Invited Editorial on “Frequency and magnitude of intermittent hypoxia modulate endothelial wound healing in a cell culture model of sleep apnea”:

The Double Edge Sword of Intermittent Hypoxia - Can intermittent hypoxia be both deleterious and protective in OSA?

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1 Figure
Whether intermittent hypoxia – the hallmark of obstructive sleep apnea (OSA) - is detrimental, protective, or both, is a fundamental question. The literature associating OSA with cardiovascular and other co-morbidities 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 co-morbidities, particularly cardio-/cerebro-vascular diseases in OSA. However, IH also elicits sleep fragmentation, and activates underlying mechanisms which promote atherosclerosis through oxidative stress, inflammation and sympathetic activation, all leading to endothelial dysfunction, hypertension and eventually to cardio- and cerebro-vascular 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 in order 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 the
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
investigating IH *in vitro*, which can be implemented and standardized throughout labs.
In this issue, 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% O₂. In the IH regimens, the
amplitude ranged from 13 to 4% or from 20 to 1% O₂ 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% O₂ and at 13% O₂. However, wound healing was
increased by about 2-fold at the hypoxic condition of 4% O₂. The lack of difference in the
repair capacity between 20% and 13% O2 prompted the authors to use 13% O2 as the
control normoxia level, to simulate the actual O2 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% O2) rather than
the hyperoxia (20% O2) which is mostly used in standard culture conditions.
In the moderate IH cycles ranging from 13%-4% O2, at the lower frequencies of 0.6 and 6
cycles/h, endothelial wound closure was accelerated by about 2-fold compared to the
13% O2 SH (control). In contrast, using the higher IH amplitude (20% to 1% O2), wound
closure was similar to control, at both low and high frequencies (0.6, 60 cycles/h), but
was lowered than control at 6 cycles/h. At the high-frequency IH patterns of 60 cycles/h
mimicking 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 O2 oscillations affected wound healing repair, and
also emphasize the complexity of the cells’ response to various hypoxic patterns, not
necessarily in a severity dependent order.
Although the paper 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 in order 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, leukocytes etc.), as implemented in this study, is
imperative for elucidating underlying mechanisms. Exposing a specific cell type to the physiologic 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. 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 (7). 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 to 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 EPCs functions, by increasing the proliferative and angiogenic
properties of EPCs derived from healthy individuals (Figure 1). Taken together, these results and the present findings of improved pattern-specific capacity 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.

REFERENCES

Legend to figure

Figure 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 to 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 hrs on ECM-coated plates with EC-CFUs conditioned media from a healthy subject after treatment with NOX, IH, and SH in culture.
