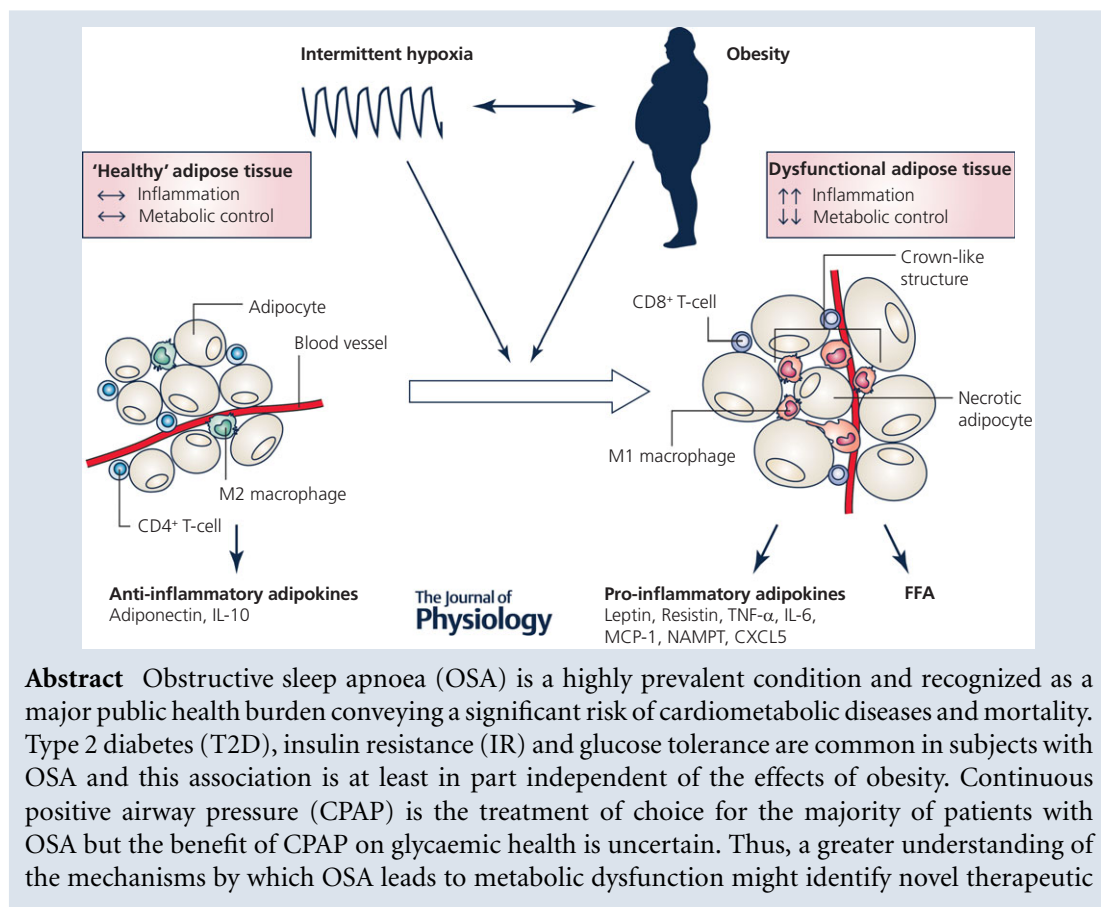SYMPOSIUM REVIEW

Adipose tissue inflammation by intermittent hypoxia: mechanistic link between obstructive sleep apnoea and metabolic dysfunction

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Abstract Obstructive sleep apnoea (OSA) is a highly prevalent condition and recognized as a major public health burden conveying a significant risk of cardiometabolic diseases and mortality. Type 2 diabetes (T2D), insulin resistance (IR) and glucose tolerance are common in subjects with OSA and this association is at least in part independent of the effects of obesity. Continuous positive airway pressure (CPAP) is the treatment of choice for the majority of patients with OSA but the benefit of CPAP on glycaemic health is uncertain. Thus, a greater understanding of the mechanisms by which OSA leads to metabolic dysfunction might identify novel therapeutic

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approaches. Intermittent hypoxia (IH), a hallmark feature of OSA, likely plays a key role in the pathogenesis and experimental studies using animal and *in vitro* models suggest that IH leads to pancreatic β-cell dysfunction and to insulin resistance in the insulin target organs liver, skeletal muscle and adipose tissue. In particular, IH induces a pro-inflammatory phenotype of the visceral adipose tissue with polarization of adipose tissue macrophages towards a M1–pro-inflammatory subtype, upregulation and secretion of numerous pro-inflammatory adipokines and subsequent impairment of the insulin-signalling pathway, changes which bear a striking similarity to adipose tissue dysfunction seen in obesity. In this review, the available evidence linking IH with metabolic dysfunction is explored with a special emphasis on the adipose tissue in this process.

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Abstract figure legend Phenotypic modulation of the adipose tissue by intermittent hypoxia (IH). The schematic diagram shows how IH in interaction with obesity leads to pro-inflammatory changes and dysfunction of the adipose tissue. Healthy adipose tissue is characterized by small adipocytes and infiltration of M2–anti-inflammatory macrophages and CD4^{+} regulatory T-cells leading to the production of anti-inflammatory adipokines. With IH treatment, CD8^{+} cytotoxic T-cells are recruited and macrophages shift towards a M1–pro-inflammatory phenotype forming crown-like structures (CLS) surrounding necrotic adipocytes. The consequence of these alterations is the upregulation and secretion of numerous pro-inflammatory adipokines and lipolysis with free fatty acid flux into the circulation collectively promoting impairment of the insulin-signalling pathway leading to metabolic dysfunction. (IL, interleukin; TNF-α, tumour necrosis factor alpha; MCP-1, monocyte chemoattractant protein-1; NAMPT, nicotinamide phosphoribosyltransferase; CXCL5, C-X-C motif chemokine ligand 5; FFA, free fatty acids).

Abbreviations BMI, body mass index; CLS, crown-like structure; CPAP, continuous positive airway pressure; ET-1, endothelin 1; FFA, free fatty acid; HIF, hypoxia inducible factor; HFD, high-fat diet; IH, intermittent hypoxia; IKK, inhibitor of nuclear factor kappa B kinase; IL, interleukin; iNOS, inducible nitric oxide synthase; IR, insulin resistance; IRS, insulin-receptor substrate; JNK, c-Jun N-terminal protein kinases; MCP-1, monocyte chemoattractant protein-1; MetS, metabolic syndrome; NF-κB, nuclear factor kappa B; OSA, obstructive sleep apnoea; PKR, protein kinase R; ROS, reactive oxygen species; SVF, stromal vascular fraction; T2D, type 2 diabetes; TNF-α, tumour necrosis factor alpha; WAT, white adipose tissue.

Introduction

Obstructive sleep apnoea (OSA), characterized by repeated pharyngeal collapse during sleep leading to intermittent hypoxia (IH), sleep fragmentation and excessive daytime sleepiness, represents an increasing public health burden. It is a common disorder affecting about 14% of men and 5% of women (Peppard *et al.* 2013) and prevalence is rapidly rising due to the strong association of OSA with obesity, with at least 60% of OSA patients being obese (Peppard *et al.* 2000). Furthermore, OSA represents a significant risk factor for the development of numerous cardiovascular diseases, such as systemic arterial hypertension, coronary artery disease, arrhythmias and heart failure leading to increased morbidity and mortality (Ip *et al.* 2002; Punjabi *et al.* 2002; McNicholas & Bonsignore, 2007; Kent *et al.* 2014). There is also compelling evidence of an independent association of OSA with metabolic dysfunction, and in particular with alterations in glucose metabolism leading to type 2 diabetes (T2D), insulin resistance (IR), glucose intolerance and metabolic syndrome (MetS), and this association seems to be at least in part irrespective of the degree of obesity (Ip *et al.* 2002; Punjabi *et al.* 2002; Kent *et al.* 2014). Moreover, OSA and obesity may exert synergistic negative effects on glucose metabolism.

The pathophysiology underlying metabolic dysfunction in OSA is incompletely understood but a large body of data indicates that the particular form of intermittent hypoxia (IH) observed in OSA, with repetitive short cycles of desaturation followed by rapid reoxygenation, plays a pivotal role. Experimental studies indicate that IH exacerbates its detrimental effect on glucose metabolism through various mechanisms. It impairs pancreatic insulin secretion but also acts directly on insulin target organs such as liver, skeletal muscle and adipose tissue. Given the close link of OSA with obesity, the latter has attracted specific attention and indeed, studies in animals and *in vitro* indicate that IH leads to adipose tissue inflammation with the downstream consequence of decreased insulin sensitivity. This review summarizes the evidence of IH-induced glucose metabolic dysfunction, focusing primarily on the role of the adipose tissue in this process.

Intermittent hypoxia and glucose metabolism

Various clinical and population studies have revealed an independent association of OSA with impaired glucose haemostasis including T2D, IR, glucose intolerance and MetS and this topic has been extensively reviewed elsewhere (Levy *et al.* 2009; Bonsignore *et al.* 2013; Kent *et al.* 2015). Notably, in many of these studies, measures of nocturnal hypoxaemia had the most robust relationship with impaired glycaemic health, suggesting a key role of intermittent hypoxia in the pathophysiology (Punjabi *et al.* 2004; Sulit *et al.* 2006; Polotsky *et al.* 2009). Over the last two decades, detailed insight into the effects of IH has been provided by animal studies. In the majority of these studies, rodents are housed in specific chambers and intermittently breathe air with low oxygen concentration. Protocols of the IH treatment vary but a common model lowers the ambient oxygen content to 5% for 60 cycles h^{-1}, mimicking severe OSA. Exposing lean C57BL/6J mice to just 9 h of such protocol of IH already resulted in decreased whole-body insulin sensitivity as measured by hyperinsulinaemic euglycaemic clamp (Iiyori *et al.* 2007). Chronic IH applied for several weeks, which more realistically recapitulates OSA, leads to several metabolic alterations, including higher fasting glucose and insulin levels, insulin resistance, glucose intolerance and β-cell dysfunction (Wang *et al.* 2013; Poulain *et al.* 2014). A small number of studies have also investigated the effects of IH in the presence of obesity in both, leptin-deficient and diet-induced obese mice, and consistently suggested an exacerbation of the obesity-induced metabolic dysfunction by IH (Polotsky *et al.* 2003; Drager *et al.* 2011; Murphy *et al.* 2015). Moreover, in healthy volunteers, an acute protocol of IH applied for 5 h resulted in impaired insulin sensitivity without increase in compensatory insulin secretion (Louis & Punjabi, 2009).

There are several potential mechanisms by which IH mediates its effect on glucose metabolic dysfunction (Fig. 1). Activation of the sympathetic nervous system in response to IH has been extensively studied in humans and rodents and has been identified to be an important pathway in the development of systemic arterial hypertension and other cardiovascular diseases in OSA (Fletcher, 2003). IH-induced sympathetic excitation also probably impacts on glucose metabolism
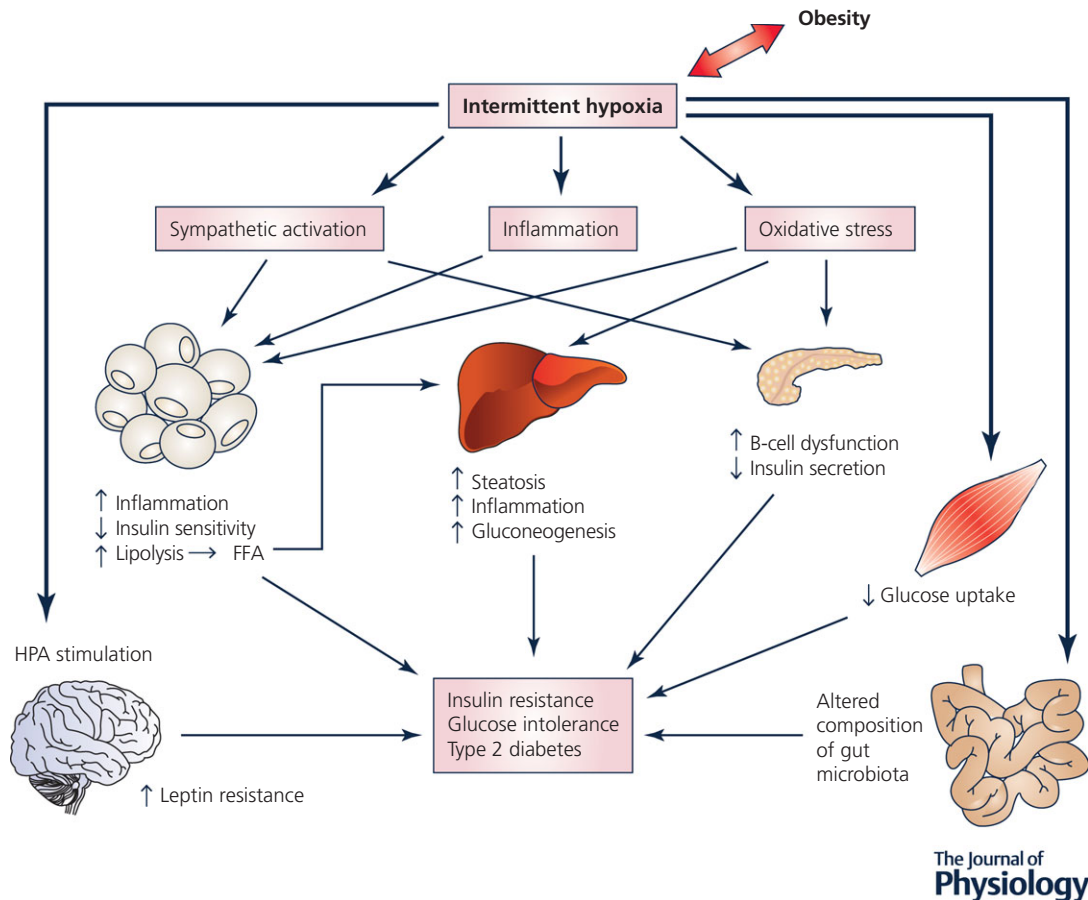
Figure 1. Potential mechanisms of alteration in glucose metabolism by intermittent hypoxia

as catecholamines are known to decrease insulin sensitivity, to reduce insulin-mediated glucose uptake and to stimulate hepatic gluconeogenesis (Deibert & DeFronzo, 1980; Dungan *et al.* 2009). Furthermore, noradrenalin (epinephrine) is a potent mediator of adipose lipolysis and through this mechanism IH may additionally impair insulin sensitivity (Carmen & Victor, 2006). However, a recent study investigated the effect of adrenal medullectomy on glucose metabolism in mice treated with IH, and although surgery led to improved insulin secretion, IH-induced hyperglycaemia and IR remained unaffected, supporting the need for further studies on this subject (Shin *et al.* 2014). Furthermore, local and systemic inflammation in response to IH is central in the pathogenesis of OSA-associated cardio-metabolic processes (Ryan *et al.* 2009). IH is a potent pro-inflammatory stimulus through activation of nuclear factor-kappa B (NF-κB), the master transcriptional regulator of inflammatory responses with the down-stream consequence of the production of numerous pro-inflammatory mediators such as tumour necrosis factor (TNF)-α, interleukin (IL)-6, IL-8 or monocyte chemoattractant protein 1 (MCP1) (Ryan *et al.* 2005; Greenberg *et al.* 2006; Ryan *et al.* 2006). These products have detrimental effects on cardiovascular and metabolic health and documented inhibitory effects on insulin sensitivity in the liver and adipose tissue (Ouchi *et al.* 2011; Cooke *et al.* 2016). Some studies have also suggested that IH directly affects the hypothalamic–pituitary–adrenal axis with a downstream-consequence of increased cortisol release, which has well-known negative consequences on glucose metabolisms, including inhibition of insulin secretion and insulin resistance (Yokoe *et al.* 2008). In addition, IH has generally been associated with increased production of reactive oxygen species (ROS) promoting oxidative stress, and studies in rodents documented increased lipid peroxidation in the liver and pancreatic β-cells (Rosa *et al.* 2011; Fang *et al.* 2014) and, hence, this pathway may also be involved in the pathogenesis of IH-induced metabolic dysregulation (Henriksen *et al.* 2011).

A recent study also points to a potential effect of IH on the gut microbiota which has gained substantial attention over the last decade playing a pivotal role in the progression of metabolic dysfunction in obesity (Schroeder & Backhed, 2016). Using a mouse model, Moreno-Indias *et al.* demonstrated substantial alteration in the microbiota composition in response to IH, but the functional implications of these changes need to be further explored (Moreno-Indias *et al.* 2015).

IH, obesity and adipose tissue dysfunction

As outlined above, OSA is strongly associated with obesity. According to epidemiological studies, the prevalence of OSA in obese subjects exceeds 30%, at least 60% of OSA patients are obese and the incidence of OSA increases incrementally with increasing body mass index (BMI) (Young *et al.* 1993; Peppard *et al.* 2000). Furthermore, both conditions lead to similar cardio-vascular and metabolic consequences, with increasing evidence of potential synergistic effects. This intimate relationship and interaction has led to the hypothesis that OSA shares various pathophysiological mechanisms of cardiometabolic complications with obesity. The pivotal role of the white adipose tissue (WAT) in obesity-mediated diseases is well described. Traditionally considered as an organ of pure energy storage, it has over the last two decades emerged as a key endocrine organ, participating in many physiological and pathophysiological processes mediated through the secretion of multiple hormones, cytokines, chemokines and other proteins, collectively referred to as adipokines (Ouchi *et al.* 2011). Obesity is characterized by an expansion of the adipose tissue which occurs via an increase in adipocyte cell size (hypertrophy), cell number (hyperplasia), or both (Jo *et al.* 2009). Whereas hyperplasia represents a healthy expansion of the adipose tissue, adipocyte hyper-trophy leads to lipid-laden, dysfunctional adipocytes that mediate inflammation and dysfunction. Furthermore, the distribution of excess adipose tissue has significant impact on pathophysiological consequences, wherein increased visceral fat mass to a greater extent than sub-cutaneous adipose tissue is associated with serious health consequences (Despres & Lemieux, 2006). In obesity, adipocyte hypertrophy and dysfunction are paralleled by quantitative and qualitative changes of the stromal vascular fraction (SVF). Immune cells are of particular relevance in this setting. Lean healthy adipose tissue is characterized by the occurrence of anti-inflammatory regulatory T cells (Tregs) and macrophages of a M2, alternatively activated, phenotype, which promote the upregulation of anti-inflammatory factors such as IL-10 or adiponectin and downregulation of pro-inflammatory cytokines, and thus are associated with tissue repair and resolution of inflammation. With increasing adipose tissue expansion, there is increased infiltration of CD8^{+} cyto-toxic T-cells, with a decrease in CD4^{+} helper and Treg cells. Macrophages polarize towards a M1, pro-inflammatory, subtype and are arranged around necrotic adipocytes in crown-like structures (CLS). M1 macrophages produce numerous pro-inflammatory cytokines such as IL-6 and TNF-α and express inducible nitric oxide synthase (iNOS) (Ouchi *et al.* 2011; Cildir *et al.* 2013). The resulting adipose tissue inflammation leads to a release of free fatty acids (FFAs) which activate various signalling pathways, including c-Jun N-terminal protein kinases (JNKs), inhibitor of nuclear factor κ-B kinase subunit β (IKKβ) and protein kinase R (PKR), collectively resulting in impairment of the insulin-signalling pathway, with

the downstream consequence of insulin resistance and metabolic dysfunction (Odegaard & Chawla, 2013).

Furthermore, there is accumulating evidence that obesity, which is characterized by excess of adipose tissue with compromised vascularity, mediates its pro-inflammatory response of WAT through hypoxia-derived pathways (Trayhurn *et al.* 2008). Hypoxia-inducible factor (HIF)-1 is the main transcriptional regulator of the hypoxia response. Recently Lee *et al.* demonstrated that genetic inhibition of HIF-1α in adipocytes protected mice from high-fat diet (HFD)-induced adipose tissue inflammation and restored insulin sensitivity not only in the adipose tissue but also in liver and skeletal muscle. In accompanying *in vitro* mechanistic studies the authors concluded that HIF-1α activation increased the expression of a wide array of pro-inflammatory adipocyte genes mediated through interaction with NF-κB (p65) gene promoters. In contrast, activation of HIF-2α in adipocytes has opposing, protective effects with attenuation of HFD-induced inflammation, glucose intolerance and IR (Lee *et al.* 2014).

Given the pivotal role of inflammation and hypoxia in obesity-induced adipose tissue dysfunction, it has long been postulated that IH as it occurs in OSA may augment this response. Indeed, various studies using rodent or reductionist models of IH have added support to this hypothesis and, moreover, have demonstrated that IH induces inflammatory changes of the visceral adipose tissue even in the absence of obesity. Using atherosclerosis-prone ApoE-deficient mice, Poulain *et al.* demonstrated increased macrophage infiltration of adipose tissue and increased expression of monocyte chemoattractant protein (MCP)-1, abnormalities which were associated with reduced insulin sensitivity and more advanced atherosclerotic lesions (Poulain *et al.* 2014). Furthermore, we and others have characterized the phenotype of adipose tissue macrophages in lean and diet-obese C57Bl/6J wild-type mice, confirming a shift of polarization towards a M1–pro-inflammatory subtype in response to IH, a response partially reversible by treatment with the anti-obesity drug resveratrol (Carreras *et al.* 2015; Murphy *et al.* 2015). Importantly, the percentage of M1 macrophages correlated directly with the level of insulin resistance and this inflammatory shift was accompanied by down-regulation of insulin-receptor substrate (IRS)-1 mRNA with subsequent alteration in IRS-1 tyrosine phosphorylation and diminished phosphorylation of Akt (Murphy AM, Thomas A, Crinion SJ, Kent BD, Tambuwala MM, Fabre A, Pepin JL, RocheHM, Arnaud C & Ryan S, unpublished data). In addition, murine adipocyte cultures in IH demonstrate greater NF-κB activation and decreased secretion of the key anti-inflammatory adipokine adiponectin than cells in control conditions (He *et al.* 2014; Carreras *et al.* 2015). We recently reported that primary human adipocytes treated with IH *in vitro* demonstrate a greater sensitivity to IH towards pro-inflammatory pathway activation, with the downstream consequence of more enhanced expression of multiple inflammatory mediators than primary cells of non-adipocyte lineage (Taylor *et al.* 2014). Collectively, these data support the critical role of adipose tissue as a potential source of pro-inflammatory mediators in response to IH.

Adipose tissue inflammation and insulin resistance give rise to lipolysis with increased and persistent FFA flux into the systemic circulation which is linked to the development of hepatic and muscle insulin resistance and β-cell dysfunction (Cooke *et al.* 2016). Studies using C57BL/6J wild-type and ApoE-deficient mice have demonstrated increased serum FFA levels with IH treatment and have proposed a pathophysiological role of this response in the development of dyslipidaemia and steatohepatitis (Savransky *et al.* 2007; Jun *et al.* 2010; Weiszenstein *et al.* 2016). Pharmacological inhibition of lipolysis by acipimox, a nicotinic acid analogue, ameliorated the detrimental effects on glucose metabolism induced by chronic IH exposure (Weiszenstein *et al.* 2016) and in a recent investigation using a combined *in vivo–in vitro* approach a potential mechanistic link between IH and lipolysis was proposed through upregulation of endothelin 1 (ET-1) (Briancon-Marjollet *et al.* 2016).

The detailed intermediate mechanisms by which IH contributes to adipose tissue inflammation are as-yet unknown. However, a hypoxia-mediated response through HIF-1α activation is plausible. In support of this hypothesis, a study in lean and diet-induced obese mice suggested that IH exposure results in a substantial decrease in adipose tissue oxygen tension with attenuation of the cyclical fluctuations (Reinke *et al.* 2011). Subsequently, adipose HIF-1 activation in response to IH has been demonstrated, associated with accelerated atherosclerosis but the detailed role of this pathway in the IH-induced metabolic dysfunction remains unknown (Drager *et al.* 2013). As outlined above, IH has been closely linked to activation of NF-κB-mediated inflammation, sympathetic excitation and oxidative stress and although the involvement of these pathways in IH-induced adipose tissue inflammation and metabolic dysfunction is likely, the exact contribution and interplay of these mechanistic links in the actual process will require further detailed investigation.

Current limitations and future directions

Despite the fast growing evidence of IH-induced adipose tissue inflammation and subsequent impairment of glucose metabolism in animals, we are as yet lacking evidence of this response in humans. The rodent models as currently used have significant limitations and thus

results have to be interpreted with caution. Firstly, the epididymal fat pads are the largest and best characterized visceral adipose tissue depot in mice but there is no true equivalent in humans. In contrast, the largest visceral adipose tissue organ in humans is omental fat which is essentially absent in rodents. Omental adipose tissue shows considerably greater vascularity and also drains blood into the portal circulation unlike the murine visceral depots and hence the relevance of the pathophysiological responses seen in mice in response to obesity or IH for the human condition of OSA is as yet unclear. Given the difficulties in routine sampling of visceral adipose tissue in humans, studies are generally limited to the morbidly obese patient group undergoing bariatric surgery, which, however, only represents a limited proportion of OSA subjects. Investigating such a cohort, no relationship between the severity of IH and the total number of macrophages in omental and subcutaneous fat depots was observed but the phenotypes of adipose tissue macrophages or other metabolic indices were not investigated and thus it remains unclear if the observed adipose tissue inflammation in response to IH mimics the process in subjects with OSA (Aron-Wisnewsky *et al.* 2012). Secondly, the IH rodent model has several caveats. It does not recapitulate all the effects of OSA, as besides IH there are also other potential pathophysiological triggering factors for cardiometabolic diseases including sleep fragmentation, intrathoracic pressure swings and hypercapnia. In addition, the pattern of IH in the majority of these models resembles very severe OSA and hence caution is advised in translating these findings universally to clinical OSA. Furthermore, rodents do not sleep continuously for 6–8 h as humans do. Although systems have been developed to trigger IH during sleep only, most models use IH unlinked to sleep.

In conclusion, despite fast-emerging evidence of metabolic dysfunction in response to IH arising predominantly from animal and reductionist models, careful translation of these findings into the clinical setting is essential. Thus, future clinical, basic and translational studies defining the detailed mechanisms and consequences of IH-induced adipose tissue inflammation are warranted and will potentially identify novel therapeutic targets.

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