CHEST

# Sleep Apnea Morbidity
## A Consequence of Microbial-Immune Cross-Talk?



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OSA has emerged as a highly prevalent public health problem that imposes important mid- and long-term consequences, namely cardiovascular, metabolic, cognitive, and cancer-related alterations. OSA is characterized by increased upper airway resistance, alveolar hypoventilation, and recurrent upper airway obstruction during sleep. Recurrent collapse of the upper airway develops with sleep onset and is associated with both intermittent hypoxemia and sleep fragmentation. The microbiome is a vast and complex polymicrobial ecosystem that coexists with the human organism, and it has been identified as playing significant roles in the development of host immunologic phenotypes. In humans and animal models, changes in gut microbial communities occur with lifestyle behaviors, such as smoking, long-distance travel, dietary preferences, physical exercise, and circadian rhythm disturbances. In parallel, diseases previously attributed in part to lifestyle such as obesity, coronary heart disease, depression, and asthma (also associated with OSA) are now claimed as microbiota related. We therefore posit that altered patterns of sleep and oxygenation, as seen in OSA, will promote specific alterations in gut microbiota that in turn will elicit the immunologic alterations that lead to OSA-induced end-organ morbidities. The present article assesses the potential mechanistic links between OSA-induced changes in gut microbiota and its morbid phenotypes.

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OSA imposes major neurocognitive, cardiovascular, and metabolic sequelae.[1,2] However, not every patient with OSA will develop end-organ morbidity, which suggests that both environmental and genetic factors are critically involved in the phenotypic variability that is inherently apparent in the disease.[3,4] However, only a relatively very small proportion of the variance in OSA morbid phenotype is accounted for, even when the genetic and environmental factors are included in the models. As such, it is possible that other elements that were not previously considered may play a major role. Among such elements, the microbiome emerges as a very likely contender, and the present article examines the emerging evidence linking

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**ABBREVIATIONS:** IH = intermittent hypoxia; LPS = lipopolysaccharide; SF = sleep fragmentation

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OSA, the microbiome, and morbidity. In this setting, the major hypothesis being formulated is that the hallmark perturbations of OSA, namely intermittent hypoxia (IH) and sleep fragmentation (SF), will affect the structure and functional properties of the gut microbial community and the latter changes in turn will mediate the altered host phenotype, manifesting as end-organ morbidity.

## The Microbiome and Human Disease

The microbiome is a vast and complex polymicrobial community that coexists with the human organism and is inordinately plastic to a variety of intrinsic or extrinsic changes while playing significant roles in the development of host metabolism-related and immunologic phenotypes (pertinent reviews are given elsewhere[5,6]). Alterations in gut microbial communities are linked with adiposity in humans and animal models, potentially via greater energy harvest and regulation of host energy metabolism at a supra-organismal level.[7] Gut microbiota mediate the association between excessive energy intake, sedentary lifestyle, and obesity, and are critically involved in the development of the "metabolic phenotype."[7,8] Multiple lifestyle-related activities such as smoking, long-distance travel, dietary preferences, and physical exercise have been shown to affect gut microbiota.[9,10] Furthermore, diseases previously attributed to lifestyle such as obesity, coronary heart disease, depression, and cognitive dysfunction are all now claimed as microbiota related.[11–13] Therefore, because OSA has been associated with compelling epidemiologic links to many, if not all, of these gut microbiota functional or morbid abnormalities (especially the cardiovascular and metabolic comorbidities), it is legitimate to expect that either OSA, or at least one of its two major constitutive elements (namely IH or disrupted patterns of sleep), will also be linked to such phenotypes via induced changes in gut microbial communities.

## Circadian Rhythm Alterations and SF

Initial studies indicated that the gut microbiota change as a function of circadian drivers and melatonin levels.[14] In turn, such changes in gut microbiota provide important signals to the circadian pacemakers that may modify circadian rhythm in what appears to be a closely interdependent relationship.[15,16] Furthermore, experiments leading to circadian clock misalignment are fraught with changes in gut microbiota that also translate into well-established metabolic derangements.[17,18] However, no overt changes in the richness or composition of the human or rat gut microbiome emerged as induced by sleep restriction. Taken together, the results of these studies suggest that circadian disruption alters the gut microbiome but that the gut microbiome is resistant to changes when sleep restriction is imposed.[19]

As mentioned earlier, SF is an important contributor to several of the major morbid phenotypes of OSA even when sleep duration is preserved.[20,21] Indeed, chronic SF in the absence of curtailed sleep duration promotes the appearance of inflammatory and oxidative stress changes in the brain, increased sleepiness, and cognitive deficits, all of which are extremely frequent clinical manifestations of OSA.[22] In addition, sustained periods of SF during the sleep period will lead to altered appetite and satiety along with increased orexigenic behaviors, ultimately promoting the emergence of increased adiposity via activation of oxidative stress and inflammatory pathways, which in turn lead to insulin resistance even before changes in body weight develop.[23-25] Notably, the metabolic changes seem to be mediated by changes in gut free fatty acid receptors,[26] suggesting that the gut microbiome may indeed be a contributor to these perturbations.

Analogously, prolonged SF is also implicated in vascular changes similar to the enhanced atherosclerosis and cardiovascular morbidities associated with OSA.[27,28] In children experiencing OSA, elevated systemic levels of lipopolysaccharide (LPS)-binding protein, a surrogate marker of underlying low-grade LPS endotoxemia from the gut,[29] were independently associated with BMI and with measures of OSA severity, as well as with established measures of insulin resistance. In addition, C57BL/6J mice subjected to SF followed in a subset by 2 weeks of recovery, along with a sleep control group,[30] uncovered that the structure of gut microbiota was significantly altered after 2 weeks of SF treatment, and such effects persisted for as long as the sleep perturbation was implemented. Bacteria from phylum *Firmicutes* increased their abundance after SF treatment by 20%, whereas bacteria from phyla *Bacteroidetes* and *Actinobacteria* lost 20% and 50% of abundance, respectively. SF exposures were further associated with growth of bacteria from families *Lachnospiraceae* and *Ruminococcaceae*, as well as several lineages from class *Clostridia* and order *Clostridiales*. On the contrary, bacteria from families *Lactobacillaceae* and *Bifidobacteriaceae* were suppressed, approximately twofold by SF. Metabolites were also extracted from

feces and analyzed by Gas Chromatography/Mass Spectrometry (GC/MS) identified 14 metabolites, including sucrose, elevated (more than twofold, $P < .01$) in fecal samples of SF mice. We also detected a decreased level of adenosine in fecal samples of SF mice (more than twofold, $P < .01$). Thus, both the gut microbial and metabolomics composition are altered by SF. Furthermore, SF and control-derived "fecal water" were applied to the confluent monolayers of human normal colon cells and Caco-2 cells; this fecal water includes a water-soluble fraction of gut metabolome along with extracellular fraction of gut proteome and therefore represents a combination of functionally active molecules affecting the gut surface. Rapid decreases in resistance induced by contact with fecal water samples from SF but not in the sleep control mice emerged; this finding indicates increased permeability, the latter being further reflected by increased plasma levels of LPS. Finally, we performed transfer of microbiota experiments in germ-free mice receiving fecal pellets from SF-exposed animals. These mice recapitulated several of the SF behaviors, namely increased food intake, and also developed significant decreases in adipose tissue insulin sensitivity along with increased presence of leukocytes, compared with transfaunation of control mouse fecal samples. Taken together, these initial experiments support the concept that the metabolic alterations associated with SF may result from concurrent changes in gut microbiota.

## IH and the Gut Microbiome

The gut capillaries are considerably perfused and exhibit high permeability because of their physiological transport functions across their wall. As a consequence, oxygen (which is at a high concentration in arterial blood) diffuses into the extracapillary compartment, establishing a gradient of oxygen concentration that decreases from the gut capillaries through the intestinal lumen. Accordingly, the gut bacteria will experience varying levels of oxygenation depending on their distance to the gut capillaries. However, there are scarce, yet convincing data showing that the level of oxygenation modifies the composition of gut microbiota and its interactions with the host through alterations in intestinal epithelial permeability; this scenario leads to increased transport of molecules secreted by the microbiota into the blood and hence into the host.

Maity et al[31] subjected rats to simulated hyperbaric air (122 kPa and 170 kPa) for periods of 10, 20, and 30 days for 5 h per day. The investigators found that total aerobes and facultative anaerobes increased compared with normoxic control subjects. Adak et al[32] investigated the fecal microbiota in 15 soldiers at base sea level and after a 15-day acclimatization to 3,500 m while keeping the same diet. At the high altitude, total aerobes decreased significantly, and total and facultative anaerobes increased. More recently, Albenberg et al[33] observed that oxygen diffused from the intestinal tissue and established a radial oxygen gradient from the mouse gut tissue interface into the lumen, also showing that oxygenation affects gut microbiota.

The events of recurrent arterial hypoxemia caused by upper airway obstruction/collapse are locally sensed by all perfused organs in the body.[34] Interestingly, these events per se could negatively impair the gut function because hypoxia/re-oxygenation directly impairs cellular function via increases in permeability and bacterial translocation and decreases in tight junction integrity.

As a result of the dynamic changes in arterial blood oxygenation in sleep breathing disorders (particularly sleep apnea), it is expected that the intermittent hypoxemia in the blood entering the gut capillaries is transmitted to the intestinal lumen by a process of simple gas diffusion. In fact, Moreno-Indias et al[35] observed oscillations in the $P_{O_2}$ within a range of approximately 0 to 200 μm from the mouse intestinal epithelial surface in animals subjected to IH mimicking sleep apnea. Accordingly, relevant portions of the microbiota (closer to the epithelium and hence the fraction with more direct interaction with the host) are indeed subjected to oscillatory events of IH, thereby inducing significant changes in the gut microbiome. These changes include increases in *Prevotella, Paraprevotella, Desulfovibrio,* and *Lachnospiraceae* and decreases in *Bacteroides, Odoribacter, Turicibacter, Peptococcaceae,* and *Erysipelotrichaceae* compared with normoxic control subjects. The relative enrichment found in obligate anaerobes suggests that reductions in oxygen content inside the gut would allow them to be more competitive and enable them to overgrow. These authors also found that animals under IH exhibited increased bacterial diversity and clustered separately compared with normoxia, potentially facilitating protection of the gut against hypoxia in the intestinal lumen. Augmented concentrations of endogenous LPS, a death product of gram-negative gut bacteria that translocates into blood capillaries through a TLR4-dependent mechanism,[36] detected in the systemic circulation were also found in this sleep apnea model.[37]

With a clinical perspective focused on the treatment of OSA, an interesting question is to what extent the changes induced by IH in the gut microbiome and in circulating LPS are reversible by eliminating apneas with an effective treatment (eg, weight loss to normalize BMI, CPAP, mandibular advancement devices). Mouse model data[37] showed that a relatively long period of 6 weeks' normoxic recovery following 6 weeks of IH exposure did not completely normalize the proportion of obligate anaerobic gram-negative bacteria; rather than replacing community members, normoxia shuffled the abundances at the phylum, family, and genus levels. Interestingly, plasma LPS levels in the mice pre-exposed to IH were considerably higher than in control mice. Therefore, alterations in the composition of gut microbiota and associated endotoxemia induced by IH mimicking sleep apnea could be irreversible, partially reversible, or requiring an extended period of normoxic recovery for normalization.

## Sleep Apnea, Microbiome, and Cardiovascular Morbidity

The association between OSA and cardiovascular disease is well established.[38] In addition to the direct consequences that OSA imposes on the cardiovascular system, current evidence suggests that gut microbiota can also modulate cardiovascular risk.

Gut microbiota is emerging as a new risk factor for cardiovascular risk.[12] A recent meta-analysis that enrolled 19,256 participants and 3,315 incident cases from the 19 data points showed that elevated concentrations of gut microbial metabolites (trimethylamine *N*-oxide and its precursors) were associated with increased risks of major adverse cardiovascular events and with all-cause mortality, independently of traditional risk factors in a variety of cardiovascular diseases.[39] Moreover, significant dysbiosis of the gut microbiota has been found in patients with ischemic stroke compared with control subjects.[40] Finally, in an animal model, both the administration of antibiotics and probiotics decreased the severity of an induced myocardial infarction in vivo.[41]

The severity of chronic heart failure has been associated with increased intestinal permeability. Edematous patients had the highest blood concentrations of endotoxins and LPS, and endotoxins decreased after re-compensation, suggesting a cause-and-effect relationship with the edematous gut wall, epithelial dysfunction, and translocation of LPS across the gut epithelial barrier. Trimethylamine *N*-oxide levels were higher in patients with heart failure compared with control subjects and were independently associated with higher mortality in chronic[42] and acute heart failure. Studies in patients with chronic heart failure have also shown that these patients have significant gut dysbiosis,[43] and treatment with antibiotics is associated with a reduction in biochemical and inflammatory biomarkers.[44]

Several clinical studies suggest that OSA alters the gut epithelial barrier, which can lead to inflammation and metabolic dysfunction.[45,46] However, evidence from clinical studies linking OSA, major cardiovascular events, and the microbiome is scarce.[47] In an animal model of OSA, Xue et al[48] examined the potential role of OSA-induced gut microbiota alterations in the development of atherosclerosis. These authors administered 3,3-dimethyl-1-butanol, an inhibitor of microbial trimethylamine production, to a group of mice, and they found that this intervention attenuated the IH and intermittent hypercapnia-induced pulmonary artery atherosclerosis, suggesting the potential involvement of gut microbiota on the OSA-induced atherosclerosis. Unfortunately, there are no clinical studies assessing the interplay between OSA, cardiovascular diseases, and microbiota.

## Sleep Apnea, the Microbiome, and Hypertension

In the last few years, mounting evidence suggests a strong relationship between gut microbiota and hypertension. Indeed, studies conducted in animal models have shown a different composition of the gut microbiome in hypertensive rats, with a decrease in microbial richness and diversity and an increase in the *Firmicutes/Bacteroidetes* ratio.[49] Even more interesting, after animals were administered antibiotics to reduce native microbiota, those animals who were gavaged with pellets from hypertensive animals developed hypertension. Notably, these results have also been replicated in human studies. Patients with hypertension and pre-hypertension exhibited a less rich and diverse gut microbiome compared with control subjects.[50] Furthermore, elevated blood pressure was observed following fecal transplantation from hypertensive human donors to germ-free mice.[51] Finally, a meta-analysis showed that administration of probiotics significantly reduced blood pressure levels, with a more significant improvement in patients with hypertension.[52]

There is little evidence regarding the role of the gut microbiome in OSA-induced hypertension. Durgan

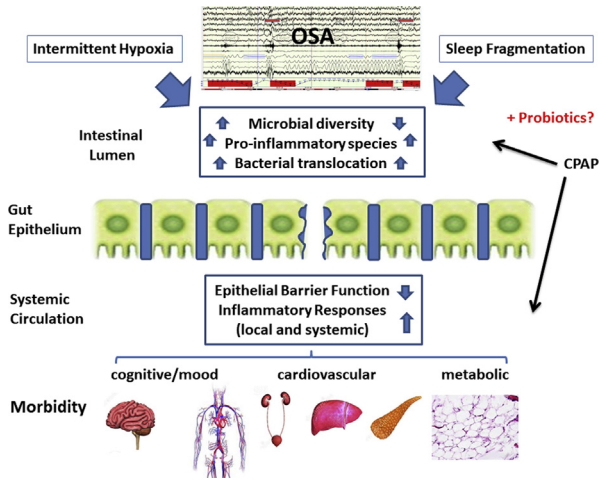


Figure 1 – *Putative effects of OSA, which is characterized by intermittent hypoxia and sleep fragmentation on gut microbiota, and the downstream effects of such changes on the emergence of systemic inflammatory processes that promote cognitive, cardiovascular, and metabolic morbidities. Institution of CPAP, even if adherent, may not sufficiently reverse the chronic impact of OSA on the microbiome, and to achieve the desired downstream end-organ outcomes, addition of targeted probiotic interventions may be needed.*

et al[53] found that, in an animal model, IH had no effect on blood pressure in rats fed normal chow diet, whereas blood pressure increased in rats fed a high-fat diet after 7 and 14 days of IH. High-fat diet and IH led to significant alterations of the gut microbiota, and transplantation of dysbiosis cecal contents from these animals into normotensive IH-exposed recipient rats on normal chow diet resulted in systemic hypertension, with blood pressure levels reaching values similar to those of the donors. Taken together, these studies indicate the likely presence of a mechanistic contribution to the relationship between gut microbiota alterations and hypertension, and further suggest that targeted modifications of the gut microbiome might become a novel form of therapy for patients with hypertension and with OSA.

## Conclusions

The evolving evidence, albeit very preliminary, indicates that OSA and its corollary hallmark manifestations (IH and SF) promote alterations in gut microbiota that modify gut permeability, change intestinal content of several important microbial-derived biologically active metabolites, and promote translocation of bacterial toxins to the systemic circulation. These perturbations, in turn, will elicit low-grade chronic immunologic alterations that ultimately initiate and propagate the emergence of OSA-induced end-organ morbidities in susceptible individuals (Fig 1). Accordingly, exploration of microbiota-modifying strategies as targeted therapeutic interventions aimed at reducing or abrogating OSA-associated deleterious consequences and adverse outcomes, albeit hypothetical at this stage, seem justified and worthwhile.

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