypoxia on circulating lipids showed marked increases in the levels of circulating FFAs, triglyceride-rich lipoproteins, fasting VLDL and postprandial chylomicrons. The extent of these changes depended on the severity of hypoxia in the model[65–68]. Results from these initial studies suggest that some of the components of CIH-induced hyperlipidaemia result from lipoprotein lipase inhibition via hypoxia-inducible factor (HIF) 1α-mediated pathways involving angiopoietin-like 4 in adipose tissues with concomitant induction of stearoyl coenzyme A desaturase activity. In a further study, the lipid-related consequences of CIH, such as liver steatosis and insulin resistance, were exacerbated in mice given an *ad libitum* high-fat diet[69]. Thus, CIH in animal models elicits the emergence of pro-atherogenic dyslipidaemia, with increased plasma levels of FFAs and modulation of lipoprotein content in chylomicrons. However, it remains unclear how the chronic increases in sympathetic activation that are associated with CIH (discussed later) modulate the dysregulation of circulating lipids, or how manipulations of diet and physical activity enhance or dampen these effects[70]. More importantly, a system-wide, in-depth exploration of metabolomics is lacking in patients with OSA and in mouse models of the disease[71].

Tissue-specific effects of CIH

Effect of CIH on adipose tissue

Chronic exposure to intermittent hypoxia in mice induces alterations in visceral white adipose tissue (vWAT), an important organ in the pathophysiology of the metabolic syndrome and T2DM. Moderate-to-severe CIH, defined as a blood oxygen saturation nadir of 75–80%, is associated with reductions in total body weight[72] and visceral fat mass (Gileles-Hillel, A. and Gozal, D., unpublished work) in lean animals. OSA in otherwise healthy children is similarly associated with failure to thrive or reduced weight gain, and treatment leads to increased somatic growth[73]. By contrast, adult individuals with OSA have increased BMI and visceral adiposity, as compared with people who snore but do not have OSA[74], although the effects of intermittent hypoxia are difficult to isolate from the effects of fragmented sleep, prior obesity and diet, all of which strongly contribute to the accumulation of fat. The CIH-induced adipose tissue alterations in animal models strikingly resemble those observed in obesity and metabolic dysfunction in humans, such as smaller, more abundant adipocytes, reduced expression of lipogenic genes, lower adipose tissue oxygen tension levels and increased expression of angiogenesis-promoting genes[75–79].

The 'hypoxia theory' of obesity and metabolic dysfunction in vWAT has become increasingly popular. This theory suggests that increased adipocyte size, coupled with unchanged vascularity of the tissue, leads to increased diffusion distances for oxygen, and therefore reduced vWAT oxygen tension. Evidence from studies in animal models[79] and in humans[80] implicates the activation of HIF-mediated transcription in the development of obesity-related vWAT metabolic phenotypes, namely inflammation, insulin resistance, lipolysis and adipocyte proliferation, with potentially divergent functions for HIF-1α and HIF-2α[81–86]. However, because the activation of HIF-1α might be the result of increased cellular energy consumption rather than hypoxia[87,88], the role of hypoxia in vWAT expansion and metabolic dysfunction is still unclear[79]. Furthermore, the presence of hypoxia and the transcriptional activation by HIF-1α would be anticipated to promote angiogenesis in obese states rather than the vascular rarefaction that generally

accompanies increased adipose tissue mass[89–92]. The presence of arteriolar and vascular dysfunction could account for the reduced vascular supply and perfusion of vWAT in human obesity[93]. In addition, high-fat diets and other obesogenic insults promote inflammatory processes within the vWAT in mice[94], comprising the coordinated interaction of various innate immune responses that potentially involve the activation of both HIF-1α and nuclear factor κB (NF-κB)[95–102]. Evidence for obesity-associated metainflammation within adipose tissue has been ascribed to interactive networks involving macrophages, natural killers cells and T cell lymphocytes[99,100,103]. Several lines of evidence suggest that although vWAT mass is affected differently by CIH and obesity, the physiological response of vWAT to both conditions is remarkably similar, and might account for the similar metabolic derangements elicited by these conditions (FIG. 1). CIH induces adipose-tissue inflammation characterized by an influx of macrophages and skewing of the macrophage polarity towards the proinflammatory M1 phenotype, resulting in increased production of proinflammatory cytokines and downstream insulin resistance in mice[72]. Results published in 2014 further demonstrate the importance of visceral adipose tissue changes following CIH and the implications of these changes for CIH-induced atherosclerosis, by showing that visceral lipectomy in nonobese male mice exposed to intermittent hypoxia resulted in a lower level of atherogenesis than was seen in sham-operated controls[104]. The role of the immune system has also been studied in *Tlr4*$^{-/-}$ knockout mice, which do not exhibit CIH-induced metabolic dysfunction, in contrast to wild-type mice[105]. The induction of a systemic insulin resistant state by CIH, with or without concurrent feeding with obesogenic diets, has now been repeatedly documented in mice[106–108]. This process differs from the metabolic alterations elicited by long-term, sustained hypoxic exposures, which conversely reduce vWAT inflammation[106,109]. The exact mechanisms underlying the differences in vWAT responses between acute and chronic hypoxia and between sustained and intermittent hypoxia are still under investigation, and might be related to activation of divergent signalling pathways that follow differential temporal trajectories[106,107,110–112].

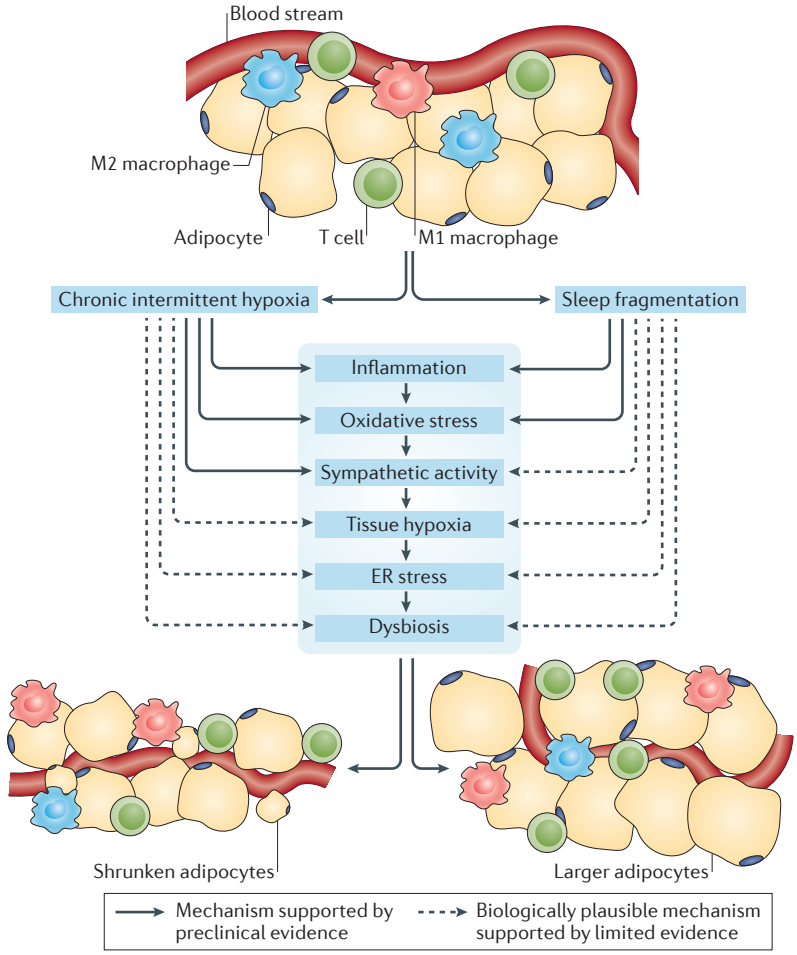

Figure 1 | **Mechanisms of adipose tissue dysfunction resulting from chronic intermittent hypoxia and sleep fragmentation based on animal models.** Chronic intermittent hypoxia (CIH) and sleep fragmentation induce a number of shared cellular responses in adipose tissue, although experimental evidence for a direct association is lacking for some of these links. However, whereas CIH results in adipose tissue lipolysis and vascular rarefaction, sleep fragmentation leads to proliferation and expansion of adipocytes and increased vasculogenesis. ER, endoplasmic reticulum.

In a genome-wide, unbiased, transcriptomic analysis of mouse vWAT, >3,000 genes manifested changes in expression patterns during the time course of CIH exposures; the majority of enriched pathways mapped to metabolic processes, mitochondrial function and oxidative stress responses[113]. These findings suggest that CIH fosters a maladaptive systemic and vWAT metabolic phenotype, which is characterized by robust inflammatory and oxidative stress responses that cause shifts in homeostatic loops involved in adipocyte function and glycaemic control, and which results in a lipodystrophic insulin-resistant phenotype. Notably, CIH induces reductions in levels of circulating adiponectin (similar to those seen in obesity)[114,115], but the effect of intermittent hypoxia on leptin levels seems to be dependent on the nature of the exposure. Models of acute intermittent hypoxia show increased systemic leptin concentrations[116], whereas decreased leptin levels have been reported in CIH models[106], potentially reflecting the parallel reductions in whole-body fat mass, as leptin is produced by adipose tissue. The role of oxidative stress in CIH-induced adipose tissue inflammation has not been directly examined thus far. Theoretically, the episodic reductions in tissue oxygenation (and subsequent reoxygenation events) should result in increased production of reactive oxygen species (ROS), although whether this alteration occurs in CIH models is an important unanswered question. Indirect evidence, such as increased lipid and protein peroxidation in response to CIH, as well as activation of specific transcriptional pathways, suggests that oxidative stress has a role in CIH-induced adipose-tissue dysfunction[113]. Studies that have focused on other end organs, such as the brain, skeletal muscle and adrenal medulla, have provided evidence for unique contributions of pro-oxidative and antioxidative stress systems to the pathophysiology of CIH, and these approaches merit further exploration[117–119]. An important and still unanswered question related to oxidative stress is the source of ROS in vWAT during CIH.

The fact that CIH induces marked increases in sympathetic activity along with reductions in parasympathetic inputs[120–122] relative to normoxia should not be disregarded. Increased sympathetic outflow and ensuing lipolysis, potentially as a result of neuro–adipose connections[123], are important contributors to the insulin resistance induced by CIH. FFAs released into the circulation from adipose tissues that have undergone lipolysis are known to induce insulin resistance in other insulin target tissues, including muscle, liver and pancreas (as reviewed elsewhere[124]). Similar to its effects in other tissues, CIH exposure promotes increased tonic and reactive afferent chemoreceptor outputs from the carotid body compared with normoxia, probably via increased local ROS production, which leads to increased synthesis and release of catecholamines and vice versa[125–127]. Elevated circulating catecholamines act directly on vWAT to promote lipolysis and the release of FFAs. Abolishment of sympathetic activity through pharmacological methods (such as antagonists against β-adrenergic or α-adrenergic receptors) or techniques (adrenal medullectomy) prevents CIH-induced lipolysis and peripheral insulin resistance[128,129]. However, the requirement for enhanced sympathetic activity to elicit insulin resistance in models of CIH has been questioned[20], indicating that multiple pathways are probably involved in the modulation of insulin sensitivity. Many of these pathways warrant further investigation for their potential roles in mediating CIH-induced insulin resistance (BOX 1). For example, the role of the NACHT, LRR and PYD domain-containing protein 3 (NLRP3) inflammasome response, which has been shown to mediate macrophage activation and insulin resistance in the context of obesity, is unexplored in the context of sleep-disordered breathing[130]. Similarly, an attractive area for research that has yet to be explored is the role of NAD-dependent protein deacetylase sirtuin-1 (SIRT1), activation of which in obesity improves several of the metabolic perturbations that also occur in CIH, such as impaired mitochondrial respiration and systemic insulin resistance[131]. Resveratrol, a putative activator of SIRT1, has been shown to improve CIH-induced systemic insulin resistance and adipose-tissue inflammation[72,132].

Box 1 | **Unanswered questions in OSA and metabolic health**

- How do the gut microbiota changes that are associated with OSA (particularly increased gut permeability and transfer into the bloodstream of metabolites or bacterial products that elicit innate immune responses) alter end-organ morbidity or changes in cellular function in the end organ?
- What are the combined effects of chronic intermittent hypoxia and sleep fragmentation in human OSA and in animal models of OSA, and are these effects reversible in various clinical scenarios, such as in patients who differ in age at disease onset, duration of disease and presence of obesity?
- What are the long-term effects of end-organ morbidities induced by paediatric OSA, such as endothelial dysfunction and systemic inflammation, on the onset and progression of adult diseases, such as atherosclerosis and type 2 diabetes mellitus, and are epigenetic and genetic susceptibilities involved?
- What are the mechanisms that potentially lead to weight gain and overall changes in cellular and organismal bioenergetics associated with OSA treatment?

OSA, obstructive sleep apnoea.

In summary, preliminary evidence supports the coordinated interaction of multiple perturbations, including oxidative stress, inflammation and sympathetic activity, as the primary drivers of vWAT dysfunction in CIH.

CIH and the liver

CIH induces several perturbations in liver morphology and hepatic function in animal models. Morphologically, CIH alone promotes mild hepatitis. When high-fat diet feeding is combined with CIH exposures, the adverse effects are markedly enhanced, and include steatosis, fibrosis and apoptosis, mimicking the increased prevalence of nonalcoholic steatohepatitis (NASH) seen in patients with both obesity and OSA compared with those with only obesity[69,133,134]. In the liver, CIH activates gluconeogenesis, which leads to elevated hepatocyte glucose output, and promotes lipid peroxidation and inflammation, as shown by the activation of transcription by NF-κB and the production of proinflammatory cytokines[135–137]. However, the mechanisms underlying the CIH-induced changes in hepatic function have been poorly explored. One possible mechanism could involve hepatic uptake of FFAs released into the circulation by adipose tissue undergoing CIH-induced lipolysis, equivalent to the uptake of FFAs in obesity-associated hepatitis[138], and a potential link between adipose-tissue dysfunction and nonalcoholic fatty liver disease (NAFLD) in CIH models. Another potential mechanism underpinning CIH-induced liver injury is oxidative stress and the activation of HIF-1α[139,140]. Both of these processes can promote NAFLD, and partial deficiency of HIF-1α prevents triglyceride accumulation in the livers of CIH-exposed animals[141]. The possible pathophysiological links between OSA and NAFLD and/or NASH have been substantially reviewed elsewhere[142].

CIH and the pancreas

In animal models, CIH induces insulin resistance as measured by HOMA, and impairs glucose-stimulated pancreatic insulin secretion. Several studies have demonstrated that CIH leads to pancreatic β-cell dysfunction, as evidenced by insufficient glucose-induced elevations in levels of insulin and neuroendocrine convertase 1 (also known as prohormone convertase 1)[143–149]. Generalized measures of pancreatic oxidative stress and islet cell mitochondrial ROS contents, specifically aconitase activity and malonyldialdehyde levels are increased in CIH-exposed mice, and ROS scavenging by pharmacological means (via administration of mito-TEMPOL or *N*-acetylcysteine) or in transgenic animal models (via overexpression of mitochondrial superoxide dismutase in pancreatic β cells) restores normal β-cell function[137,144,148–151]. Even fairly short 4 day exposures to intermittent hypoxia result in β-cell apoptosis and proliferation[148]. In keeping with findings showing that adrenomedullectomy in wild-type mice prevents pancreatic dysfunction[152], CIH in a genetically-induced T2DM mouse model increases both pancreatic and plasma levels of FFAs, impairs glucose tolerance, induces β-cell apoptosis and dampens β3-adrenergic-receptor-mediated insulin secretion[143]. Overall, although limited in number, the available studies assessing the effect of CIH on

pancreatic function underscore the adverse effect of this hallmark feature of sleep-disordered breathing on β-cell homeostasis, and further stress the importance of oxidative stress and altered sympathetic activity.

CIH and muscle

CIH seems to induce insulin resistance in skeletal muscle, but little empirical data supports this impression. Short-term exposures to intermittent hypoxia lasting several hours induce insulin resistance characterized by reduced glucose utilization by skeletal muscle during hyperinsulinaemic–euglycaemic clamps in mice[20]. Prolonged CIH exposures, which elicit whole-body insulin resistance, also reduce levels of solute carrier family 2, facilitated glucose transporter member 4 (GLUT4) in the membranous fraction of skeletal muscle cells in mice[106], which suggests a mechanism underlying the impaired glucose uptake and reduced insulin signalling observed in skeletal muscle in CIH models.

CIH, OSA and the gut microbiota

The importance of the gut microbiota in the pathogenesis of obesity and the metabolic syndrome has gained substantial attention in the past decade, and a number of studies have demonstrated the importance of gut microbial communities in modulating not only the absorption of nutrients but also the control of appetite and organ-specific changes that contribute to glucose homeostasis[153–158]. However, very little is known about the changes to the gut microbiota that occur in patients with OSA or in animal models of CIH or sleep fragmentation, or the effect of such changes on the metabolic dysregulation associated with these exposures. In two paediatric studies, OSA was associated with low-grade endotoxaemia and impaired gut-barrier integrity, which correlated with insulin resistance[159] and with severity of NAFLD[160]. We reported in 2015 that CIH in mice is accompanied by considerable and reproducible changes in gut microbial communities, but the functional implications of such changes are only now being actively investigated[161]. Further studies are needed to corroborate those findings and explore potential mechanisms (BOX 1).

Sleep fragmentation

Repetitive arousal from sleep resulting from respiratory events, and the resultant fragmentation of normal sleep architecture, are common features among many adult individuals and children with OSA. Until the past decade, substantial technical difficulties existed in the implementation of an animal model of sleep fragmentation that would prevent social isolation, require no direct human interaction during testing, enable unrestricted access to food and water, and utilize a minimally stressful stimulus to awaken the animal. Most early studies of the metabolic effects of sleep fragmentation involved the use of stressful stimuli, such as the inverted water pot technique[162]. In the past decade, a number of groups including ours have reported on improved methodologies that largely fulfill the abovementioned criteria for an animal model of the sleep fragmentation component of human OSA, and achieve reproducible sleep fragmentation for prolonged periods of time[163–170]. This experimental model has thus far been instrumental in enabling understanding of the contribution of sleep fragmentation to OSA-related conditions such as obesity[171], metabolic dysfunction[172], neurocognitive dysfunction[173] and cardiovascular alterations[174]. Notably, owing to ethical constraints, chronic sleep fragmentation imposition on humans is impossible.

Sleep fragmentation and appetite regulation

Intriguing findings from human studies examining leptin and ghrelin signalling and appetite regulation in adult individuals and children with OSA show that OSA shifts food preferences towards increased consumption of fat and carbohydrate, and reduces satiety[175–178]. These observations have prompted the development of a conceptual framework in which OSA, similar to restriction or deprivation of sleep, induces changes in leptin signalling that lead to reductions in satiety, along with cravings for high-energy foods[179]. Results from experiments with the mouse sleep-fragmentation model described previously have demonstrated the emergence of hyperphagic behaviours in awake mice exposed to chronic sleep fragmentation, resulting in gains in body weight and fat mass over time, and leading to obesity[171,180]. This profound effect of sleep fragmentation on the amount and pattern of food intake in a setting of standard chow was mediated by increased endoplasmic reticulum stress in the hypothalamus and tyrosine-protein phosphatase non-receptor type 1 (PTP-1B)-mediated resistance to the hypothalamic leptin receptor, as evidenced by decreased hypothalamic leptin signalling, despite increases in plasma levels of leptin[171,180]. Notably, a mechanistic link between endoplasmic reticulum stress and leptin resistance has been previously described in the context of a high-fat diet in normally sleeping mice[181]. No study to date has dissected the role of ghrelin or other appetite and satiety modulators in animal models of sleep fragmentation.

Sleep fragmentation, insulin sensitivity and vWAT

Mice chronically exposed to sleep fragmentation develop systemic insulin resistance (as measured by both glucose-tolerance and insulin-tolerance tests), even before increases in body weight occur[172]. Furthermore, long-term sleep fragmentation in mice is associated with major changes in the transcriptional networks in visceral fat cells, which leads to systemic and adipose-tissue-specific metabolic disturbances that seem to be orchestrated by vWAT microRNA–mRNA pathways[182]. The adipose tissue alterations in this model are similar to those found in other animal models of obesity, namely recruitment of macrophages and adipocyte progenitor cells from the bone marrow to adipose tissue, and increased production of proinflammatory mediators, resulting in local and systemic insulin resistance[172,183]. In contrast to animals exposed to CIH, animals exposed to sleep fragmentation do not exhibit increased vWAT lipolysis, but rather a progressive proliferation of adipocytes and expansion of fat depots via increases in both cellular number and cellular size[183] (FIG. 1). To date, oxidative stress has emerged as a major mechanism

underlying adipose tissue inflammation induced by chronic sleep fragmentation. Male mice with a heterozygous deletion of the gene encoding cytochrome b-245 heavy chain (a subunit of NADPH oxidase 2 (NOX2), a major source of cellular ROS) that were exposed to sleep fragmentation manifested the same changes in food consumption as wild-type mice exposed to sleep fragmentation, but showed no weight gain or expansion of adipose tissue depots[172,183]. Furthermore, vWAT inflammation and insulin sensitivity were unaltered in mutant mice[172,183], which provides support for the role of oxidative stress in metabolic dysfunction induced by sleep fragmentation. The results of preliminary experiments by our group have found that treatment with resveratrol, an activator of SIRT1 and an inhibitor of oxidative stress, in mice exposed to sleep fragmentation prevents the development of insulin resistance[184]. However, the effects of oxidative stress and inflammation induced by sleep fragmentation on metabolic regulation in muscle, liver and pancreas have not yet been examined.

Conclusions

Animal models of OSA have expanded our understanding of the potential mechanisms mediating the metabolic dysfunction associated with this condition in humans. Oxidative stress, elevated sympathetic activity and inflammation have emerged as leading candidate pathways underlying disruption of homeostatic metabolic processes in several critical target organs. The deleterious metabolic effects of CIH and prolonged sleep fragmentation in lean animals are further exacerbated by the presence of obesity or high-fat diets, emphasizing the bidirectional relationships and interactions between OSA and obesity in metabolic health. However, many unanswered questions still remain (BOX 1), and we lack a clear understanding of how CIH and sleep fragmentation promote metabolic dysfunction in human OSA. An improved understanding of how alterations in the gut–brain axis and end-organ metabolic tissue in animal models of OSA translate to the human disease is necessary before targeted interventions can be developed.

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Author contributions

A.G-H, L.K-G and D.G. researched data for the article, contributed to discussions of the content, wrote the article and reviewed and/or edited the manuscript before submission

Competing interests

The authors declare no competing interests.