Hypoxia-induced endothelial dysfunction: Could targeting oxidative stress provide protection?

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Experimental Physiology

DOI:
10.1113/EP091276

Published: 26/05/2023

Peer reviewed version

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Hypoxia can cause disturbances to vascular homeostasis and endothelial dysfunction, and is believed to contribute to pathophysiology of atherosclerosis. Over the past 10 years, a growing body of evidence implicates oxidative stress and diminished nitric oxide (NO) bioavailability as a key mechanistic pathway to explain the deleterious effects of acute and chronic hypoxia on endothelial function. In this connections article, we highlight three key papers which combine to characterize the effect of hypoxia on endothelial function, support the mechanistic role of oxidative stress and NO bioavailability, and set the scene for targeted interventions to prevent endothelial dysfunction in the presence of hypoxia.

The endothelium is a heterogeneous tissue for structure and function; therefore, a comprehensive assessment of endothelial function is crucial to understand the significance of hypoxia-induced endothelial dysfunction. In the first (and to date, only) article to examine the effect of hypoxia on microvascular and large-vessel endothelial function in the same study, we observed hypoxia-induced reduction in endothelium-dependent microvascular function (43% reduction in perfusion response to acetylcholine) and endothelium-dependent large vessel vasodilatation (18% reduction in flow-mediated dilatation of the brachial artery [FMD]) (Jones *et al.*, 2021). Notably, the extent of the decrease was approximately two-fold higher in the microcirculation compared with the large vessels. This finding is interesting in the context of an oxidative stress model of hypoxia-induced endothelial dysfunction since the microvasculature is generally more sensitive to oxidative stress than large blood vessels. This is due to a greater surface-area-to-volume ratio, making it more exposed to reactive oxygen species (ROS), which can cause oxidative stress. Further, a greater prevalence of adhesion molecules in microvascular endothelial cells (compared to endothelial cells in large blood vessels) increases susceptibility to infiltration of inflammatory molecules, which can activate endothelial cells and diminish NO bioavailability.

In addition to intra-individual comparisons across vascular beds (microvasculature versus large blood vessels), we demonstrated that individuals with greater cardiorespiratory fitness better preserve microvascular endothelial function during hypoxic exposure. One of the well-known benefits of cardiorespiratory fitness is an improved redox status (the balance between ROS production and antioxidant defence). Regular exercise and physical activity have been shown to increase antioxidant levels and reduce the production of ROS. In our study, we observed that those with superior cardiorespiratory fitness had the smallest hypoxia-induced reduction in microvascular function, which may be due to a beneficial redox status and greater NO bioavailability in these individuals.

Early evidence for the role of oxidative stress and NO bioavailability in hypoxia-induced endothelial dysfunction came from Bailey et al., (2013). Bailey and colleagues investigated blood oxygen saturation, redox status, NO bioavailability, and endothelial function in lowlanders under three experimental conditions of increasing oxidative stress (normoxia, acute hypoxia, and exhaustive exercise) and compared to well-adapted (healthy) and maladapted (chronic mountain sickness) high altitude residents. They identified that oxidative stress measured by the presence of free radicals was mildly elevated in adapted highlanders, but substantially increased in maladapted highlanders. Further, maladapted highlanders exhibited worse hypoxaemia (lower oxygen saturations), which was accompanied by greater concentrations of free radicals, lower concentrations of antioxidants, lower nitrite bioavailability, and worse vascular reactivity, with significant correlations between each of these variables. Our own work (Jones *et al.*, 2021) is consistent with the original mechanism proposed by Bailey *et al.*, but rather than comparing lowlanders and genetically distinct high-altitude natives, we identified intra-individual differences between vascular beds, and inter-individual differences within lowlanders. Our finding that superior cardiorespiratory fitness is associated with protection from hypoxia-induced endothelial dysfunction begins to move the field on from the necessary work of characterizing the nature of hypoxic effects, to identifying protective countermeasures and possible preventative interventions. However, we reported only a cross-sectional correlation, and did not conduct an experimental intervention.

Though the application in hypoxia is novel, the idea that exercise training status can protect against endothelial dysfunction in physiological states of elevated oxidative stress is not new. Oxidative stress is generally increased with older age, and has been implicated in the aging process, and the development of age-related diseases. Indeed, more than 20 years

ago, Taddei *et al*., (2000) used strain-gauge venous occlusion plethysmography to investigate the combined effects of older age and exercise training status on forearm blood flow response to acetylcholine with and without intravenous vitamin C. The age-associated decline in response to acetylcholine was ameliorated by exercise training status in older adults. In addition, older sedentary participants showed age-associated increased inhibition with L-NMMA (NO inhibitor) that was ameliorated in older athletes. The administration of vitamin C, which donates electrons to neutralize ROS and other free radicals, corrected the L-NMMA-induced inhibition in older sedentary individuals but not in older athletes. Since administering vitamin C does not improve endothelial function in populations with a 'healthy' redox status (young adults and older athletes), the observed vitamin C improvement in endothelial function, supports the potential for targeted interventions to reverse endothelial function impairment specifically in the context of oxidative stress (e.g., in hypoxia).

In a recent study, Stone *et al.,* (2022) utilized the same methodology as Taddei and colleagues (strain-gauge plethysmography) to investigate the effects of intravenous vitamin C administration on hypoxia-induced endothelial dysfunction. Forearm blood flow response to acetylcholine decreased by ~30% at high altitude (4300m) compared to sea level but was largely restored with the addition of vitamin C infusion. Further, consistent with the original work of Bailey *et al.,* (2013), the magnitude of both the reduction in endothelial function and the response to vitamin C, were positively associated with the severity of hypoxaemia. In other words, those with the lowest oxygen saturations appeared to experience the greatest reduction in endothelial function that was explained by greater oxidative stress. Taken together these papers support the notion that endothelial function impairment in hypoxia is at least in part due to oxidative stress, and that oxidative stress may be a modifiable target for protection.

While Bailey and colleagues (2013) highlighted those at exacerbated risk due to this pathway, our own work (Jones *et al*., 2021) identified individuals protected from vascular decline, possibly due to this pathway, and subsequently Stone *et al.,* (2022) have built on this to demonstrate targeted experimental manipulation of the pathway overcomes the impairment. Interventions to target each point of the pathway (hypoxaemia, oxidative stress, NO bioavailability), may protect against hypoxia-induced impairment in vascular dysfunction in both lowlanders sojourning to high altitude, and in high-altitude natives. However, there is currently no evidence that supplemental antioxidants provide effective protection for vascular function. To date, studies using oral supplementation of antioxidant cocktails (e.g., 1 g of ascorbic acid; 400 IU of alpha-tocopherol acetate, and 600 mg of alpha-lipoic acid) haven't

been consistently successful at improving oxygen saturations, and importantly, have not assessed endothelial function. Equivocal results in the efficacy of oral antioxidant supplementation to improve oxygen saturations may be due to different ascent profiles, dosages and intervention durations, or due to the method of administration (oral versus intravenous). Oral administration may not reach the endothelium, and intravenous injection is used as an acute experimental manipulation to investigate mechanisms; it is not proposed as a feasible prophylactic treatment. Indeed, Stone is careful to not overstate whether vitamin C is a viable intervention (particularly if it requires intravenous injection). The evidence is not yet clear enough to provide practical recommendations and future studies are needed to determine the feasibility and efficacy of specific antioxidant interventions. Though our own study implies that easily practicable interventions to improve sea-level cardiorespiratory fitness may provide protection (Jones *et al.*, 2021), we used a cross-sectional approach. Experimental research is needed to confirm the efficacy of any practical recommendations relating to exercise training.

A consistent finding across all these papers is that hypoxia, and other conditions associated with oxidative stress (ageing and a sedentary lifestyle), reduce endothelial-dependent vascular reactivity (measured by FMD and response to acetylcholine), but do not affect endothelial independent vascular reactivity (measured by NO donors GTN or SNP). This is regardless of the method of assessment, or the vascular bed being assessed (microvasculature or large vessels). Combined, this provides compelling evidence that it is NO-dependent endothelial function that is impaired by hypoxia, and not the capacity of smooth muscle to respond to NO. These papers provide diverse evidence but consistently support the interpretation that the hypoxia-induced impairment in endothelial function is, at least in part, caused by elevated oxidative stress and impaired NO bioavailability. Further, individual differences appear to influence susceptibility to this effect; individuals experiencing worse hypoxaemia, worse oxidative stress, and/or lower bioavailability of NO, will experience greater impairments in vascular function. Methods to reduce oxidative stress and improve endogenous NO bioavailability (e.g. exercise training and antioxidants) may protect against hypoxia-induced impairments in vascular reactivity. These findings have implications not only for those travelling or residing at high altitude. Since hypoxia is characteristic of several diseases at sea level (e.g., COPD), and implicated in the progression of cardiovascular disease, which itself exacerbates vascular hypoxia, resulting in a positive feedback loop. The research progression outlined in this article sets the scene for targeted interventions to protect against hypoxia-induced endothelial dysfunction at high altitude, and in pathological states.

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Conflict of interest

None of the authors has any conflicts of interest.

Funding

No funding was received for the preparation of this article.