Cardiovascular morbidities of obstructive sleep apnea and the role of circulating extracellular vesicles

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***Abstract*:** Obstructive sleep apnea (OSA) is characterized by recurrent upper airway collapse during sleep resulting in impaired blood gas exchange, namely intermittent hypoxia (IH) and hypercapnia, fragmented sleep (SF), increased oxidative stress and systemic inflammation. Among a myriad of potential associated morbidities, OSA has been particularly implicated as mechanistically contributing to the prevalence and severity of cardiovascular diseases (CVD). However, the benefits of continuous positive airway pressure (CPAP), which is generally employed in OSA treatment, to either prevent or improve CVD outcomes remain unconvincing, suggesting that the pathophysiological mechanisms underlying the incremental CVD risk associated with OSA are not clearly understood. One of the challenges in development of non-invasive diagnostic assays is the ability to identify clinically and mechanistically relevant biomarkers. Circulating extracellular vesicles (EVs) and their cargos reflect underlying changes in cellular homeostasis and can provide insights into how cells and systems cope with physiological perturbations by virtue of the identity and abundance of miRNAs, mRNAs, proteins, and lipids that are packaged in the EVs under normal as well as diseased states, such as OSA. EVs can not only provide unique insights into coordinated cellular responses at the organ or systemic level, but can also serve as reporters of the effects of OSA in CVD, either by their properties enabling regeneration and repair of injured vascular cells or by damaging them. Here, we highlight recent progress in the pathological CVD consequences of OSA, and explore the putative roles of EVs in OSA-associated CVD, along with emerging diagnostic and therapeutic opportunities.

The reviews of this paper are available via the supplemental material section.

Keywords: CVDs, exosomes, extracellular vesicles, pathology of OSA, OSA

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Obstructive sleep apnea and cardiovascular morbidity and treatment

Obstructive sleep apnea (OSA) is a highly prevalent condition characterized by repetitive episodes of partial (hypopnea) or complete (apnea) obstruction of the upper airways, resulting in episodic reductions in oxyhemoglobin saturation followed by reoxygenation upon upper airway opening during sleep,[1] a phenomenon usually denoted as intermittent hypoxia (IH). OSA is also associated with recurrent intermittent hypercapnia, increased intrathoracic pressure swings, and with sleep fragmentation, as illustrated by the recurring arousals triggered by the respiratory perturbations. The actual prevalence of OSA varies tremendously between studies, with more conservative estimates of 3% in women and 10% in men between the ages of 30–49 years, and 9% in women and 17% in men between the age of 50–70 years.[2–8] Among myriad reported associations on OSA morbidity, untreated OSA has been particularly reported to exert adverse consequences, such as excessive daytime sleepiness, cardiometabolic complications, neurocognitive

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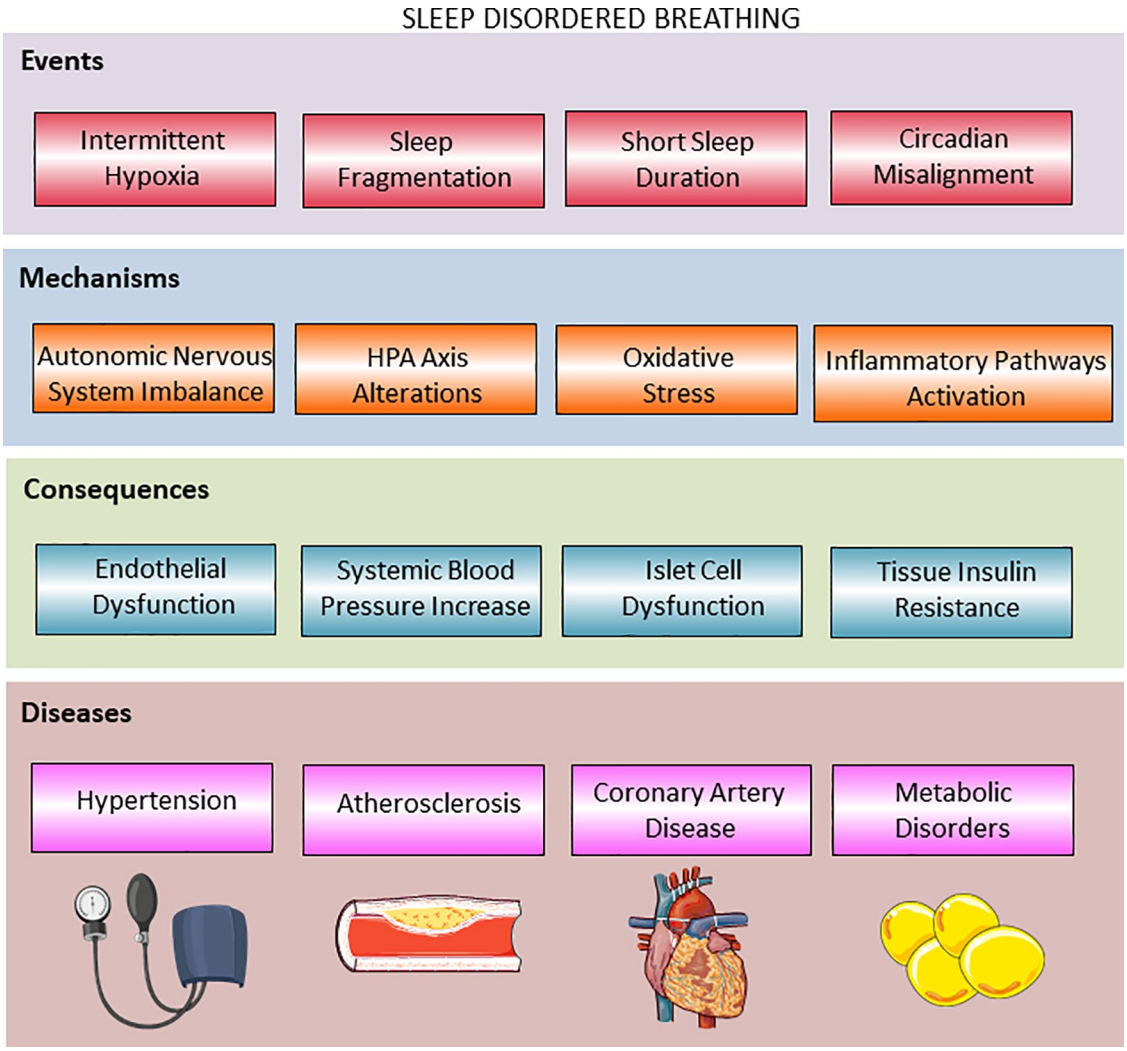

Figure 1. Schematic diagram illustrating the systems-based approach to the pathology of OSA, by enunciating the major physiologic alterations in OSA, including four different categories of events (intermittent hypoxia, sleep fragmentation, short sleep duration, and circadian misalignment), mechanisms (sympathetic activation, HPA axis alterations, oxidative stress, and inflammation), consequences (endothelial dysfunction, increased blood pressure, islet cell dysfunction, and insulin resistance), and diseases (hypertension, atherosclerosis, coronary artery disease, and metabolic disorders).
HPA, hypothalamic-pituitary-adrenal; OSA, obstructive sleep apnea.

and mood disorders, and an increased incidence of motor vehicle accidents, along with poor overall quality of life and increased overall mortality.[9–13] In Figure 1, we show the impact of OSA on end-organ function as being mediated by interactions of four different categories (events, mechanisms, consequences, and diseases), ultimately leading to unique personal fingerprints of OSA in each individual.[14] Intermittent hypoxia, sleep fragmentation, short sleep duration, and circadian misalignment, either individually or in combination, can lead to the activation of several pathogenetic pathways, ultimately resulting in oxidative stress and inflammation, along with recruitment of autonomic nervous system imbalance with increased sympathetic outflow and reactivity, as well as vagal withdrawal in the context of major activation of stress pathways as reflected by the recruitment of the hypothalamic-pituitary-adrenal (HPA) axis. As consequences, increased systemic blood pressure, endothelial dysfunction, hypercoagulability, dyslipidemia, and insulin resistance are representative elements of end-organ dysfunction that ultimately lead to

systemic hypertension, atherosclerosis, and associated ischemic cardiovascular and cerebrovascular diseases, and metabolic disorders.[15–18] It has been proposed that stiffness of endothelial cells is closely linked to the function of the vasculature, as it regulates the release of vasoactive substances such as nitric oxide (NO) and reactive oxygen species.[19] OSA has been linked with increased oxidative stress characterized by increasing superoxide anion release from circulating leukocytes, leading to reduced nitric oxide bioavailability and increased lipid peroxidation.[20,21] Associations between sleep disturbances, circadian dysfunction, and adverse outcomes affecting the cardiovascular and metabolic systems, in addition to multiple other deleterious consequences, have been reported, and the evidence continues to gain credibility toward causative biological plausibility. For example, sleep restriction or disruption impact molecular mechanisms in peripheral tissues, such as innate immune cells and metabolically active organs.[22–27] Recently, we have shown that alternating dark–light cycles mimicking shift work in mice altered their fecal microbiota and colonic epithelium permeability, ultimately leading to metabolic dysfunction.[28] Several studies have explored the diurnal and evening-to-morning differences in circulating microvesicles, including in OSA, and, conversely, the potential role of circulating microvesicles in regulating peripheral clocks has also been investigated.[29–33]

To better understand the implications of OSA and its associated morbidities, a substantial search for generation of adequate animal models that reliably mimic the human disease has been the focus of major research efforts for over several decades.[34–45] A wealth of accumulated evidence suggests that chronic intermittent hypoxia (CIH), generated during repetitive apneic episodes, is one of the major key causal factors linking OSA and CVD.[46] OSA is clearly an independent mechanistically associated factor in the development of systemic hypertension, with the risk increasing as the severity of OSA increases.[47] In light of the chronicity of OSA, it becomes apparent that CIH exposures mimic OSA more closely than acute IH exposures, and the physiological responses to acute IH or acute sleep fragmentation can differ markedly from the responses to chronic comparable exposures, thereby lending the temporal domain of complexity to an already quite complex array of responses to either IH or fragmented sleep.[48–50] Indeed, the IH profiles can markedly vary in severity, cycle frequency, and duration of hypoxemia.[35] The choice of frequency and pattern of the stimulus results in markedly different saturations of oxyhemoglobin, with usual reported ranges from 60% to 80% in mice exposed to cycles with an inspired fraction of oxygen (F_IO_2) of 5% every 30 s, and from 83% to 86% in mice exposed to cycles with F_IO_2 of 6–10%. In this regard, different experimental protocols can be generated to simulate different degrees of severity of the disease, corresponding to mild, moderate, or severe OSA.[51]

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 or the presence or absence of concurrent physical activity or nutritional supplements, emphasizing the multidirectional relationships and interactions between OSA and obesity in metabolic health.[39] Several of our previous studies showed that mice exposed to a well characterized sleep-fragmentation model have demonstrated the emergence of hyperphagic behaviors in awake mice when exposed to chronic sleep fragmentation,[45,52,53] resulting in accelerated body weight and visceral fat mass accruals over time, and ultimately leading to frank obesity.[42,54,55] Epidemiologic evidence has also identified the presence of a strong association between untreated OSA and the morbidity and mortality of various prevalent cardiovascular diseases.[16,56] Cardiovascular disease (CVD) is the leading global cause of death, and accounts for approximately one of every four deaths annually, thereby posing a great economic burden to both society and healthcare systems (see: https://www.cdc.gov/nchs/fastats/leading-causes-of-death.htm).[57] Identifying and developing new diagnostic or therapeutic strategies may provide multiple opportunities for reductions in the mortality increases associated with OSA in the context of CVD. Furthermore, coronary artery disease (CAD) is one of the most prevalent chronic CVD, and represents a leading cause of mortality worldwide.[58] More recent implementation of coronary computed tomography angiography (CTA) has allowed for rapid, noninvasive and direct assessment of the burden of CAD, and is a valid and increasingly employed tool in the assessment of CAD in the OSA population.[59–61]

Many human diseases, including OSA, are the end-result of many dynamic and lifelong gene–environment interactions that are modulated by

multilevel biological networks.[62] CPAP treatment of OSA, particularly if adhered to, can improve cognitive function, reduce insulin resistance, and is the most effective approach to lower blood pressure in OSA patients with refractory hypertension. However, and to the substantial surprise of the sleep research community, the use of CPAP was not associated with reduced risks of cardiovascular outcomes, diabetes mellitus, or death for patients with OSA in recent randomized controlled trials.[63–68] Therefore, it is essential to develop novel pharmacological agents to counteract the pathophysiological mechanisms responsible for OSA-related adverse consequences, namely oxidative stress, sympathetic activation, and low-grade inflammation.[63] Beneficial effects of CPAP therapy on the level of microparticles (MPs) were reported in a small trial that consisted of the withdrawal of CPAP therapy for 2 weeks. Discontinuation of CPAP and re-emergence of OSA were associated with a significant increase in endothelial MPs levels, providing evidence that MPs formation may be causally linked to OSA, and may be promoting endothelial activation and dysfunction in these patients.[69] The increased CVD risk of patients with OSA might be due to elevated platelet-derived MPs (PDMPs), as illustrated by decreases in plasma levels of PDMPs following treatment with CPAP.[70] Of note, PDMPs can be released *via* platelet activation by various agonists, including inflammatory cytokines, adenosine degradation products, or high shear stress.[70–75] Microvesicles (MVs) have been implicated in the pathogenic mechanisms of OSA; however, the data have been somewhat inconsistent, possibly due to circadian and temporal variation in the levels of circulating MVs.[29] Furthermore, it has been reported that, in OSA patients, circulating MPs can induce endothelial dysfunction by promoting reductions in endothelial-derived NO production.[71] It has also been reported that OSA is associated with upregulation of circulating sCD40L levels and increased propensity for platelet-monocyte to aggregate that may account for the increased incidence of cardiovascular events in this population.[76]

Noteworthy, circulating levels of extracellular vesicles (EVs) derived from blood vessel cells are increased in CVD, including acute myocardial infarction (AMI), leading to the assumption that such EVs may serve not only as prognostic or diagnostic biomarkers, but in addition due to their ability to carry and transfer biological information at the level of the organism they present the potential to serve as biological vectors.[77,78] As such, and as a corollary to the major objectives of the present review, a succinct description of EVs and their potential usefulness in OSA will be developed.

Circulating extracellular vesicles

EVs were initially described over 30 years ago when two independent groups observed that multivesicular bodies in reticulocytes released such vesicles into the extracellular space.[79,80] Since then, EVs have been purified from nearly all mammalian cell types. Importantly, the secretion of EVs is not restricted to mammalian cells, but has also been identified in lower eukaryotes and prokaryotes.[81–83] EVs have been classified based on their cellular origin and their biological function(s). There are three main classes of EVs as determined based on their biogenesis: exosomes (30–120 nm), microvesicles (100–1000 nm), and apoptotic bodies (1000–5000 nm). Exosomes are classified as a well specified subtype of EVs, and are distinct from other types of EVs as they contain a lipid bilayer on their envelope secreted by many cell types.[84,85] Exosomes carry a large diversity of cargos including messenger RNA (mRNA), micro-RNA (miRNAs), proteins, and lipids, and play key roles in intercellular communication.[86–88] Exosomes can be isolated from many types of body fluids and conditioned cell culture medium.[86,90] Due to the important role of exosomes in intercellular communication, exosomes have the potential to be used clinically in a variety of different ways, such as to be harnessed as pharmacological delivery agents, refined as noninvasive biomarkers for early diagnosis of disease states or disease-associated consequences, and as biologic reagents to treat diseases as well as to enhance tissue repair and regeneration.[90,91] Several methods that have been proposed as providing optimal approaches to EVs isolation, but each of them is fraught with limitations, particularly in the context of epidemiological studies, where thousands of samples need to be analyzed. Therefore, further improvements in EV purification, isolation, and content characterization are required to refine their applicability and minimize interassay variability.[92–95] In addition, current isolation technologies make it difficult to distinguish different EV subpopulations. Furthermore, contamination from protein aggregates, RNA–protein complexes, and other particles may affect the EV quantification and characterization results.[96]

Therefore, further research is crucial to develop simple technologies that carry a reasonable cost to isolate highly purified EVs for downstream application analysis (transcriptomics, proteomics, and lipidomics).[96] We and others believe that further improvements of EVs isolation and characterization methods, and in Omics technologies including transcriptomics, proteomics, and lipidomic analyses of EVs biological contents, will enable clinicians to adopt and refine the use of EVs and their cargos to diagnose and monitor CVDs.[97] The composition and the quantity of EVs would provide additional information on the severity of the disease.[85,98,99] Recently, we showed exosome isolation and characterization procedures as a general application pipeline that incorporates several methods for isolation, validation, and characterization.[100]

Physiological and pathological functions of extracellular vesicles

As mentioned, EVs act as important mediators of intercellular communication that influence both physiological and pathological conditions to change cell phenotype. Most of the studies regarding the possible physiological roles of EVs have been based on indirect *in vitro* evidence, especially in the context of immune system and cell-to-cell communication.[101] Furthermore, several physiological functions of exosomes have been identified *in vitro* when different types of mRNAs, miRNAs, or lncRNA change their abundance inside the vesicles.[102,103] Exosomes also exhibit proangiogenesis, procoagulant and pro- or anti-inflammatory effects as well as altering effects on vascular tone and vessel wall, most likely related to exosome capability of transporting and cell–cell transferring of proteins, mRNAs, and miRNA, among others.[104] All these features make EVs strong candidates as reporters and effectors of disease. In the context of CVD, EVs are involved in cell proliferation and differentiation, inflammation, stress response, angiogenesis, senescence, stem cell maintenance, tissue repair, and cardiovascular remodeling,[105–110] which are associated with many cardiovascular pathologies such as cardiac hypertrophy, heart failure, hypertension, atherogenesis, and diabetic cardiomyopathy.[111–116]

Extracellular vesicle uptake and function

EVs from donor cells can be taken up by recipient cells. The unique structure and outer envelope of EVs protects their cargo from enzymatic degradation during transit through the extracellular environment.[85,117] The most common method for detecting EV uptake involves the use of fluorescent lipid membrane dyes (lipophilic dyes), including PKH67, PKH26, rhodamine B, DiI, and DiD, to stain EV membranes.[50,118,119] EVs have been suggested to be internalized into target cells by various uptake mechanisms, including membrane fusion and different endocytic pathways including phagocytosis, receptor-mediated endocytosis, lipid raft–mediated endocytosis, caveolin-mediated endocytosis, clathrin-mediated endocytosis, and micropinocytosis.[85,100,120–122]

EVs derived from mononuclear blood cells (MBCs) have been involved in horizontal mRNA transfer and induce proangiogenic effects *in vitro* and *in vivo*.[123] In addition, EV-mediated crosstalk between endothelial cells (ECs) depends on miR-214, which was shown to activate angiogenic programming in target cells while EC senescence was suppressed.[107] Moreover, increased understanding of the role of EVs in vascularization has opened up the potential use of EVs in vascular therapeutics, with emerging concepts focused on the development of EVs for pro- or antiangiogenic therapies used for organ regeneration or cancer treatments, respectively.[124] In the context of OSA, we have shown that plasma exosomal miRNAs play an important role in endothelial dysfunction in both children and in adults.[119,125,126] The mechanism by which miRNAs are received and processed by target cells in a biologically active state is, as yet, undefined.

In physiological conditions, EVs may bind to the membrane proteins of the surface of target cells through receptor–ligand interactions, resulting in intracellular stimulation of signal transduction scaffolds and gene pathways. Upon EV binding and active mRNA and miRNA loading inside the recipient cell, gene expression through *de novo* translation and post-translational regulation of target mRNAs is effectively regulated.[127–130] The ability of EVs to alter the transcriptome and signaling activity within recipient cells allows them to induce highly specific and circumscribed phenotypic changes.[131] Intravenously injected exosomes disappear rapidly from blood circulation and accumulate in the liver, spleen, and lung.[132] Furthermore, following exosomes injection *via* different routes showed that intraperitoneal

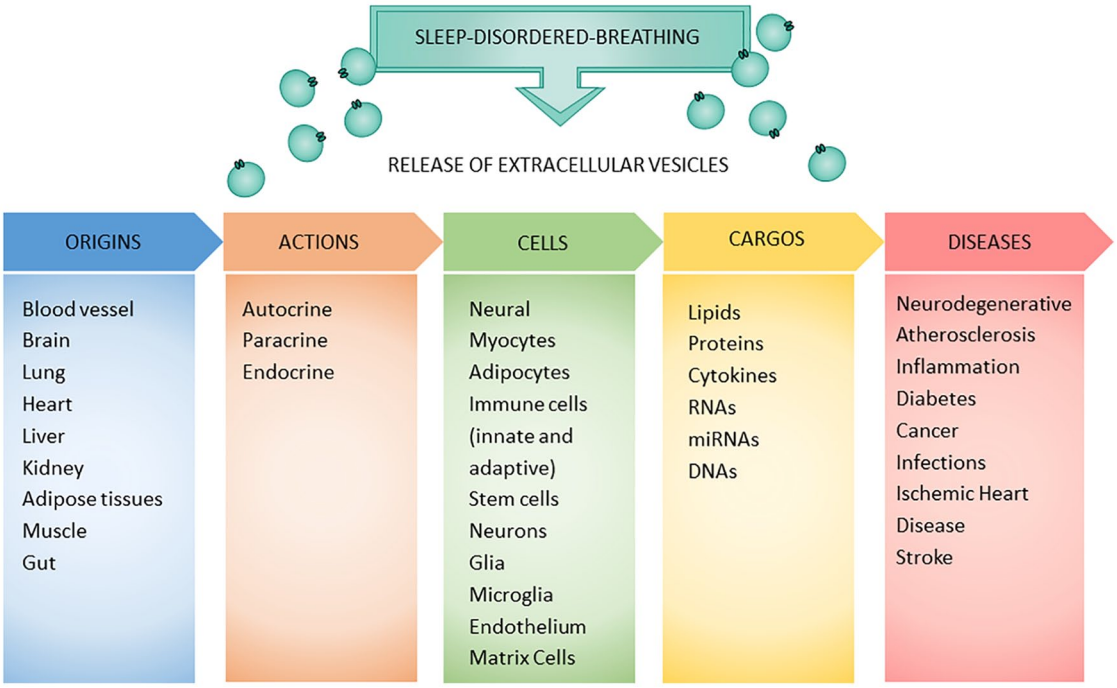

Figure 2. Schematic diagram illustrating release in sleep-disordered breathing of EVs that target many tissues through either autocrine, paracrine or endocrine processes to selectively target cells from different organs. EVs carry active component cargos, which, in turn, modulate or mediate many OSA-associated morbidities.
EVs, extracellular vesicles; OSA, obstructive sleep apnea.

injection resulted in higher accumulation in the pancreas and gastrointestinal tract compared with intravenous injection, whereas subcutaneous injection resulted in much lower accumulation of exosomes in all measured organs.[133] Figure 2 presents a schematic diagram illustrating the release of EVs in the context of the perturbations elicited by sleep-disordered-breathing that target many tissues through either autocrine, paracrine, or endocrine mechanisms and affect heterogeneous cell populations in tissues from different organs. Recently, we showed that exosomes derived from children or adult subjects were internalized by endothelial cells.[119,125,134] We also showed that exosomes from the plasma of mice exposed to either intermittent hypoxia or sleep fragmentation were internalized in mouse adipocytes *in vitro* and delivered their miRNA, protein, or lipid cargo.[50]

Dual effects of extracellular vesicles on cardiovascular diseases

A very large degree of variability in the effect of CPAP treatment on blood pressure (BP) levels has been observed, likely because of the multifactorial nature of systemic hypertension.[135] Actually, 25%–30% of patients who use CPAP treatment for >4 h/night do not experience a positive effect on BP.[136,137] Such disappointing outcomes could also reflect the fact that the criteria for adherence to therapy may not necessarily translate to optimal outcomes, since evidence supporting more extensive regular use of CPAP throughout the duration of sleep is more likely to results in better symptomatic improvements.[138,139] Furthermore, it is possible that some degree of irreversibility is present in OSA patients, particularly among those who have sustained the sleep-associated perturbations for very long periods of time before seeking treatment. Indeed, in recent studies, we and others have focused specifically on this issue. Short-term intermittent hypoxia during sleep that mimics OSA leads to structural alterations in the vasculature that appear to be reversible.[140] In contrast, long-term exposures to similar models of OSA are associated with either partial or minimal function and structural recovery.[141–143]

Over the last a few years, the role of EVs has changed from being only a marker of vascular

integrity toward being a functionally relevant effector in the context of intercellular vascular signaling.[144] The potential of exosomes as diagnostic biomarkers or therapeutic agents for CVD has attracted significant attention since the first study in isolating exosomes from cultures of cardiomyocytes grown *in vitro*.[145,146] Furthermore, EVs are released from most cell types in the circulation including erythrocytes, leukocytes, platelets, and endothelial cells, and carry a multitude of biological information to alter the pathophysiological processes of CVD.[77,147] Various studies showed that EVs act in an autocrine or paracrine fashion and mediate cardioprotection, while another study showed that stimulation with hypoxia resulted in the release of EVs that were enriched in miR-30.[148,149] EVs that were taken up by endothelial cells induced their proliferation and angiogenesis *in vitro*.[150] Patients with vascular diseases with evidence of systemic endothelial damage, such as atherosclerosis, show significantly increased levels of circulating EVs.[151] In addition, EVs from various cellular sources contribute to vascular inflammatory processes including endothelial activation, monocyte adhesion, and transmigration,[152,153] and certain subtypes of EVs can mediate vascular protection and endothelial regeneration.[77,154,155] On the one hand, EVs contribute to development and propagation of atherosclerosis by promoting endothelial dysfunction, while, on the other hand, evidence of the beneficial effects of certain EVs on vascular function and endothelial regeneration has also emerged.[154] Indeed, stem cell-derived EVs exert a protective effect against cardiac myocyte (CM) apoptosis during myocardial infarction and ischemia/reperfusion injury.[156,157] In addition, exosomes derived from heat shock protein 20 (HSP20)-overexpressing CM also protect against the hyperglycemia-induced CM death through increased levels of HSP20.[158] Furthermore, EVs secreted by stem cells play not only critical roles in repairing CM function and in restoring angiogenic potential of endothelial cells (ECs), but also mesenchymal stem cells (MSCs) exposed to hypoxia release EVs, which promote neo-angiogenesis and preserve cardiac performance after myocardial infarction.[159,160]

Recently, we showed that circulating exosomes derived from untreated patients with OSA induce significant increases in endothelial cell senescence markers with reciprocal decreases in sirtuin expression, which are only partially reversed upon long-term adherent CPAP treatment (average of 6 h CPAP use per night every night for 12 months).[161] Our findings suggest that circulating exosomes contribute to the senescence of endothelium in OSA, and are amenable to improvements, at least in part, after treatment of OSA with adherent CPAP.[161] In regards to OSA patients, plasma exosomes obtained before OSA treatment induce endothelial dysfunction in naïve endothelial cells. In obese or OSA children with evidence of endothelial dysfunction, but not among those with preserved endothelial function, plasma exosomes induce marked *in vitro* and *in vivo* functional and structural alterations in naïve endothelium that are mediated by selective components of the exosomal miRNA cargo.[126] Plasma-derived exosomes in otherwise healthy subjects exposed to 4 days of intermittent hypoxia mimicking OSA are constitutively altered in their miRNA cargo, and exhibit the ability to induce endothelial dysfunction *in vitro*. We further demonstrated that such properties are reversed upon normoxic recovery.[125] In patients suffering from the obstructive hypoventilation syndrome (OHS) (the most severe form of sleep-disordered-breathing) as well as in mice exposed to intermittent hypoxia or sleep fragmentation as seen in moderate to severe OSA, plasma exosomes lead to reduced insulin sensitivity in naïve adipocytes *in vitro*, and such effects were attenuated by CPAP treatment, particularly after long-term adherent therapy. In contrast, the beneficial effects of CPAP on exosome-induced insulin resistance were undetectable among OSA patients who opted not to receive any treatment.[50,162] Figure 3 shows how during untreated OSA, EVs can be released from different cell types and effect endothelial dysfunction. At this point, we have not tested if EVs can induce endothelial protection either *in vitro* or *in vivo* studies if they are released from specific progenitor cells or are generated under specific circumstances afforded by age, gender, genetic variance, severity of hypoxia, etc.[163–166]

Potential roles of extracellular vesicles as therapeutic applications and biomarkers

EVs convey biological cargos derived from parent cells to the destination cell targets and their functions are intrinsically dependent on the functional status of the original cells.[85] The bilayer lipid membrane of EVs acts as an efficient protection barrier for their inner molecules, thus contributing to their stable measurements in body fluids.[85,100,167] EVs

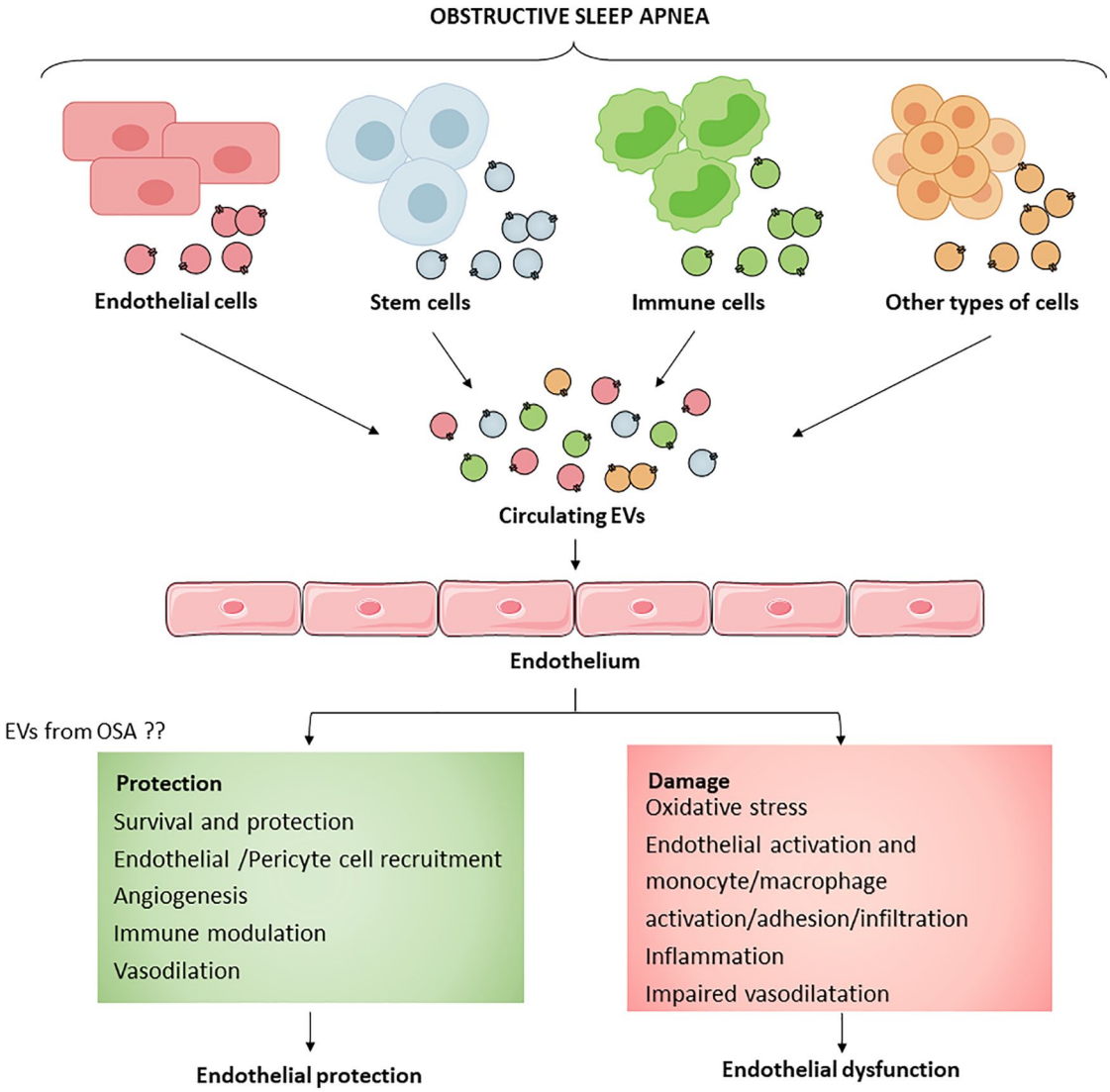

Figure 3. Schema illustrating the potential for dual effects of EVs whose cargoes have been modified in patients suffering from OSA. EVs can be released from several cell types, including endothelial cells, stem cells, immune cells and other cell types, which can then promote damage to cardiovascular systems, resulting in the induction or acceleration of cardiovascular diseases. Alternatively, the effects of EVs derived from OSA patients can confer protection of cardiovascular targets. Studies are needed to delineate more specifically the major operators of these two EVs-related effects.
EVs, extracellular vesicles; OSA, obstructive sleep apnea.

can readily become novel minimally invasive (using blood samples) or noninvasive diagnostic markers (based on analysis of urine and saliva) to overcome the current setbacks of traditional needle or excision biopsies.[168] In addition, EVs provide several unique advantages over the use of cells for therapeutic applications, which include the absence of conservation of activity between species, lower immunogenic potential, and theoretically improved tissue- or cell-targeting potential.[169,170] The use of EVs for therapy of human disease is becoming a central focus of nanomedicine for their ability to deliver biologically active material to target cells.[90,171] Several investigators demonstrated that overexpression of miRNAs enhanced the therapeutic effects of exosomes, and also showed that overexpression of proteins that can change the expression profile of targeted miRNAs and proteins may improve the therapeutic profile of exosomes.[172,173] Furthermore, EVs from MSCs have been used to stimulate tissue

repair following myocardial infarction, and EVs derived from tumor antigen-pulsed dendritic cells (DCs) have been exploited for cancer immunotherapy, suggesting that these vesicles serve as potential drug delivery vehicles.[174,175] EVs can be engineered to have certain tissue- or cell-type-specific targeting ligands present on their surface by expressing plasmid fusion constructs comprising targeting ligands fused to extracellular vesicle transmembrane proteins. Loading cargoes into EVs can be divided into two basic approaches: exogenous loading (with incorporation of small molecules/proteins/RNA into or onto isolated EVs) and endogenous loading (providing cells with the means to incorporate small molecules/proteins/RNAs into EVs during their biogenesis).[88] The ability of EVs to shuttle proteins and nucleic acids from one cell to another may be applied for therapeutic purposes, particularly as certain EVs preferentially bind to specific cells and could thus deliver a drug, a ligand mediating a receptor-induced signal, an altered protein, or an RNA, in order to affect the desired cellular processes.[176]

Several studies have reported increased sensitivity for EVs-based biomarkers compared with whole serum or other body fluid biomarkers.[176,178] Furthermore, repeated evidence has emerged of EVs enrichment with specific molecular components (RNAs, proteins, and lipids) that reflect the status of the parental cell or tissue source,[177] and, as indicated, EVs might represent not only robust vehicles of disease-specific biomarkers, but may also be engineered as uniquely effective therapeutic targets. However, efforts in that direction have not yet been specifically addressed to date.

Conclusion

OSA is a major public health concern attributable mainly to its significant link with cardiovascular morbidity and mortality. The benefit of CPAP therapy on cardiovascular outcomes remains uncertain, and it is unclear whether such benefits can be potentiated *via* precision-based selection of the candidate patients most likely to benefit from such intervention rather than apply a one therapy fits all approach. EVs are important players of exchanges between cells, through the transmission of various proteins, bioactive lipids and genetic information to alter the phenotype and function of recipient cells. Thus, EVs have not only been implicated in numerous biological and pathological processes but are emerging as robust candidates for integration with sleep disorders such as OSA in the quest to develop improved predictive biomarkers and innovative targeted therapies, ultimately enabling attenuation or reversal of OSA effects on the cardiovascular system and other end-organ systems affected by this condition.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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Supplemental material

The reviews of this paper are available via the supplemental material section.

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