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EDITORIAL FOCUS

The double-edged sword of intermittent hypoxia—can intermittent hypoxia be both deleterious and protective in OSA? Focus on "Frequency and magnitude of intermittent hypoxia modulate endothelial wound healing in a cell culture model of sleep apnea"

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WHETHER INTERMITTENT HYPOXIA (IH)—the hallmark of obstructive sleep apnea (OSA)—is detrimental, protective, or both, is a fundamental question. The literature associating OSA with cardiovascular and other comorbidities is overwhelming, yet it is not clear whether IH also exerts protective effects. To address this question, the large number of IH profiles associated with different severity-dependent changes in frequency, magnitude, and durations should be considered. These may initiate diverse and sometimes opposing outcomes.

Intermittent hypoxia has been implicated in the development of various comorbidities, particularly cardiovascular/cerebrovascular diseases in OSA. However, IH also elicits sleep fragmentation and activates underlying mechanisms that promote atherosclerosis through oxidative stress, inflammation, and sympathetic activation, all leading to endothelial dysfunction, hypertension, and eventually to cardiovascular and cerebrovascular diseases (8, 10). It is also evident that not all patients with OSA develop cardiovascular or other comorbidities, which led to the hypothesis that IH may also exert protective mechanisms in the form of ischemic preconditioning (9). Although several human and animal studies have provided supportive evidence for this hypothesis, in these studies, the effects of IH per se could not be studied independently from other well-known consequences of OSA, such as sleep fragmentation and sympathetic activation. For instance, an attempt has been made to determine the contribution of sleep fragmentation to endothelial dysfunction and cardiovascular disease in an animal model (6). Yet, sleep fragmentation in animal models, as in humans, is confounded with sympathetic activation. Thus, in vitro studies focusing on IH are imperative to delineate its independent effects. Moreover, human and animal studies investigating the effects of OSA on the cardiovascular system mostly focused on severe forms of OSA. Yet, in clinical practice, a large number of OSA patients have a mild or moderate severity of the syndrome. On the other hand, most of the in vitro studies published are limited by the use of IH patterns that do not resemble the fast IH cycles typical of OSA, mainly due to the slow diffusion rates in culture between the air phase and the medium. In the few studies conducted with IH patterns resembling OSA, the systems are cumbersome and not easily implemented (1). Therefore, there is a need for a simpler method that enables the investigation of IH in vitro, which can be implemented and standardized throughout all laboratories.

In this issue of *Journal of Applied Physiology*, Campillo et al. (4) used a novel in vitro technique to investigate the impact of IH on the healing capacity of human aortic endothelial cells in culture. By employing their newly described well-controlled IH system in vitro (5), they were able to investigate the effects of various frequencies and amplitudes, thus, mimicking distinct fast IH cycles, as evident in diverse OSA severities.

Cultured human primary aortic endothelial cells were wounded by scratching and were subjected to 24 h of either sustained or intermittent hypoxia. Specifically, sustained hypoxia (SH) was maintained at 1%, 4%, 13%, or 20% O_2. In the IH regimens, the amplitude ranged from 13 to 4% or from 20 to 1% O_2, with frequencies of 0.6, 6, or 60 cycles/h, mimicking mild and severe OSA patients and controls. The repair capacity of the wounded endothelial cells markedly differed among treatments. In SH, a similar repair capacity was observed at 20% O_2 and at 13% O_2. However, wound healing was increased by approximately twofold at the hypoxic condition of 4% O_2. The lack of difference in the repair capacity between 20% and 13% O_2 prompted the authors to use 13% O_2 as the control normoxia level, to simulate the actual O_2 level to which aortic endothelial cells are exposed in vivo. The authors should be commended for choosing a baseline control value simulating the in vivo conditions for aortic endothelial cells (13% O_2) rather than the hyperoxia (20% O_2), which is mostly used in standard culture conditions.

In the moderate IH cycles ranging from 13 to 4% O_2, at the lower frequencies of 0.6 and 6 cycles/h, endothelial wound closure was accelerated by approximately twofold compared with the 13% O_2 SH (control). In contrast, using the higher IH amplitude (20% to 1% O_2), wound closure was similar to control, at both low and high frequencies (0.6, 60 cycles/h), but was lower than control at 6 cycles/h. At the high-frequency IH patterns of 60 cycles/h that mimic severe OSA, endothelial wound closure was not significantly modified, regardless of the regimen of IH treatment. Altogether, these findings clearly demonstrate that both amplitude and frequency of O_2 oscillations affected wound healing repair, and also emphasize the

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complexity of the cells' response to various hypoxic patterns, not necessarily in a severity-dependent order.

Although the article by Campillo et al. (4) depicts limited findings, its importance goes far beyond the data presented. In vitro studies focusing on IH are crucial to clarify the complex effects of IH on specific cellular functions, which cannot be addressed in animal models or in patients with OSA. In vitro studies focusing on IH allow separating the IH component from sleep fragmentation or sympathetic activation to determine its independent contribution to cardiovascular morbidity. In this context, employing in vitro models by using distinct IH paradigms and specific cell types (such as endothelial cells, cardiac cells, smooth muscle cells, and leukocytes), as implemented in this study, is imperative for elucidating underlying mechanisms. Exposing a specific cell type to the physiological partial oxygen pressure, and to IH regimens with OSA-relevant frequencies characteristic to its microenvironment in vivo, is fundamental, and is likely to provide relevant findings to OSA. A given IH profile may induce diverse cellular and functional responses, specific for each cell type. Moreover, by using various inhibitors and molecular biology methods, altered oxidative, inflammatory and transduction pathways and changes in DNA and proteins specific to each cell type can be detected.

As shown in the present study, such an approach can help to determine which IH patterns might be favorable and which might be unfavorable, and to which specific cellular system. Indeed, as shown by varying the frequency and the magnitude of the hypoxic cycles, both parameters have a crucial impact on the outcome of a given cellular response. These results are in line with earlier findings by Jackman et al. (7), demonstrating dichotomous effects of different severities of chronic IH in a mouse model in vivo, in which severe IH was deleterious to focal ischemic cerebral injury, while mild IH exerted protective effects. Similarly, in an acute IH model of rats, a moderate IH pattern decreased cardiac infarct size, while a severe IH paradigm increased infarct size as compared with controls (2).

The present results may have clinical implications as well. Previous results from our laboratory (3) demonstrated that coexistent mild-to-moderate OSA in patients with acute myocardial infarction (MI) increased the mobilization, proliferative, and angiogenic properties of endothelial progenitor cells (EPCs) compared with patients with acute MI without OSA. Additionally, although using a different cyclicity from OSA, IH in vitro had similar effects on EPC functions, by increasing the proliferative and angiogenic properties of EPCs derived from healthy individuals (Fig. 1). Taken together, these results and the present findings of improved pattern-specific capacity

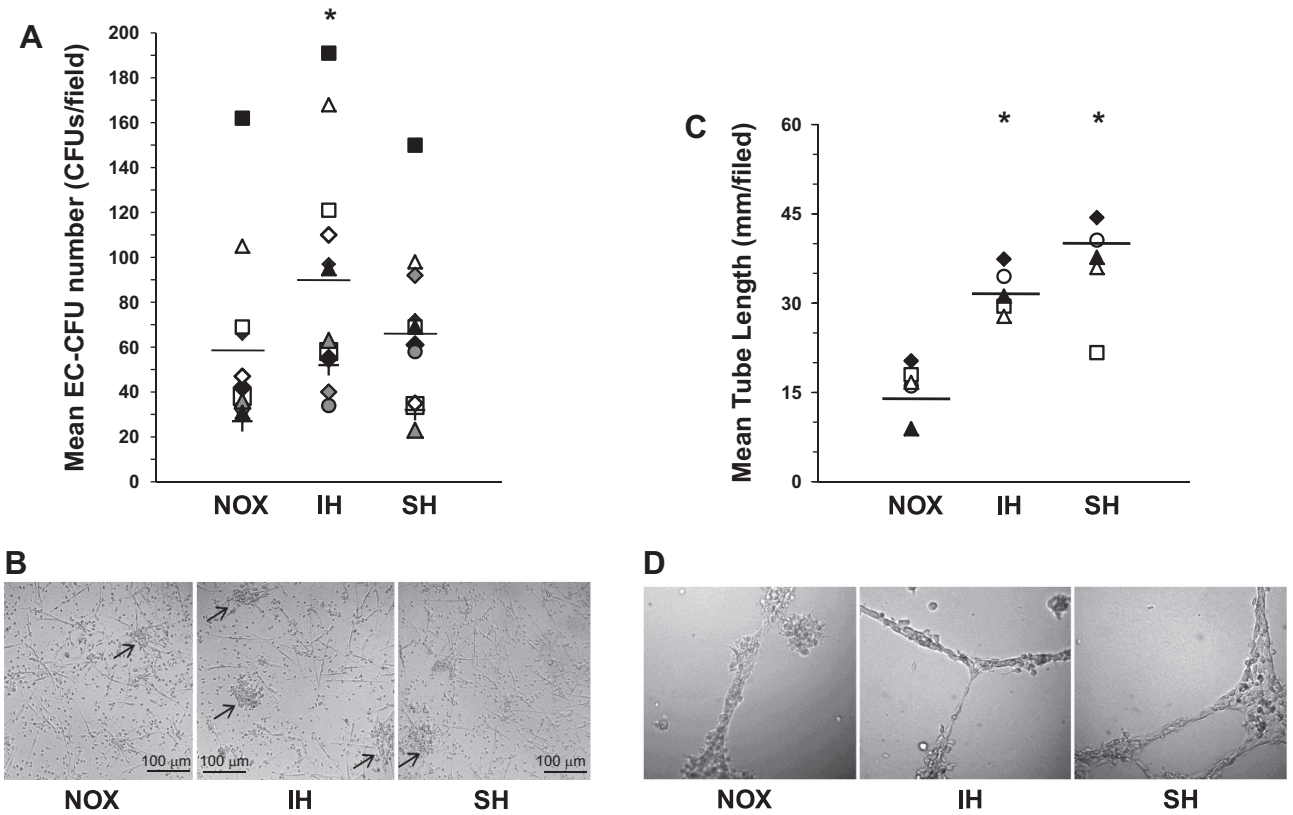

Fig. 1. Effects of intermittent hypoxia in vitro on endothelial cell colony-forming unit (EC-CFUs) numbers and tube formation. *A*: individual and mean EC-CFUs numbers on the 7th day in culture. Cells were exposed to intermittent hypoxia (IH) and to sustained hypoxia (SH) in vitro and compared with normoxia (NOX). *$P < 0.017$, IH vs. NOX. *B*: representative photomicrographs of EC-CFUs for NOX, IH, and SH are shown by arrows. *C*: individual and mean tube length after treatment with EC-CFUs conditioned media from NOX, IH, and SH. *$P < 0.017$, IH and SH vs. NOX. *D*: representative photomicrographs of endothelial tube formation by EAhy926 endothelial cells grown for 24 h on ECM-coated plates with EC-CFU conditioned media from a healthy subject after treatment with NOX, IH, and SH in culture. Reprinted with permission of the American Thoracic Society. Copyright © 2017 American Thoracic Society. Berger S, Aronson D, Lavie P, and Lavie L., 2013. Endothelial progenitor cells in acute myocardial infarction and sleep-disordered breathing. *American Journal of Respiratory and Critical Care Medicine*, 187: 90–98 (3). The *American Journal of Respiratory and Critical Care Medicine* is an official journal of the American Thoracic Society.

of endothelial wound healing, suggest that, indeed, in some instances, IH may play a protective role in OSA, as implicated in a number of animal and clinical studies (8).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

L.L. and P.L. drafted manuscript; L.L. and P.L. edited and revised manuscript; L.L. and P.L. approved final version of manuscript.

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