Understanding the role of antioxidant therapy for intermittent claudication; good, bad, or both?

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PERIPHERAL ARTERIAL DISEASE (PAD) is a global health issue, affecting 202 million people worldwide (12, 18). The most common symptom of PAD is intermittent claudication. Although claudication patients have a limited progression to chronic limb ischemia, PAD is associated with increased risk of cardiovascular morbidity and mortality (8). This fact, along with the impact on lifestyle and the large population affected, has caused PAD to be identified as an area where new therapies are urgently needed (15, 22). Currently, the only medications approved by the US Food and Drug Administration for treating PAD-associated walking impairment are the phosphodiesterase inhibitors pentoxifylline and cilostazol (22). However, these agents have been shown to produce only minimal improvement in maximal treadmill walking distance (33), are expensive, and have multiple side effects. There have been many clinical trials with various molecular and cell-related therapies with varying degrees of success [reviewed by Cooke and Losordo (7)] for both critical limb ischemia and intermittent claudication, but among the most effective therapies in claudicants have been those impacting vascular redox and inflammatory status (2, 4). Much evidence exists for a role of oxidant stress and inflammation in cardiovascular disease in general, and PAD patients have high circulating levels of inflammatory (6, 27) and oxidant stress markers (13, 20). Thus therapies targeted to address an abnormal redox state may have potential as mitigators of PAD.

In the current issue of the American Journal of Physiology-Heart and Circulatory Physiology, da Silva Jr et al. (9) present results of a double-blind, randomized, crossover study designed to determine effects of acute administration of N-acetylcysteine (NAC) in patients with intermittent claudication on walking capacity, postocclusive reactive hyperemia in the leg, and circulating levels of inflammatory and angiogenic mediators. The rationale for the study was based, in part, on previous data that glutathione infusion improved pain-free walking distance (3), along with studies showing that NAC mitigated the inflammatory response to exercise (23, 31) and improved fatigue resistance in soleus muscle in a mouse model of PAD (30). NAC was administered in five doses over 4 days and found to increase the plasma ratio of reduced to oxidized glutathione. Unexpectedly, results were negative for increased walking tolerance and leg blood flow/reactive hyperemia, and NAC had no measureable effect on exercise-induced increases in soluble vascular cell adhesion protein-1, monocyte chemotactic protein-1, or endothelin-1. The authors examined the expression of several circulating angiogenic mediators, including microRNA (miRNA)-126, because it is known to be markedly reduced in patients with PAD (32). A novel finding was that miRNA-126 expression increased following maximal exercise in claudication patients. However, NAC prevented maximal exercise-induced increases in circulating miRNA-126, along with the angiogenic mediators VEGF, endothelial nitric oxide synthase, and phosphatidylinositol 3-kinase regulatory β-subunit, suggesting that miRNA-126 signaling is redox sensitive in intermittent claudication patients. Thus, contrary to the initial hypothesis, treatment with NAC did not result in improved walking capacity and could be detrimental in light of the reduced angiogenic response.

The da Silva results are consistent with other studies (14, 29) using chronic administration of the antioxidants vitamin C or E that found these agents to limit beneficial effects of exercise on muscle function, but are in marked contrast with recent clinical studies administering ramipril in claudicants, which demonstrated a significant positive outcome on pain-free and maximal walking distance, improved perfusion, reduced inflammatory markers, and increased angiogenic markers (1, 2). Because ramipril also impacts vascular redox status, these studies raise fundamental questions regarding antioxidant therapies to improve functional capacity in PAD patients. Among the most central points to consider are questions related to dose/duration and therapeutic targets.

In the present NAC study, there was only acute (4 day) administration of drug and the effect on the ratio of reduced to oxidized glutathione was likely not sustained between treatments. The study with ramipril was 6 mo in duration. Both NAC and ramipril as administered might provide acute benefits in terms of vascular redox status or endothelial function, but chronic administration is likely necessary to affect compensatory vascular remodeling, which could significantly increase blood flow, as collateral resistance is the primary limitation to tissue perfusion in PAD patients (21, 25). As suggested by Drummond et al. (11), dose and biological availability are important factors to consider for effects of antioxidants on cardiovascular events. Although common to all studies using systemic antioxidants, it is unknown what effect systemic administration of NAC had in the present study on oxidant stress at the level of the vasculature. While NAC clearly improved the reduced-to-oxidized glutathione ratio and impacted circulating angiogenic mediators, whether it affected the primary source of reactive oxygen species (ROS) that altered vascular function is unclear. Antioxidant dose also is a potential issue because too high a concentration of antioxidant may suppress acute and chronic compensatory processes, consistent with the concept of an optimal redox window where some level of ROS is necessary for the function of physiological signaling pathways (16, 17, 19, 28). Preclinical studies with animal models may be useful in gaining additional insight into this issue, particularly with agents not yet tried in humans.

Another issue central to all studies of PAD mitigators is the mechanism by which they potentially alter vascular function, particularly in relation to sources of ROS. The cause of the
impairment must be known to design effective therapies to treat arterial insufficiency, and such therapies ideally should target the source of pathological ROS while not affecting physiological, beneficial sources (5). Angiotensin-converting enzyme inhibitors (ACEI) such as ramipril have been known for some time to provide vascular protection by decreasing oxidative stress and improving endothelial function (26), a key component of flow-mediated dilation and compensatory remodeling. The benefit of ACEI results in part through decreasing angiotensin II activation of NADPH oxidase (Nox), a primary source of vascular ROS (10). In addition, elevated serum-soluble Nox2 is associated with endothelial dysfunction (flow-mediated dilation) and increased oxidative stress in patients with PAD (20), and carriers of Nox2 deficiency have shown a reduced atherosclerotic burden (34). Drummond et al. (11) have pointed out that preventing formation of ROS, for instance via inhibition of Nox, may be a more effective strategy than attempting to scavenge existing species, perhaps accounting in part for the efficacy of ACE inhibition on intermittent claudication. ACEI thus have pleiotropic effects beyond blood pressure lowering, and, although associated with antioxidant actions, possibly act via a different mechanism or on different targets than other types of antioxidants. The second study by Ahimastos et al. (1) addressed mechanisms of ramipril and intermittent claudication in PAD patients and showed that it is associated with an increase in the biomarkers of angiogenesis/angiogenesis and reduction in the markers of thrombosis, inflammation, and leukocyte adhesion in patients with intermittent claudication. Reducing inflammation is an important component of treating PAD and claudication because an endothelial proinflammatory state is known to impair vasodilatory capacity in resistance vessels and to suppress flow-mediated outward remodeling/collateral growth (35).

Much work remains to be done to address the numerous questions to be answered concerning the possibility of altering redox balance to treat intermittent claudication. One area that could improve outcome for clinical trials is prescreening of patients to determine their capacity for flow-mediated dilation (35) and ideally the systemic and tissue levels and sources of oxidant stress, which could allow for optimized therapies. The inclusion of appropriate patients, whether due to genetic background or other factors, perhaps explains success of some trials and failures of others. Selecting a subpopulation of patients most likely to benefit from antioxidant therapy has been shown to be a viable approach (24). There remains a critical need to understand mechanisms, with a large body of human and preclinical data suggesting that endothelial dysfunction is a primary contributor to vascular dysfunction and intermittent claudication. Because the molecular mechanisms regulating endothelial dysfunction are likely not isolated to a single pathway, more clinical studies that include molecular measurements are needed. The present study by da Silva et al. (9) and the work by Ahimastos et al. (1, 2) show the value of such experiments in conjunction with clinical outcomes to gain insight into potential mechanisms regulating vascular function. In summary, it will be important to delineate the multiple mechanisms that mediate PAD-related vascular impairments and determine whether they vary by risk factor and patient makeup. If oxidant stress is indeed central to mediating impaired vasodilatory and growth responses, it will be critical to target the responsible source(s) of pathological and not beneficial ROS to develop truly effective claudication therapies.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: S.J.M. conception and design of research; S.J.M. drafted manuscript; S.J.M. and J.L.U. edited and revised manuscript; S.J.M. and J.L.U. approved final version of manuscript.

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