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Circadian Dysregulation: The Next Frontier in Obstructive Sleep Apnea Research

Douglas C. von Allmen, MD[1], Lauren J. Francey, MS[2], Garrett M. Rogers, MS[3], Marc D. Ruben, PhD[2], Aliza P. Cohen, MA[4], Gang Wu, PhD[2], Robert E. Schmidt[2], Stacey L. Ishman, MD, MPH[1,4,5], Raouf S. Amin, MD[5,6], John B. Hogenesch, PhD[2,6], and David F. Smith, MD, PhD[1,4,5]

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Abstract

Objective. To review the effects of the circadian clock on homeostasis, the functional interaction between the circadian clock and hypoxia-inducible factors, and the role of circadian dysregulation in the progression of cardiopulmonary disease in obstructive sleep apnea (OSA).

Data Sources. The MEDLINE database was accessed through PubMed.

Review Methods. A general review is presented on molecular pathways disrupted in OSA, circadian rhythms and the role of the circadian clock, hypoxia signaling, crosstalk between the circadian and hypoxia systems, the role of the circadian clock in cardiovascular disease, and implications for practice. Studies included in this State of the Art Review demonstrate the potential contribution of the circadian clock and hypoxia in animal models or human disease.

Conclusions. Molecular crosstalk between the circadian clock and hypoxia-inducible factors has not been evaluated in disease models of OSA.

Implications for Practice. Pediatric OSA is highly prevalent and, if left untreated, may lead to cardiopulmonary sequelae. Changes in inflammatory markers that normally demonstrate circadian rhythmicity are also seen among patients with OSA. Hypoxia-inducible transcription factors interact with core circadian clock transcription factors; however, the interplay between these pathways has not been elucidated in the cardiopulmonary system. This gap in knowledge hinders our ability to identify potential biomarkers of OSA and develop alternative therapeutic strategies. A deeper understanding of the mechanisms by which OSA impinges on clock function and the impact of clock dysregulation on the cardiopulmonary system may lead to future advancements for the care of patients with OSA. The aim of this review is to shed light on this important clinical topic.

Keywords

obstructive sleep apnea, sleep apnea, hypoxia, circadian clock, circadian biology, circadian medicine.

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Pediatric obstructive sleep apnea (OSA) is highly prevalent. Upper airway collapse associated with OSA results in fragmented sleep and intermittent hypoxic episodes, with or without increases in carbon dioxide levels; these episodes lead to an increase in sympathetic nervous system activity.[1] Even among young children, OSA was linked to altered blood pressure regulation,[2] systemic hypertension,[3,4] and hypertrophy of the left ventricle.[5,6] If these complications are not addressed, they worsen over time and can become life threatening. Early pathophysiologic changes may lead to a lifetime burden of cardiovascular, pulmonary, and metabolic disease. Also important, the long-term significance of milder forms of OSA on the heart and lung are

[1]Department of Otolaryngology–Head and Neck Surgery, College of Medicine, University of Cincinnati, Cincinnati, Ohio, USA
[2]Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA
[3]College of Medicine, University of Cincinnati, Cincinnati, Ohio, USA
[4]Division of Pediatric Otolaryngology–Head and Neck Surgery, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA
[5]Division of Pulmonary and Sleep Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA
[6]Department of Pediatrics, College of Medicine, University of Cincinnati, Cincinnati, Ohio, USA

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Corresponding Author:
David F. Smith, MD, PhD, Divisions of Pediatric Otolaryngology and Pulmonary and Sleep Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, 3333 Burnet Ave, OH 45229, USA.
Email: David.Smith3@cchmc.org

not known. These gaps in knowledge hinder our ability to identify potential management strategies aimed at the prevention and treatment of lifetime disease burden.

Several mechanisms are thought to contribute to the cardiovascular and pulmonary sequelae of OSA in children and adults. OSA involves dysregulation of a variety of physiologic processes, including upregulation of a systemic inflammatory response,[7] heightened levels of oxidative stress,[8] and endothelial dysfunction.[9,10] Dysregulation of the circadian clock may also play an important role. This transcriptional/translational regulatory system coordinates the 24-hour timing of physiologic rhythms through a group of endogenous oscillators that regulate tissue-specific outputs in gene expression, protein synthesis and degradation, and metabolism.[11] Multiple processes, including body temperature, blood pressure, and hormone secretion, exhibit daily rhythms under the control of this internal timing mechanism.[12]

Clock dysfunction can promote or even cause disease. For example, in mouse models, eating exclusively during the inactive (sleeping) phase promotes obesity and fatty liver disease.[13] Night-eating disorder among humans is likewise associated with metabolic syndrome.[14,15] Chronic jet lag in mice can promote nonalcoholic fatty liver disease, which can progress to fibrosis and hepatocellular carcinoma.[16] Furthermore, genetic ablation of clock genes in mice exacerbates this progression. Shift work disorder among humans has been labeled a class 2 carcinogen by the International Agency for Research on Cancer.[17] Recent work also showed that abrogated clock function promotes neurodegeneration, and circadian disruption was observed preclinically among patients with Alzheimer's disease.[18,19] Despite this literature, the role of clock disruption in lung or cardiopulmonary disorders in general has received relatively little attention.

The presence of disease can alter the timing, strength, and impact of these rhythms, affecting the body's ability to maintain homeostasis. Although the role of the circadian clock has been described in many other diseases, otolaryngology research has not yet entered this new frontier. Research within the field of sleep medicine demonstrated that circadian oscillation of inflammatory biomarkers are altered in adults with OSA.[20] Compared with healthy children, children with OSA exhibit changes in the diurnal rhythm of inflammatory biomarkers.[21] These findings imply that the circadian clock may play an important role in OSA.

This State of the Art Review describes key aspects of the circadian clock and the overlap between this complex pathway and hypoxia-inducible transcription factors. In addition, it offers a rationale for the role of the circadian clock in the cardiopulmonary sequelae seen among patients with OSA. A more in-depth understanding of this complex interplay will ultimately lead to the development of better diagnostic and therapeutic techniques.

Methods

We present a State of the Art Review that encompasses a broad range of topics related to OSA and circadian dysregulation. These topics include molecular pathways disrupted in OSA, circadian rhythms and the role of the circadian clock, hypoxia signaling, and crosstalk between the circadian and hypoxia systems. All articles cited in our review were identified via an extensive search for all terms relating to each topic; however, only articles published in English were included. We further restricted our review to relevant articles published since 2010, with the exception of earlier articles that are considered seminal work in the fields of circadian biology and models of intermittent hypoxia, as they provide pertinent and essential background knowledge.

To identify articles related to circadian dysregulation of the cardiopulmonary system in animal models and human studies of OSA, we used the PubMed database with the following search terms: (*obstructive sleep apnea* OR *OSA* OR *sleep apnea* OR *hypoxia* OR *daytime variation*) AND (*circadian clock* OR *circadian rhythm* OR *clock*). With these broad search terms, we identified 2359 potentially relevant articles. These abstracts were reviewed by the senior author (D.F.S.) and the first author (D.C.V.A.). No articles could be identified that specifically discussed our topic. In view of the paucity of studies pertaining to this broad subject, articles were included in this review that discussed the relationship between dysregulation of the molecular components of the circadian clock and hypoxia in human disease or animal disease models. After initial evaluation, 23 abstracts were selected for formal review. Five relevant articles pertaining to the aforementioned content are discussed in the sections titled ''Crosstalk between the Circadian and Hypoxia Systems,'' ''The Role of the Circadian Clock in Cardiovascular Disease,'' and ''Implications for Practice: The Importance of the Circadian Clock in Otolaryngology.'' Although several articles are presented that discuss the role of the circadian clock in hypoxia models, to date no studies have examined the molecular contribution of circadian dysregulation in models of OSA. Institutional review board approval was not required for this study.

Discussion

Molecular Pathways Disrupted in OSA

Many studies investigated molecular pathways that contribute to cardiopulmonary disease among children with OSA, thus laying the foundation for current and future research. Nevertheless, an in-depth understanding of all mechanisms involved in OSA remains elusive. To date, multiple studies sought to demonstrate the molecular consequences of untreated OSA. Previous results suggest that progression of disease is directly or indirectly mediated by disruption of neural control (ie, changes to the sympathetic nervous system),[22-24] increased oxidative stress,[25-28] or endothelial dysfunction.[10,29-31] Upregulation of a systemic inflammatory response was strongly implicated in OSA disease progression,[32-36] and evaluation of inflammatory biomarkers provided insight into the potential role of the circadian clock in the disease process.

One of the hallmarks of OSA is an elevation of systemic inflammatory markers (cytokines and acute-phase reactants).

Research showed that changes exist in an array of inflammatory markers, including interferon gamma and interleukin 8, among patients with OSA.[33] We recently demonstrated that children with OSA exhibit changes in the normal diurnal variation (circadian rhythm) of biomarkers seen in healthy children and that groups of cytokines appear to be positively or negatively associated with vascular stiffness, which is an indication of cardiovascular dysfunction.[21] Although the downstream signaling pathways activated by intermittent hypoxia are thought to be involved in the progression of OSA, the impact of the circadian clock in OSA has not been studied.

Circadian Rhythm and Role of the Circadian Clock

The circadian clock is a network that affects multiple systems throughout the body. As a means of adapting to daily environmental cues, animals—from flies to humans—have evolved internal clocks that follow the 24-hour light-dark cycle.[37] This autoregulatory feedback loop is tightly controlled to prepare the body for various activities (ie, exercise, feeding and digestion, fasting, and sleeping) that regularly occur at specific times of the day. Circadian rhythms, including the sleep-wake cycle in mammals, are organized hierarchically.[38,39] Although the central oscillator of the circadian clock, the suprachiasmatic nucleus, lies in the hypothalamus,[40] autonomous oscillators exist in peripheral tissues throughout the body.[41-43]

At its heart, the circadian clock is a cell-autonomous transcriptional/translational feedback loop. The transcriptional activators brain and muscle arnt-like 1 (BMAL1) and circadian locomotor output cycles kaput (CLOCK) and the transcriptional repressors period 1 (PER1), PER2, PER3, cryptochrome 1 (CRY1), and CRY2 constitute the core ''E-box'' molecular clock.[11] The BMAL1/CLOCK activators activate the PER/CRY repressors. The repressors are translated in the cytoplasm, where they complex with each other and ancillary factors, and the entire complex then translocates to the nucleus. Eventually, the repressors interact with the BMAL1/CLOCK complex and repress their transcription. They destabilize, fall off BMAL1/CLOCK, and the cycle of BMAL1/CLOCK activation starts anew. The whole cycle takes ~24 hours. These endogenous molecular timers orchestrate transcriptional networks for thousands of clock output genes in all cells of the body throughout the light-dark cycle.[11,38]

Hypoxia Signaling

Mechanisms that allow cells to respond to low-oxygen conditions are critical for metabolic homeostasis.[44] The hypoxia-inducible factors (HIF1A, HIF1B, HIF3B, HIF2/ARNT) are critical regulators in this process.[45,46] Under normoxic conditions, HIF1A is constantly transcribed, translated, and quickly targeted for degradation by prolyl hydroxylases via the proteosome. Under hypoxic conditions, HIF1A, HIF2A, and HIF3A are stabilized and translocate to the nucleus, where they interact with their partner, HIF1B (aka ARNT), to activate gene expression. These programs can operate at the cellular level (eg, hypoxia triggering an increase in glycolysis), at the organ level (eg, skeletal muscle after vigorous exercise), or at the level of the whole organism (eg, upregulation of erythropoietin and production of red blood cells at altitude). Changes in the activity of these transcription factors were implicated in a number of diseases, including OSA.[47-53]

Crosstalk between the Circadian and Hypoxia Systems

Hypoxia-inducible factors are members of the same bHLH-PAS transcription factor family as BMAL1 and CLOCK. Hogenesch et al[54] demonstrated that BMAL1 could directly bind HIF1A and HIF2A. Additionally, this clock/hypoxia complex was shown to interact with DNA and drive transcription, demonstrating that these heterodimers could respond to cellular hypoxia.[54] Later, Saito et al demonstrated that HIF2A could interact with BMAL1 to activate gene expression in chondrocyte development,[55] proving that these interactions occurred in vivo. More recent work by Adamovich et al[56] demonstrated a daily rhythm of oxygen levels in mouse tissue. Furthermore, these rhythmic cycles of oxygen levels can synchronize cellular clocks in a HIF1A-dependent manner. Even more significant, short exposures of low oxygen levels can reset the internal clock.[56] Additionally, research conducted by Wu et al[57] demonstrated that BMAL1 interacts with HIF1A, exhibiting crosstalk at the genome level. PER2 recruits HIFs to DNA enhancer sequences,[44] thus affecting transcription (**Figure 1**). Based on these collective findings, this type of interaction could play a critical role in diseases such as OSA that develop as a result of changes in hypoxia-inducible transcription factors.

The Role of the Circadian Clock in Cardiovascular Disease

Myocardial infarction, occurring as a result of acute episodes of cardiac ischemia, leads to changes in hypoxia-inducible transcription factors in cardiovascular tissue. Ventricular arrhythmias and sudden death are more likely to occur at certain times of the day among patients with ischemic heart disease.[58-60] For example, ST-segment elevation myocardial infarctions most frequently occur in the morning.[61] Knockout mice with altered expression of circadian genes demonstrate changes in tolerance to episodes of myocardial ischemia and reperfusion.[50,61,62] Together, these findings suggest that rhythmic patterns could affect outcomes after exposure to hypoxia and that morbidity and mortality could be better or worse depending on the time of day of these episodes.

Montaigne et al[63] recently examined differences in the outcomes for patients undergoing aortic valve replacement for valve stenosis at various times of the day. In this large prospective study (n = 596),[63] major postoperative cardiac events among patients followed for more than a year were significantly reduced for those who undergo surgery in the

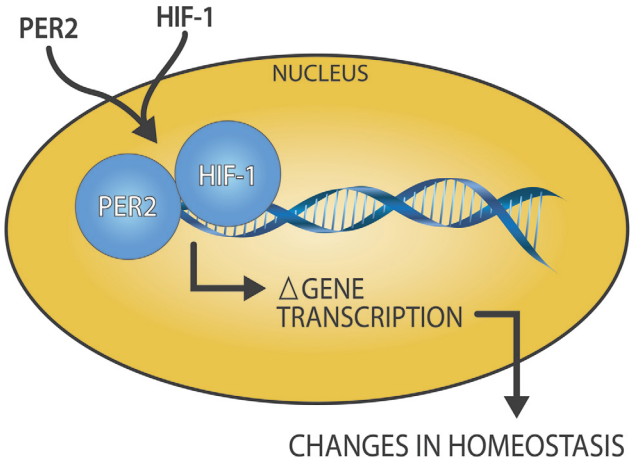

Figure 1. Circadian clock members (PER) and hypoxia-inducible factors (HIF-1) can work together to change transcription, affecting cellular homeostasis. Created based on work described in Kobayashi et al.[44]

afternoon versus the morning. Ex vivo molecular studies of human myocardial tissue revealed an intrinsic ability of the samples to better tolerate hypoxic exposures in the afternoon, a time when a specific circadian clock protein is at the lowest level.[63] These authors further demonstrated that deletion of this circadian clock gene in a murine ischemia-reperfusion model reduces injury.[63] They concluded that hypoxic injury of the myocardial tissue is influenced by the circadian clock, with certain times of hypoxic exposure being more harmful to cardiovascular tissue.[63] Together, these findings suggest that time of day can affect outcomes after cardiovascular surgery and that pharmacologic therapy directed at clock proteins or their outputs may reduce postoperative injury. Furthermore, these results have broad implications on our understanding of the molecular mechanisms involved in other disease models, such as those of OSA.

The Importance of the Circadian Clock in Otolaryngology

Epidemiologic data demonstrated a substantial increase in the prevalence of OSA over the last several decades.[64] This increase in turn resulted in a rapidly escalating burden for patients and the health care system. Treatment strategies for affected patients have primarily comprised methods of maintaining patency of the airway during sleep (eg, adenotonsillectomy, site-directed secondary surgery, continuous positive airway pressure, use of an oral appliance). However, it is evident that a more comprehensive understanding of the upstream molecular pathways that are disrupted and that may lead to OSA is essential.

Transcription factors upregulated in the presence of hypoxic conditions and those that control the circadian clock are intimately involved at the genome level. It is thus feasible that hypoxic exposure leads to dysregulation of the molecular clock. OSA is a disease that also results in sleep fragmentation[65] independent from the effects of hypoxia-inducible factors. Furthermore, sleep disruption seen with untreated OSA is believed to play a role in the pathophysiologic consequences of OSA, including cognitive dysfunction, respiratory instability, and cardiovascular disease.[65,66] However, the exact key molecular players are unknown, and the role of the circadian clock as a protective versus injurious network has not been delineated. This leaves researchers with several important but as of yet unanswered questions: Does clock dysregulation occur from hypoxic exposure or from sleep fragmentation? Is sleep fragmentation alone playing a fundamental role in cardiovascular disease, or does sleep fragmentation combined with hypoxic exposure dramatically increase the risk for pathophysiologic consequences?

Intermittent obstruction of the upper airway in the setting of OSA leads to arousals and sleep fragmentation.[65] Sleep fragmentation can in turn disrupt regulation of the cardiovascular system[67,68] and increase the risk for cardiovascular disease.[69,70] In animals, hypertension occurs from sleep fragmentation and intermittent hypoxia and recovery (as a model of OSA).[66] The risk of consequences to the cardiovascular system from both was shown to be similar, suggesting that the risks associated with OSA are directly related to sleep fragmentation rather than hypoxia.[66] Other work evaluating a large patient population demonstrated a more significant risk for cardiovascular events in the presence of short sleep duration and OSA together, as compared with either one alone.[71] It is clear that cardiopulmonary risks associated with sleep fragmentation are very similar to those associated with OSA; however, we do not currently understand how these 2 processes are linked.[72]

A better understanding of the molecular and cellular mechanisms that link the circadian clock with those processes seen in the presence of OSA (ie, systemic inflammation, autonomic dysregulation, metabolic dysfunction) could substantially affect our advancements in the care of patients with this disease. To date, the key ''molecular players'' are unknown. Research focused on these players has the potential to identify molecular targets that could serve as OSA biomarkers—work that could be used to develop other methods to test for the presence or persistence of OSA. The application of circadian medicine could also be used to refine current treatments. Specifically, exposure to oxygen desaturations during ''time-critical'' windows of sleep may inappropriately reset the clock, resulting in circadian misalignment. In contrast, appropriately timed use of continuous positive airway pressure may improve outcomes. Last, molecular-based pharmacologic therapies could be developed to limit pathologic progression of disease, as suggested for patients undergoing aortic valve repair.[63]

Research in the field of chronobiology has already had a significant impact on multiple medical disciplines and the treatment of many diseases, including cancer,[73-75] hypertension,[76-79] and asthma.[80-82] Given the high prevalence of OSA among children[83] and adults[84] and the fact that OSA is one of the most common reasons for clinic visits to or

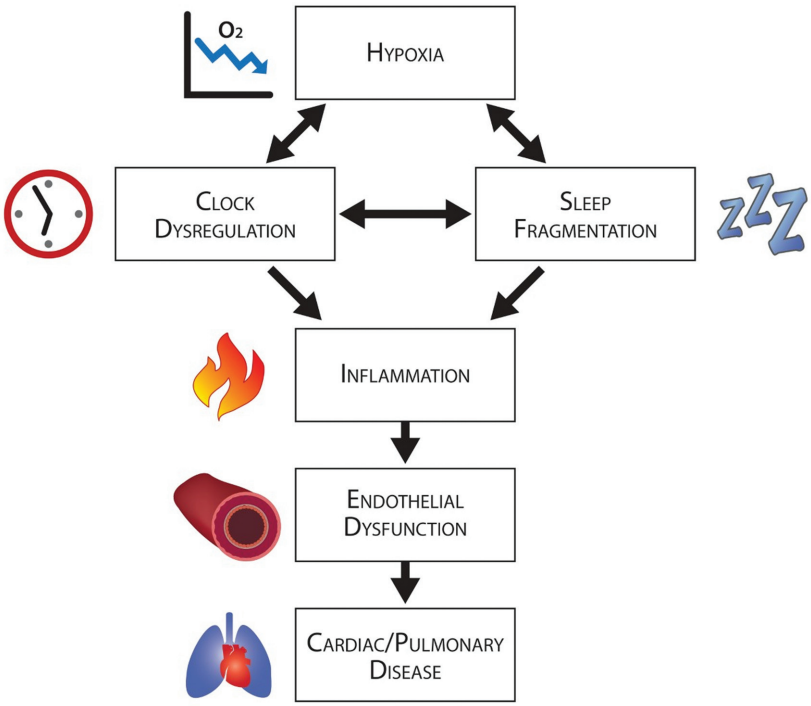

Figure 2. A proposed mechanism for the role of circadian clock dysregulation, sleep fragmentation, and hypoxia in the pathophysiologic progression of cardiopulmonary disease seen in untreated obstructive sleep apnea.

surgical intervention by otolaryngologists, it is incumbent on us to enter this next frontier of research. Studies aimed at identifying the role of circadian dysregulation in the initiation or progression of OSA have the potential to expand our diagnostic approaches and therapeutic interventions for this commonly encountered and significantly burdensome disease. A proposed mechanism is outlined in **Figure 2**.

Conclusions

Despite significant advances in research relating to the development and progression of OSA, it is our belief that future work examining the role of circadian dysregulation is required to clearly delineate the contributory pathways responsible for the development of OSA. Identification of these upstream pathways that may be responsible for cardiopulmonary disease may lay the foundation for the development of new diagnostic techniques for OSA and the generation of new medical therapies. In view of the progress being made in other fields of medicine and the far-reaching implications that research in circadian biology may have on OSA, we are presently investigating the molecular crosstalk that exists between the circadian clock and hypoxia-inducible factors in a murine model of OSA. It is our hope that this translational research will interest clinicians and that the future findings will result in significant advancements in the understanding of OSA and ultimately its clinical management and the well-being of affected patients.

Author Contributions

Douglas C. von Allmen, design of the work, drafting and revising, final approval of the version, agreement to be accountable; **Lauren J. Francey**, design of the work, drafting and revising, final approval of the version, agreement to be accountable; **Garrett M. Rogers**, design of the work, drafting and revising, final approval of the version, agreement to be accountable; **Marc D. Ruben**, design of the work, revising, final approval of the version, agreement to be accountable; **Aliza P. Cohen**, design of the work, drafting and revising, final approval of the version, agreement to be accountable; **Gang Wu**, design of the work, revising, final approval of the version, agreement to be accountable; **Robert E. Schmidt**, design of the work, revising, final approval of the version, agreement to be accountable; **Stacey L. Ishman**, design of the work, revising, final approval of the version, agreement to be accountable; **Raouf S. Amin**, design of the work, drafting and revising, final approval of the version, agreement to be accountable **John B. Hogenesch**, design of the work, drafting and revising, final approval of the version, agreement to be accountable; **David F. Smith**, design of the work, drafting and revising, final approval of the version, agreement to be accountable.

Disclosures

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