Understanding the pathophysiological mechanisms of cardiometabolic complications in obstructive sleep apnoea: towards personalised treatment approaches

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The pathogenesis of cardiometabolic complications in obstructive sleep apnoea is poorly understood and this perspective summarises the key learning points of an ERS research seminar discussing the interaction of potential pathophysiological triggers http://bit.ly/2vANqBi

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ABSTRACT In January 2019, a European Respiratory Society research seminar entitled "Targeting the detrimental effects of sleep disturbances and disorders" was held in Dublin, Ireland. It provided the opportunity to critically review the current evidence of pathophysiological responses of sleep disturbances, such as sleep deprivation, sleep fragmentation or circadian misalignment and of abnormalities in physiological gases such as oxygen and carbon dioxide, which occur frequently in respiratory conditions during sleep. A specific emphasis of the seminar was placed on the evaluation of the current state of knowledge of the pathophysiology of cardiovascular and metabolic diseases in obstructive sleep apnoea (OSA). Identification of the detailed mechanisms of these processes is of major importance to the field and this seminar offered an ideal platform to exchange knowledge, and to discuss pitfalls of current models and the design of future collaborative studies. In addition, we debated the limitations of current treatment strategies for cardiometabolic complications in OSA and discussed potentially valuable alternative approaches.

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Introduction

Obstructive sleep apnoea (OSA) is a highly prevalent disorder which has rapidly evolved into a major global public health burden [1, 2]. It is independently linked with the development and control of numerous cardiovascular and metabolic conditions including hypertension, coronary artery disease, stroke, heart failure, type 2 diabetes or nonalcoholic fatty liver disease (NAFLD), leading to substantial morbidity and mortality [1, 3–5].

Despite significant efforts, the pathophysiological mechanisms underlying cardiometabolic disease processes in OSA remain incompletely understood. OSA is a complex disorder and clinical studies have often been limited by the coexistence of frequent comorbid conditions, heterogeneity in clinical presentation and large variability in duration of the disease prior to diagnosis. Thus, much of our knowledge of the pathogenesis has been conducted by translational studies using experimental models. The hallmark features of OSA, namely intermittent hypoxia and sleep fragmentation, have been identified as key players in cardiometabolic processes in OSA [6]. However, the interaction between these two pathophysiological triggers and the relative contribution of other potential traits are poorly defined.

Over the past few years, the detrimental effects of sleep disturbances in general on morbidity and mortality have been increasingly recognised and important pathophysiological roles have been attributed not only to sleep fragmentation but also to sleep deprivation or circadian misalignment. However, these factors remain inadequately explored in OSA. Furthermore, recent evidence supports important immune and inflammatory modulation in response to hypercapnia [7] and although it is a well-recognised feature in OSA, it has gained little attention as a potential contributor to adverse consequences.

Unravelling the detailed mechanisms underlying cardiovascular and metabolic disease processes in OSA is of major importance and is likely to lead to the identification of novel treatment strategies. Continuous positive airway pressure (CPAP) therapy is the treatment of choice in OSA, but its benefits on cardiometabolic health remain uncertain. In order to advance this important field, the European Respiratory Society (ERS) research seminar entitled "Targeting the detrimental effects of sleep disturbances and disorders", held in Dublin (Ireland) in January 2019, brought together international experts and research groups from different areas of sleep medicine to promote exchange of knowledge, to identify specific research questions and to facilitate future collaborative projects. This perspective summarises the key learning points, conclusions and proposals from the seminar.

Intermittent hypoxia: past, present and future

Experimental models of intermittent hypoxia: benefits and limitations

Intermittent hypoxia plays a key role in the pathophysiology of cardiometabolic disease processes in OSA, and various clinical studies have identified the superiority of markers defining the severity of intermittent hypoxia in the prediction of cardiovascular outcomes over the traditional apnoea–hypopnoea index which predominantly reflects airflow limitations [8–10]. Recent studies recognised the hypoxic burden, a single marker capturing the frequency, duration and depth of obstructive events-associated oxygen desaturation, as potentially valuable metric in this setting; however, its superiority over other traditional polysomnographic intermittent hypoxia-related parameters such as the oxygen desaturation index will require further longitudinal studies [11, 12]. Detailed insight into the effects of intermittent hypoxia has been provided predominantly by studies using experimental models. The noninvasive rodent model of intermittent hypoxia, in which the animals alternately breathe nitrogen-enriched air to simulate hypoxia and air or oxygen for the reoxygenation phase has been fundamental to the field and there have been excellent reviews summarising the contribution of this model to our current knowledge [13–15]. However, the model has also considerable limitations and hence, results of those animal studies cannot automatically be extrapolated to the human condition of OSA. Firstly, the rodent model does not accurately reflect the oxygenation pattern seen in OSA patients with the latter being characterised by a considerable intra-and inter-subject variability and generally less severe desaturations than that of the rodent model [16]. Secondly, rodent sleep is not as consolidated as that of humans, and hence a significant proportion of the intermittent hypoxia occurs while the animal is awake. Although systems have been developed to trigger the intermittent hypoxia during sleep only [17, 18], given the labour- and cost-intensive nature of this approach, most models use intermittent hypoxia unlinked to sleep. Thirdly, an increasing body of evidence supports the potentially very significant confounding effects of external stressors such as cold temperature, diet or housing conditions on inflammatory and immune responses in rodents [19]. Furthermore, as mentioned above, the intermittent hypoxia model does not recapitulate all the effects of OSA and new approaches to noninvasively reproduce upper airway obstruction may be more suited to study detrimental effects in OSA [20, 21].

Cell culture models of intermittent hypoxia have been utilised to complement animal studies and have allowed the investigations of detailed intermittent hypoxia-induced cellular responses and signalling

mechanisms in different cell lines [22–25]. These models have advanced continuously over recent years. Growing cells on ultrathin semipermeable membranes allows rapid oscillations in the partial pressure of oxygen and fully automated systems facilitate the application of an adequate control exposure in parallel to the intermittent hypoxia treatment with tight control of potentially confounding parameters such as temperature or carbon dioxide tension [24]. Thus, many limitations associated with earlier approaches have been overcome and cell culture studies are likely to grow in their importance to the field. However, the specific intermittent hypoxia patterns at the cellular level in relevant tissues in humans with OSA remain largely unknown and therefore, may not be accurately reflected in *in vitro* studies, which remains a significant limitation.

In order to bridge the gap between animal models and clinical studies, human models exposing healthy volunteers to intermittent hypoxia have been developed and have provided substantial additional insight into the acute effects of intermittent hypoxia [26]. Experiments have shown that several weeks of intermittent hypoxia for 8 h each night lead to blood pressure elevation and a sustained increase in sympathetic tone [27]. These changes are coupled with an alteration in arterial baroreflex sensitivity and an increase in hypoxic chemosensitivity, illustrating the impact of intermittent hypoxia exposure on autonomic activity, which is one of the key mechanisms of neurogenic hypertension exhibited by OSA patients [27, 28]. So far, the usage of such models is limited due to the substantial costs and ethical considerations. Furthermore, they are similarly limited to intermittent hypoxia and sleep fragmentation, but do not include other pathophysiological traits of OSA such as hypercapnia or respiratory efforts. Combining such factors and exposing not only healthy humans but patients without OSA in the future might provide us with invaluable benefit understanding the very detailed mechanisms of cardiometabolic diseases in OSA.

Adaptive and maladaptive responses to intermittent hypoxia: where is the threshold?

Depending on the frequency of cycles, the depth of the hypoxic period and the duration of the exposure, intermittent hypoxia can induce both beneficial and detrimental effects, and hence is considered a "double-edged sword". Short exposures to low-frequency intermittent hypoxia with mild hypoxia improves endurance performance of athletes, facilitates high-altitude acclimatisation and may be cardio- and neuroprotective through pre-and post-conditioning effects [29–35]. Furthermore, recent studies have suggested that exposure to mild intermittent hypoxia initiates long-term facilitation of upper airway muscle activity, and thus may be a therapeutic modality to reduce treatment pressure of CPAP in OSA patients [36]. In contrast to these effects of mild intermittent hypoxia, the chronic intermittent hypoxia pattern typically associated with moderate or severe OSA is associated with numerous deleterious consequences [37, 38]. Furthermore, as suggested by various animal studies, the duration of intermittent hypoxia exposure may determine the potential reversibility of its consequences [39, 40]. Hence, a key question in the field relates to the determination of the actual threshold between these opposite effects. It is increasingly recognised that this threshold can be modified by various factors. Particularly, the impact of age has gained increasing attention stimulated by clinical observations suggesting blunted cardiovascular outcomes in elderly OSA subjects [41–43]. Intriguingly, programmed cell death-1 receptor and its ligand are upregulated in OSA subjects in comparison to healthy controls; however, this is not evident in older patients attributed to an impaired activation of hypoxia inducible factor (HIF)-1α, and this differing response may account at least in part for the increased cancer mortality seen in younger OSA subjects [44]. Recent animal studies lend support to this hypothesis, demonstrating a blunting of vascular remodelling in older in comparison to younger mice [45]. Other factors which may contribute to the shift from an adaptative to a maladaptative response in OSA include genetic susceptibility, sex, age, smoking habits, physical activity and the presence of comorbidities such as obesity, established cardiovascular disease, diabetes or others [37] (figure 1). It will be essential to study these modifying factors and their interactions with intermittent hypoxia in future preclinical experiments in greater detail, and these results may assist predicting the consequences of OSA and the potential benefits of its treatment for specific patient phenotypes.

Novel insights into the detrimental cardiometabolic effects of intermittent hypoxia

Despite limitations of the current models of intermittent hypoxia there is continuously growing evidence of the detrimental effects of this hallmark feature in cardiometabolic disease processes in OSA. Table 1 highlights important findings of recent publications. Clinical and experimental studies have shed further insight into the mechanistic pathways of intermittent hypoxia-mediated hypertension. Although previous studies failed to demonstrate benefit of supplemental oxygen on blood pressure [46, 47], in a recent randomised controlled trial in patients with moderate-to-severe OSA, supplemental oxygen delivered at a high flow rate, but not sham treatment, abolished morning blood pressure rise following CPAP withdrawal for 2 weeks, supporting the key role of intermittent hypoxia in this process [48]. Furthermore, this

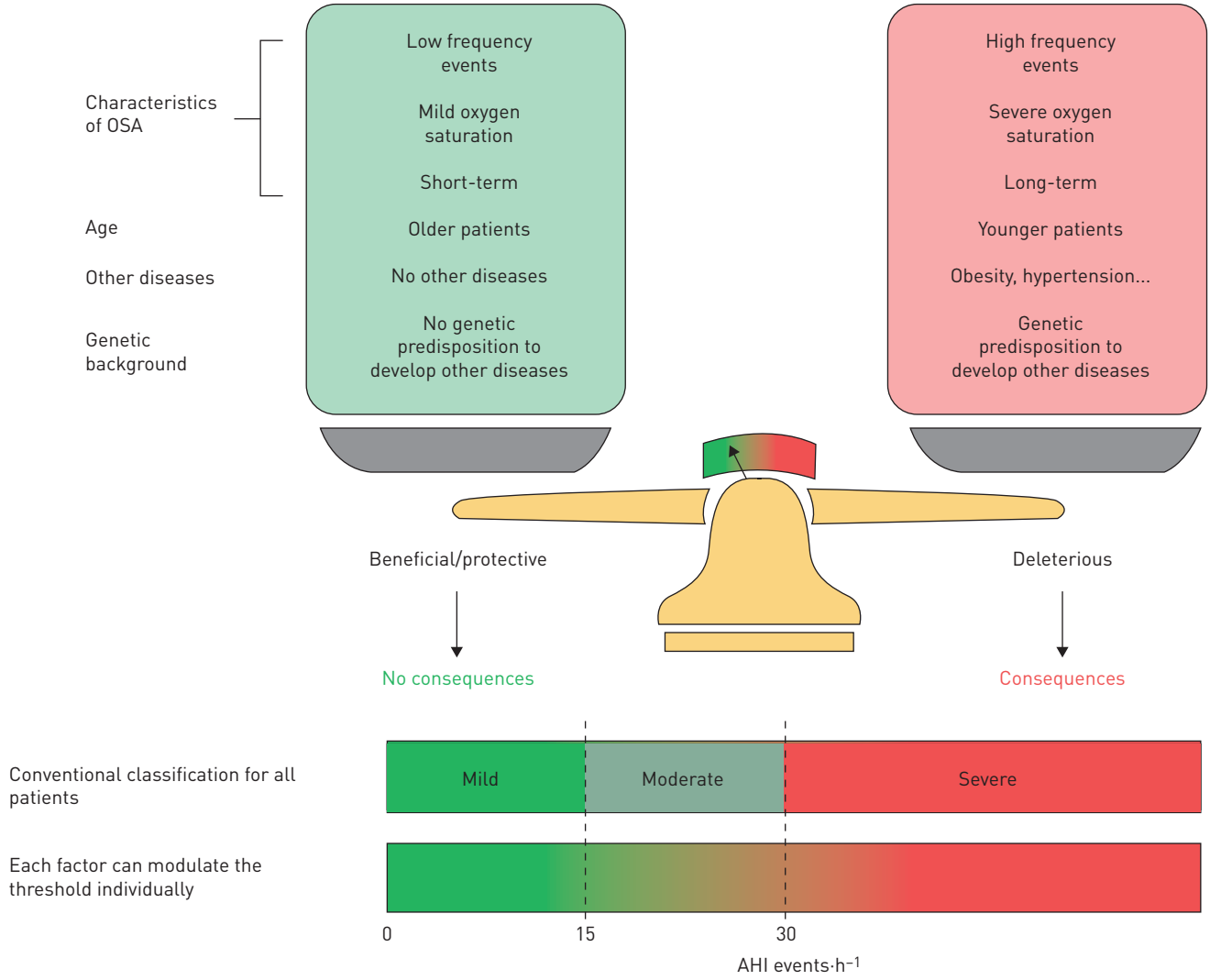

FIGURE 1 Protective and detrimental effects of intermittent hypoxia depending on the characteristics of the pattern of obstructive sleep apnoea (OSA) and other modifying factors. AHI: apnoea–hypopnoea index.

response was associated with upregulation of genes of the NF-κB-associated proinflammatory pathway in circulating leukocytes, providing further evidence of the pivotal contribution of inflammatory processes in cardiovascular complications in OSA [25, 49, 50]. In addition, the blood pressure increase in response to intermittent hypoxia is probably mediated by the renin–angiotensin–aldosterone system, and both renal denervation and pharmacological blockage of angiotensin II have been shown to prevent intermittent hypoxia-mediated blood pressure increases in rodents [51, 52]. Intermittent hypoxia also promotes atherosclerosis [53, 54] and associated diseases, and a recent study reported the promotion of a vasoconstrictive profile in human cultured coronary artery cells in response to this stimulus [55]. The importance of intermittent hypoxia-mediated proinflammatory pathway activation in detrimental vascular diseases was again highlighted by Song *et al.* [56], who reported significant attenuation of atherosclerotic processes in atherosclerotic-prone *ApoE*$^{-/-}$ mice with an additional genetic inhibition of NF-κB in comparison to standard *ApoE*$^{-/-}$ mice. Interestingly, younger animals appear to be more susceptible to intermittent hypoxia-induced cardiac remodelling than older mice [45] and normoxic recovery may reverse these changes [57], raising the possibility that CPAP therapy, if provided early in the course of OSA, may lead to attenuation of this process. Furthermore, murine studies suggest that intermittent hypoxia affects the fetus during maternal gestation and may predispose the adult male offspring to vascular disease, which is an important topic requiring further detailed investigations [58, 59].

Furthermore, considerable advances have been made in our understanding of the mechanisms of intermittent hypoxia-induced insulin resistance, type 2 diabetes and other glucose disorders and their relevance to OSA [60]. A randomised crossover trial of CPAP *versus* CPAP withdrawal revealed that OSA

TABLE 1 Important contributions from recent experimental studies investigating the effects of intermittent hypoxia (IH) on cardiovascular and metabolic outcomes

First author, year [ref.]	Characteristics of IH	Species, cells	Main message
Cardiovascular disease			
Castro-Grattoni, 2016 [57]	20–6% O_2, 60 events·h^{-1}, 6 h·day^{-1}, 6 weeks and 6 weeks recovery	6-week-old mice	IH-induced cardiovascular injury can be reversed by normoxia
Cortese, 2017 [205]	21–6% O_2, 20 events·h^{-1}, 12 h·day^{-1}, 20 weeks	22–25 g C57Bl/6 WT and CD36$^{-/-}$ mice	IH promotes the recruitment of metabolic active macrophages to the aortic wall, triggering atherogenesis, and this was absent in *CD36*$^{-/-}$ mice
Arnaud, 2018 [206]	21–5% O_2, 60 events·h^{-1}, 8 h·day^{-1}, 14 days	12-week-old WT and nmMLCK$^{-/-}$ mice	Nonmuscle myosin light chain kinase is a key mechanism in IH-induced vascular oxidative stress, inflammation and barrier dysfunction
Farré, 2018 [207]	20–6% O_2, 60 events·h^{-1}, 6 h·day^{-1}, 8 weeks	2- and 18-month-old mice	IH increases passive stiffness of myocardial extracellular matrix
Sharma, 2018 [55]	30 min 1% O_2, 30 min 21% O_2, 9 cycles	Human coronary artery endothelial cells	IH upregulates caveolin-1 and endothelin-1 expression
Song, 2018 [56]	20–6% O_2, 60 events·h^{-1}, 8 h·day^{-1}, 9 weeks	ApoE$^{-/-}$ and ApoE$^{-/-}$ overexpressing IκB mutant mice	IH activates NFκB-pathway in the aorta with progression of atherosclerosis with significant attenuation in mutant mice
Suarez-Giron, 2018 [208]	20–6% O_2, 60 events·h^{-1}, 6 h·day^{-1}, 8 weeks	6-week-old C57Bl/6J mice	Acetylsalicylic acid prevents IH-induced vascular remodelling
Takahashi, 2018 [52]	21–10% O_2, 12 events·h^{-1}, 8 h·day^{-1}, 4 weeks	8-week-old male C57Bl/6J mice	IH-induced renal sympathetic nerve activation is involved in systemic oxidative stress, endothelial dysfunction and renin–angiotensin activation
Badran, 2019 [59]	21–12% O_2, 60 events·h^{-1}, 12 h·day^{-1}, 18 days	8-week-old mice	Gestational IH promotes vascular disease in adult male offspring
Rubies, 2019 [209]	15-s upper airway obstructions, 60 events·h^{-1}, 6 h·day^{-1}, 21 days	250–300 g Sprague-Dawley rats	Mesenchymal stem cell infusion blunts OSA-related vascular changes
Metabolic alterations			
Gozal, 2017 [65]	21–6% O_2, 20 events·h^{-1}, 12 h·day^{-1}, 6 weeks *versus* continuous 8% O_2 (SH)	8-week-old C57Bl/6J mice	In contrast to SH, IH induces whitening and inflammation of adipose tissue with insulin resistance
Murphy, 2017 [24]	Mice: 21–5% O_2, 60 events·h^{-1}, 8 h·day^{-1}, 6 weeks Cells: 40 s 16% O_2, 40 s 3% O_2, 8 h·day^{-1}, 3 days	19-week-old C57Bl/6J mice fed on low- or high-fat diet 3T3-L1 murine adipocytes THP-1-derived macrophages	IH induces a pro-inflammatory phenotype of adipose tissue with polarisation of macrophages towards an M1-phenotype contributing to the pathogenesis of IH-mediated insulin resistance
Poulain, 2017 [62]	21–5% O_2, 60-s cycle, 8 h·day^{-1}, 6 weeks	8-week-old C57Bl/6J mice	IH reduces epididymal adipose tissue, but induces glucose dysregulation, thus, IH-induced inflammatory remodelling could represent the main determinant of metabolic dysfunction
Thomas, 2017 [210]	21–5% O_2, 60 events·h^{-1}, 8 h·day^{-1}, 5 days	8-week-old WT AMPKα2$^{-/-}$, muscle-specific AMPKα1α2$^{-/-}$ mice	IH impairs insulin sensitivity, but improves glucose tolerance by activating skeletal muscle AMPK
Conotte, 2018 [211]	21–6% O_2, 60 events·h^{-1}, 8 h·day^{-1}, 35 days	9-week-old mice	Alteration in energy metabolism towards anaerobic pathways Excess production of vitamin B3, liver function modulations and a stimulation of creatine synthesis
Khalyfa, 2018 [212]	21–6% O_2, 20 events·h^{-1}, 12 h·day^{-1}, 2–20 weeks	22–25 g mice	Alterations in exosomal cargo in response to IH impede insulin-signalling pathway in cultured adipocytes
Polak, 2018 [66]	21–6% O_2, 60 events·h^{-1}, 12 h·day^{-1}, 2 weeks	6–8-week-old male C57Bl/6J mice	IH led to impaired glucose tolerance and insulin resistance and these responses were partially ameliorated with pharmacological blockage of endothelin-1 type B receptor

O_2: oxygen; WT: wild type; OSA: obstructive sleep apnoea; SH: sustained hypoxia; AMPK: AMP-activated protein kinase.

recurrence promotes an increase in circulating free fatty acids (FFA) and glucose during sleep, which was associated with sympathetic and adrenocortical activation [61]. Pancreatic β-cell dysfunction and insulin resistance in the insulin target organs adipose tissue, liver and skeletal muscles have been implemented in the pathogenesis of insulin resistance in response to intermittent hypoxia. Adipose tissue inflammation is increasingly recognised to play a key role and epididymal lipectomy prevents intermittent hypoxia-induced glucose alterations in mice [62, 63]. In lean and diet-induced-obese mice as well as cultured cells, the intermittent hypoxia-mediated insulin resistance is accompanied by polarisation of adipose tissue macrophages towards a pro-inflammatory phenotype with the net consequence of metabolically dysfunctional adipose tissue [24]. In addition, intermittent hypoxia leads to release of FFAs from adipose tissue into the systemic circulation, and chronically elevated levels of FFA may further contribute to impaired glucose metabolism through promotion of insulin resistance in the liver and decreased pancreatic insulin secretion [64]. Continuing the debate of how adipose tissue perceives intermittent hypoxia, a recent study compared the effects of intermittent hypoxia and sustained hypoxia on the visceral adipose tissue in mice and identified important differences [65]. While sustained hypoxia led to a preferential activation of the HIF-1 pathway with adaptive responses to the hypoxic insult, intermittent hypoxia induced a pro-inflammatory phenotype and whitening of the adipose tissue with subsequent insulin resistance. In addition, improvement in glucose function with blockage of the endothelin-1 type B receptor has been reported, thus supporting previous results and identifying a potential therapeutic target [66, 67].

OSA is also associated with the development of NAFLD, at least partly independent of the effects of obesity or shared comorbidities [68, 69]. The degree of nocturnal hypoxia in OSA has been identified as independent predictor of this association and supporting the pivotal role of intermittent hypoxia in this process, rodents exposed to intermittent hypoxia develop impairment of hepatic lipid metabolism, steatosis and fibrosis [70–72]. The pathogenesis of intermittent hypoxia-mediated NAFLD probably includes activation of pro-inflammatory pathways and oxidative stress, but remains incompletely explored. Interestingly, a recent study utilising cell culture techniques and diet-induced-obese mice concluded that hypoxia promotes NAFLD through HIF-2α-mediated lipogenesis *via* peroxisome proliferator-activated receptor-α activation [73] and this mechanistic pathway warrants further exploration in the setting of intermittent hypoxia.

Pathophysiological responses of sleep disturbances in OSA

Sleep fragmentation

Normal sleep is essential for a person's health and wellbeing and beside sleep duration, the continuity of sleep is increasingly recognised in its importance for normal daytime function. In healthy volunteers, experimental sleep fragmentation has been shown to lead to a decrease in insulin sensitivity and to blunting of the usual nocturnal dip in blood pressure [74, 75]. Furthermore, in a population of 780 healthy elderly subjects, repeated sympathetic arousals during sleep were associated with elevated systolic blood pressure and higher risk of hypertension, after controlling for multiple potential confounders [76].

In OSA, sleep fragmentation is a characteristic feature and a consequence of recurrent arousals leading to subsequent excessive daytime sleepiness as the most debilitating daytime symptom in these patients. Arousals in OSA are associated with repetitive substantial blood pressure rises as high as 80 mmHg [77, 78]. However, the contribution of these acute changes to the development of cardiovascular and metabolic conditions is still under debate. A few studies have cross-sectionally or longitudinally evaluated the association of polysomnographic variables characterising sleep fragmentation, such as the arousal index, with cardiovascular or metabolic outcomes in OSA, and have come to different conclusions. Among 355 children evaluated for sleep disordered breathing, a high arousal index predicted the presence of endothelial dysfunction [79]. In contrast, in a large prospective study demonstrating an independent relationship between incidence of metabolic syndrome and moderate-to-severe OSA, only indices characterising intermittent hypoxia, but not sleep fragmentation, were identified as predictors of this outcome [80]. Similarly, in a cross-sectional analysis of 2055 participants from the Multi-Ethnic Study of Atherosclerosis, sleep depth as a continuous measure of arousability was not associated with higher blood pressure, in contrast to markers of hypoxic burden [81]. Adding to the debate, a Japanese study recently reported an independent association of the arousal index with the presence of carotid intima plaques. However, the study population was small and a substantial proportion of subjects suffered from various confounding comorbidities [82]. Thus, the role of sleep fragmentation in cardiometabolic disease processes in OSA remains uncertain. However, the reliability of the arousal index based on a one-night polysomnography as marker of sleep fragmentation is still uncertain and there are no clear normative values of the arousal index in various demographic groups.

The role of sleep fragmentation in cardiometabolic disease processes has been the focus of numerous animal studies. In earlier studies using canine or rat models, sleep fragmentation induced by recurrent

acoustic arousals failed to contribute to hypertension [83, 84]. However, Launois *et al.* [85] compared the haemodynamic responses of respiratory and nonrespiratory arousals in a porcine model and found that only respiratory arousals led to blood pressure surges, providing a potential explanation for the lack of response in the previous studies. Over the past decade, substantial advances in our understanding of responses to sleep fragmentation have been made by studies utilising a murine model that requires no direct human interaction and arousals are induced by recurrent minimally stressful tactile stimuli. Long-term exposure to this model of sleep fragmentation initiated the development of mild hypertension, endothelial dysfunction and early structural vascular changes in C57Bl/6J wild-type mice and led to a significant progression of atherosclerotic lesion in $ApoE^{-/-}$ mice [86, 87]. Furthermore, sleep fragmentation induces insulin resistance predominantly mediated by visceral adipose tissue inflammation [88]. In summary, sleep fragmentation in mice leads to adverse cardiometabolic consequences, but the detailed contribution of this triggering factor in OSA requires further translational studies.

Short sleep duration

In recent years there has been mounting evidence of a U-shaped association between both abnormally short and long sleep duration and numerous adverse health outcomes including mortality and diabetes [89, 90]. Evidence is particularly strong for short sleep duration, defined as a habitual sleep time of ⩽6 h, which has been linked with increased morbidity and mortality attributed mainly to adverse cardiometabolic risk including obesity, hypertension, cardiovascular disease and type 2 diabetes [89, 91–93]. This is of specific public health concern, as sleep duration has been declining in the past few decades and ∼35% of the general population sleep <6 h per night [94, 95]. In particular, various cross-sectional and prospective studies have linked short sleep duration to increased prevalence and incidence of insulin resistance and type 2 diabetes [90, 96, 97] and a meta-analysis on this topic revealed that self-reported sleeping ⩽5 h imposed a 45% increase in the risk of diabetes [97]. Furthermore, insufficient sleep is associated with poorer glycaemic control in diabetic subjects and may also be related to poorer diabetes self-care behaviours, leading to recommendations by the American Diabetes Association to include evaluation of sleep duration and pattern as part of comprehensive diabetes care [98, 99]. In support of a causal relationship between short sleep and metabolic dysregulation, experimental sleep restriction in humans leads to increased calorie intake, weight gain, insulin resistance and possibly impaired insulin secretion with sympathetic excitation, inflammation, changes in the composition of the gut microbiota and alterations of the 24-h cortisol profile with blunting of the usual nocturnal decline proposed to be the underlying mechanistic links [100–102]. However, some of these alterations have been found to be transient and how these results correlate with diabetes as a consequence of long-term sleep restriction remains unknown. In addition, studies evaluating the benefit of experimental sleep extension on glucose metabolisms have yielded conflicting results [103–107]. However, the ability of subjects to extent their sleep time greatly varies and a recent cross-over study on 21 short-sleeping healthy participants revealed that only those who could sleep >6 h during sleep extension showed improvement of fasting insulin resistance and β-cell function [106]. Thus, there are still unanswered questions surrounding the association of sleep duration with cardiometabolic diseases and we require further large-scale well-designed epidemiological and translational studies before definite conclusions can be drawn.

The specific role of short sleep in OSA and its relative contribution to the pathophysiology of cardiometabolic comorbidities are as yet unclear. Several studies using wrist actigraphy or polysomnography as tools to measure sleep length have identified shorter sleep in OSA subjects in comparison to their non-OSA counterparts [108, 109]. However, data from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) cohort, comprising >2000 participants, revealed only a significant association of OSA, but not sleep duration, with prevalent obesity, hypertension and dyslipidaemia [108]. Similarly, a recent cross-sectional analysis of the Nagahama study of >7000 subjects identified an independent association of OSA and obesity, but not of actigraphy-evaluated sleep duration, with prevalent diabetes and hypertension [110]. In contrast, Ren *et al.* [111] found that in subjects with OSA, objectively measured sleep duration of 5–6 h and <5 h increased the odds of prevalent hypertension by 45% and 80%, respectively. Furthermore, in a prospective study of >13 000 subjects with recent acute coronary syndrome, both, OSA and short sleep were identified as independent predictors of major coronary events; however, the reliance on the Berlin questionnaire and subjectively reported sleep time were substantial limitations [112].

Circadian misalignment

All life on Earth benefits from an internal timing mechanism to permit adaptations to the predictive changes from day to night, and this is termed the circadian clock. Most cells of the immune system have an intact clock, and inflammatory and immune parameters frequently show circadian control, including vaccine responses, innate inflammatory reactions, and aspects of the adaptive immune response [113–115].

Manipulations of the core clock machinery, or environmental disruptions, such as changes to the lighting schedule or fast/feed cycles result in changes to the immune response with, in most cases, an exaggerated inflammatory component [116]. In mammals, the internal circadian clock is synchronised to the external light–dark environment through a retinal pathway to the central clock in the brain. The central clock, the suprachiasmatic nucleus, then entrains peripheral clocks through neural and humoral pathways [117] (figure 2).

Circadian rhythms influence virtually all aspects of physiological architecture and chronic disruptions, for instance as seen in shift workers, have been linked to obesity, metabolic derangements, cardiovascular diseases, mood disorders or cancers [118–121]. Laboratory studies using forced circadian misalignment have lend support to this evidence, demonstrating insulin resistance, inverted cortisol rhythms and increased blood pressure in participants subjected to this protocol [122, 123].

The circadian rhythm and sleep are mutually linked in a bidirectional relationship. The sleep–wake cycle is one of the most prominent circadian-regulated behaviours and the circadian clock acts as a gating mechanism to confine sleep to specific parts of the diurnal cycle [124, 125]. Genetic disruptions of the clock lead to alterations of physiological sleep regulation. At the same time, changes in sleep timing or duration can feedback on clock function and experimental studies have demonstrated that sleep curtailment or mistiming strongly affects molecular correlates of the circadian clock [126–129].

Given the profound sleep disturbances in OSA, disruptions in clock oscillations are also likely. However, surprisingly, the prevalence of circadian misalignments in OSA is hardly investigated and the potential contribution of this trait to cardiometabolic comorbidities has been largely neglected. Some studies have demonstrated alterations of the normal circadian variations of blood pressure and arterial stiffness as well

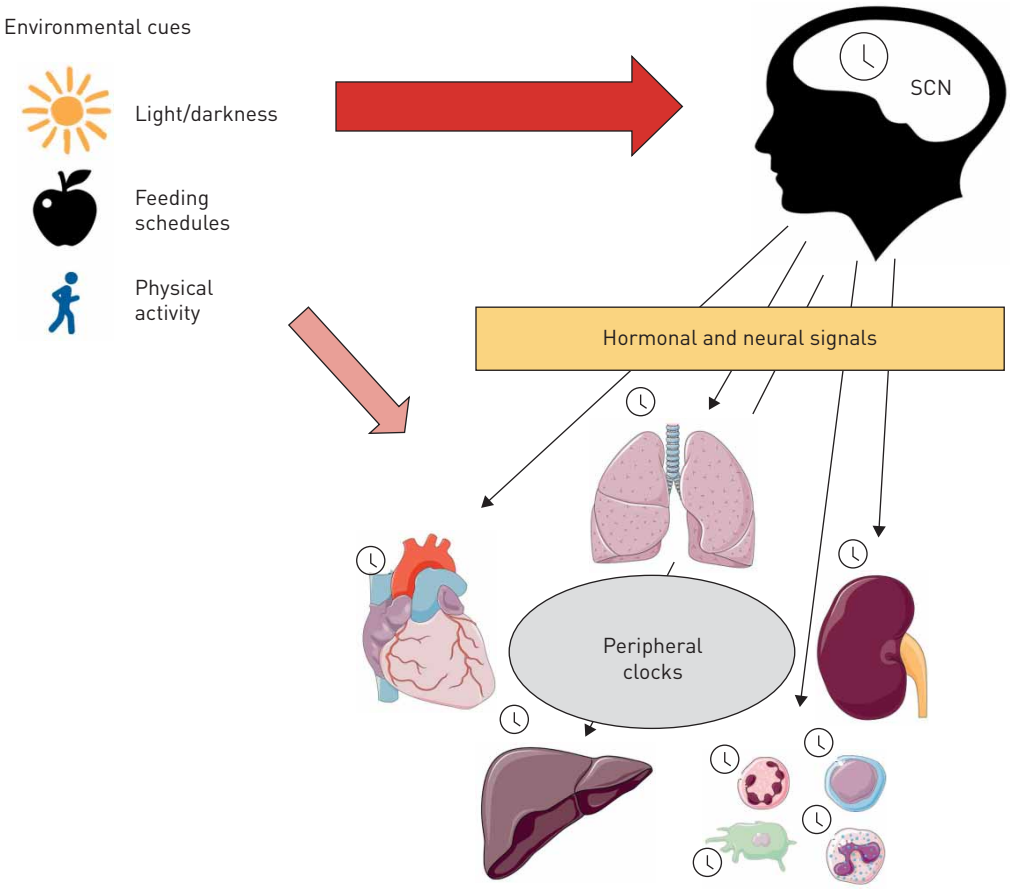

FIGURE 2 Schematic depiction of the normal mammalian circadian clock. Environmental cues such as light–dark cycle, mealtimes or physical activity influence the central and peripheral clocks. The central clock, located in the suprachiasmatic nucleus (SCN) of the hypothalamus, synchronises and entrains peripheral circadian clocks *via* neural and endocrine pathways. The resulting oscillations of clock proteins and activity translates into circadian behaviour and physiology, *i.e.* sleep/wakefulness, upper airway collapsibility, metabolic rhythms or immune responses.

as circulating cytokines, hormones, prothrombotic and fibrinolytic markers in OSA subjects compared to controls with improvement following effective CPAP therapy [130–135]. Furthermore, Martinez-Nicolas *et al.* [136] evaluated the circadian rhythm of distal skin temperature and identified significant alterations in OSA with lower temperature at night in comparison to controls; these disturbances correlated with the severity of the disease and improved with CPAP therapy. Moreover, these changes correlated with the level of daytime sleepiness and the authors suggested that the measurement of skin temperature may facilitate understanding of the pathophysiology of sleepiness in these patients.

Beside disruptions of peripheral tissue clocks there is limited evidence of an impact of OSA on central clock function. Burioka *et al.* [137] reported a blunted daily variability of the expression of the master clock-regulating gene Period1 (*PER1*) in leukocytes of OSA subjects *versus* matched controls with higher levels at night-time and restoration of the normal pattern with CPAP. In addition, OSA adversely affects melatonin secretion. Synthesised by the pineal gland, melatonin production and secretion are directly dependent on the ambient light–dark cycle, and hence melatonin is a main output signal of the central circadian pacemaker, the suprachiasmatic nucleus [138]. Alteration of the pattern of melatonin secretion with loss of the nocturnal peak has been reported in OSA subjects. However, studies so far have failed to show a benefit of this finding with CPAP therapy [139].

In summary, circadian disruptions in OSA are poorly understood and the identification of the detailed contribution of these processes to cardiometabolic disease processes is a major research priority and targeting these alterations may identify important novel treatment approaches.

Hypercapnia: a forgotten dimension

Repetitive episodes of hypoxia and reoxygenation in OSA are inextricably coupled with oscillations in the partial pressure of carbon dioxide (CO_2) ranging from hypocapnia to hypercapnia. Notably, hypercapnia in combination with hypoxia results in increased sympathetic activity, which is not seen with hypocapnic hypoxia [140]. In contrast to intermittent hypoxia, the consequences of intermittent hypercapnia are poorly understood and the potentially pathophysiological and modifying roles of this identity in OSA patients, who are predominantly eucapnic during daytime, have been grossly ignored. This is partly attributable to the fact that CO_2 monitoring is currently not routinely used in clinical practice and methods such as end-tidal or transcutaneous CO_2 measurements have significant limitations. Furthermore, most commonly used animal models have focused on intermittent hypoxia without controlling for CO_2. However, extrapolating from other respiratory conditions there is now ample evidence suggesting possibly important pathophysiological responses to hypercapnia. Depending on the clinical scenario, the consequences of high CO_2 have been referred to as a "double-edged sword" with the potential to be both detrimental and beneficial for patients. Hypercapnia is an independent predictor of mortality in COPD and in patients with acute respiratory distress syndrome (ARDS) admitted to the intensive care unit [141, 142]. Furthermore, studies in mice demonstrate that hypercapnia is damaging in the context of a bacterial challenge, probably as a consequence of CO_2-dependent immunosuppression [143]. Conversely, mild hypercapnia resulting from a protective ventilation strategy improves mortality in ARDS patients, which has led to the concept of "permissive hypercapnia" [144, 145]. In support, murine studies have demonstrated hypercapnia-associated acidosis to be markedly protective in the context of a lipopolysaccharide-induced destructive inflammatory challenge and in stretch-induced lung injury [146, 147]. Although this field is still in its infancy, there is emerging knowledge of how altered CO_2 levels affect cell signalling and gene expression and subsequent immunity and inflammation. Studies from model organisms and cell cultures indicate that hypercapnia elicits changes in cellular signalling and gene expression in a variety of different cell types, *e.g.* lung epithelium [148, 149], smooth muscle [150], skeletal muscle [151] and monocytes [152]. These changes are thought to be independent of hypercapnia-associated acidosis and reveal distinct clusters of differentially expressed genes from those elicited by hypoxia. Notably, genes associated with the immune response (*e.g.* Rel-dependent antimicrobial peptides) are differentially expressed in hypercapnia. To date, a master regulator of CO_2-dependent gene expression, analogous to HIF for the hypoxia response, has not been identified. However, work on the NF-κB pathway has revealed sensitivity of both the canonical (RelA-dependent) and noncanonical (RelB-dependent) pathway to CO_2. In particular, noncanonical NF-κB family members, such as IKKα, RelB and p100 are sensitive to CO_2, independent of an external inflammatory stimulus supporting a potentially important role of this pathway in immune regulation [149, 153, 154].

Thus, hypercapnia is much more than a simple waste product of aerobic respiration. CO_2 has the ability to elicit changes in gene expression, particularly those associated with immune and inflammatory signalling. These findings imply that CO_2 is likely a highly relevant dimension in OSA and the role of hypercapnia should be given greater consideration in the future, particularly in the context of immune regulation. As we strive to better understand the pathophysiological mechanisms of OSA-associated cardiometabolic

complications we must give due consideration to the role of circulating CO_2 levels and control for altered CO_2 levels in our models.

What next: CPAP for everyone or personalised treatments?

As extensively discussed in the research seminar and summarised in this perspective, the pathophysiology of cardiovascular and metabolic diseases in OSA is complex and remains incompletely understood. It has become increasingly clear that focusing on single mechanistic traits is too simplistic. Most research into this subject so far has been directed to intermittent hypoxia and sleep fragmentation as dominant triggering factors. Intrathoracic pressure swings as a result of forced inspiration against an occluded upper airway leading to shear stress on the heart and intrathoracic blood vessels have also been recognised to contribute to adverse consequences, in particular to atrial fibrillation and heart failure [155–157]. Other features such as alterations in sleep beyond disruptions as consequence of recurrent arousals as well as intermittent changes in CO_2 partial pressure may play important detrimental or attenuating roles and there is likely to be considerable interaction between those mechanistic pathways. Furthermore, many modifying factors such as age, sex, genetic makeup, anthropometric features, physical activity or comorbid conditions which are frequent in OSA may influence disease processes. In addition, there is probably a substantial influence of diet. Above all, cardiometabolic disease process are considerably amplified in the presence of a high-fat diet, potentially mediated through alteration of the gut microbiome, and this interaction will require further attention in future studies [158, 159] (figure 3).

While past and current experimental models have been fundamental to our understanding of the direct consequences of single pathophysiological triggering factors, these are too simplistic to reflect the clinical condition of OSA in all its complexity. Thus, unsurprisingly, results obtained from preclinical models often failed to translate into human populations and consequently, this has left us with limited knowledge

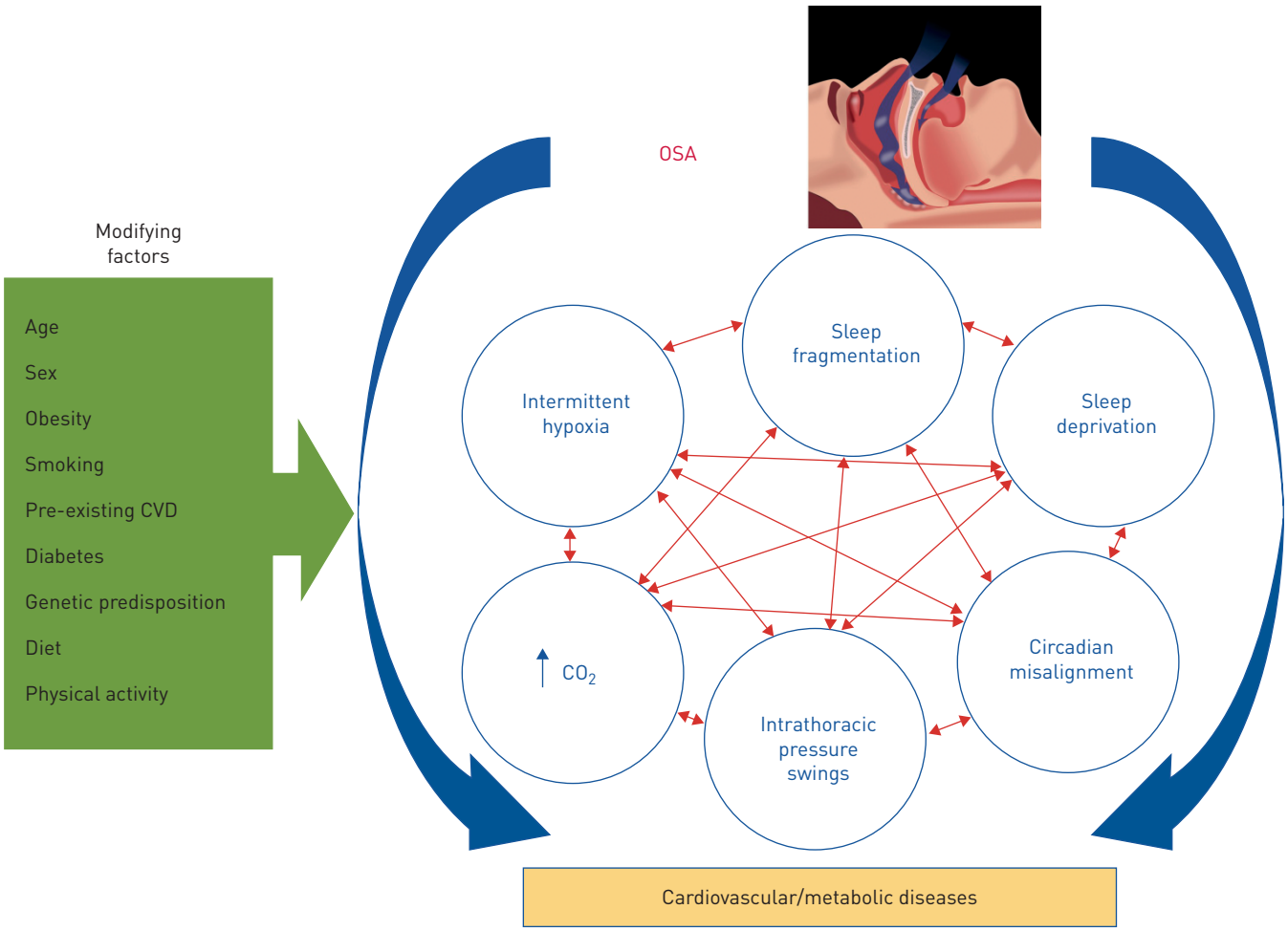

FIGURE 3 Pathophysiological traits for cardiovascular and metabolic diseases in obstructive sleep apnoea (OSA). CVD: cardiovascular disease; CO_2: carbon dioxide.

of potential therapeutic targets. Furthermore, OSA is a very heterogenous condition characterised by a wide diversity of clinical symptoms and presentations. Several cluster analyses have identified a variety of different clinical and polysomnographic phenotypes and between those OSA subgroups; there is considerable variability in age, obesity, the degree of sleepiness and burden of comorbidities [160–163]. Importantly, clusters substantially differ in their susceptibility to adverse cardiovascular complications [162] and thus, detailed understanding of the mechanisms underlying cardiometabolic disease processes with identification of these different phenotypes in OSA are crucial steps for the detection of effective treatment strategies.

CPAP therapy is the treatment of choice for the majority of OSA patients. It improves sleepiness, quality of life and neurocognitive function, but its benefit in cardiovascular and metabolic outcomes is uncertain. Controversy remains, especially regarding the usefulness of CPAP therapy in minimally symptomatic or asymptomatic OSA patients, recently highlighted in excellent pro/con debates [164–167]. Although CPAP has demonstrated its positive effects on early cardiovascular disease processes, *i.e.* endothelial dysfunction [168, 169], blood pressure control [170] or insulin resistance [171, 172], several recent randomised controlled trials, despite their limitations, have questioned its efficacy in preventing cardiac events in patients with established cardiovascular disease [173–177]. The effectiveness of CPAP may vary depending on the phenotype and, for example, 25–30% of OSA patients with adequate adherence to CPAP treatment (>4 $h \cdot night^{-1}$) do not benefit from CPAP treatment in blood pressure control and some may even experience an increase [178–180]. Following a *post hoc* analysis of the Sleep Apnea Cardiovascular Endpoints (SAVE) study, cluster analysis suggested that OSA patients with multimorbidity (OSA combined with diabetes and/or severe cardiovascular disease) may benefit most from CPAP therapy [181]. Data from the Spanish Sleep Network revealed that in OSA patients with resistant hypertension, a single cluster of cardiovascular system-related functional miRNAs distinguished CPAP responders from nonresponders, suggesting the usefulness of such epigenetic biomarkers in the guidance of personalised treatments; this field requires further exploration [182]. The benefits of available alternative single treatment strategies for OSA on cardiometabolic processes are also unclear. Mandibular advancement devices (MAD) are an effective alternative to CPAP, especially for patients with moderate disease or poor adaptation to CPAP. There is evidence of blood pressure reduction with MAD, but the effect on other disease processes remains unexplored [183]. Undoubtedly, we need to identify therapeutic approaches targeted to the needs and characteristics of individual patients through implementation of personalised medicine, and OSA-specific treatments need to be embedded in a multidisciplinary management in conjunction with lifestyle measures and optimal pharmaceutical treatments for comorbid conditions. As supported by a recent American Thoracic Society clinical practice guideline, weight reduction should be incorporated into the management of all overweight and obese OSA patients [184] and CPAP combined with weight loss leads to incremental reductions in blood pressure and insulin resistance [185]. However, weight loss is difficult to achieve with conventional measures alone and bariatric surgery, although effective in achieving weight reduction and metabolic improvements, is not a suitable intervention for everyone. A liraglutide-facilitated weight loss regimen may be an intriguing alternative [186], but further studies are needed to explore this approach in OSA. Long-term increased physical exercise is also associated with weight loss and leads to improvement in blood pressure, hence should also be part of an integrated care plan [187, 188].

An optimal treatment approach includes targeted pharmacological treatment of cardiovascular risk factors and comorbidities. This has been best studied in the setting of hypertension, and while antihypertensive drugs are far more effective than CPAP therapy in lowering blood pressure, there is added benefit with CPAP therapy [189].

Finally, targeting daytime sleepiness, which has been suggested as an independent predictor of adverse cardiovascular outcomes in OSA [162], may be an alternative treatment approach. Several pharmacological agents including modafinil, solriamfetol or pitolisant have been shown to improve sleepiness in OSA patients, and future studies evaluating the benefit of these agents on cardiometabolic end-points are required [190–192].

Concluding remarks and future directions

The future of medicine relies on the ability to assess disease risk at an individual level, the understanding of molecular mechanisms underlying progression of disease and aggregation of comorbidities and early initiation of personalised interventions [193]. The ERS research seminar "Targeting the detrimental effects of sleep disturbances and disorders" held in Dublin in 2019 was a founding event for stimulating scientific exchanges and eliciting a European research consortium at the critical mass. The common objective was to address the biological mechanisms of consequences of OSA in a continuum from molecules to cells, tissues, organs, systems, persons and impact on populations. The overarching goals for improving OSA

management include the identification of significant OSA phenotypes and actionable pathways mediating cardiometabolic consequences, and to facilitate the development of innovative therapeutic approaches.

To achieve these goals of precision and personalised medicine in OSA and to formulate advice for value-based care, the following approaches will be prerequisites.

- A first step already ongoing is the innovative use of real-world data [194, 195], well-managed cohorts and wearable monitoring [196] to enable physiological profiling and identification of homogeneous OSA groups of interest (*i.e.* main OSA clusters composed of relevant individual, social and environmental context factors and their interdependencies). The absence of definition of these relevant phenotypes might be a reason that large CPAP randomised controlled trials have shown no or modest effects on reduction of late cardiovascular events [173, 174, 197].
- Large randomised controlled trials have failed to show benefit of CPAP therapy on cardiovascular outcomes; however, they had significant limitations and in the majority of studies the most appropriate (*i.e.* sleepy) patients for treatment were excluded, leaving us with numerous questions and uncertainties. Realistically, further large randomised controlled trials will be difficult to conduct in the field as the costs are prohibitive with unacceptable delays before getting the answer. Thus, there is a crucial need to implement innovations in clinical research methods for reducing costs and increasing productivity. Recently, alternative solutions have been proposed including conducting randomised trials in existing cohorts [198], use of electronic health records to derive control arms [199], and/or use of new statistical methods such as causal inference to affirm causality from observational data [199].
- Cell-culture, animal and human models of intermittent hypoxia exposures need to be technically improved by including hypercapnia as an associated stimulus. Less severe and more clinically relevant forms of intermittent hypoxia exposure should also be considered in murine models to better understand adaptive and detrimental effects in OSA and findings should also be reproduced in different species. Organ-on-a-chip multichannel three-dimensional microfluidic cell cultures will be a step forward to simulate the activities, mechanics and physiological responses of entire organs to intermittent hypoxia, allowing the current limitations of conventional cell culture paradigms to be overcome. The combination of sleep fragmentation/deprivation and circadian misalignment with intermittent hypoxia exposures is also crucial to truly represent the multiple OSA clinical scenarios. These methodological improvements have the potential to allow identification of clinically relevant molecular pathways and to discover new pharmacological targets with their respective companion biomarkers in the near future. Such developments will contest the current paradigm for management of OSA largely reflecting a "one size fits all" approach whereby a large majority of patients are exclusively treated with CPAP without appropriate combined therapies targeting OSA-related organ-specific damage.
- Progression and accumulation of comorbidities during OSA life courses need to be better characterised and anticipated to define disease activity and delineate early and personalised interventions. This requires deep longitudinal profiling implying to make repeated deep phenotyping evaluations available and to longitudinally collect multiple biosamples analysed with multi-omics strategies. In addition, we require reliable markers predicting cardiometabolic outcomes which may guide treatment decisions. The recently identified hypoxic burden derived from overnight polysomnography is a promising indicator, but it requires further large longitudinal studies.
- Artificial intelligence will be fundamental for harnessing the dynamics and heterogeneity of OSA longitudinal trajectories [200, 201]. It may provide unique insights for organising the huge diversity of factors contributing to individual OSA patients' trajectories into a rational framework amenable for clinical decision, therapeutic intervention and reform of the health system.
- Diagnostic and therapeutic pathways will be completely reshaped by new automated methods and artificial intelligence. This will be the case for new techniques for identifying abnormal respiratory events during sleep [202] or new methods for analysing sleep electroencephalograms [203]. Finally, the interest of repeated patient profiling is supported by intrasubject night-to-night variability in OSA severity impacting on the occurrence of major outcomes [204].

To achieve these ambitious goals for OSA patients and the society, structuration of international consortiums is absolutely required and the ERS seminar was a critical step in this direction.

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