PERSPECTIVES

Exercising an option to prevent age related decline of vascular BH_4 and uncoupling of eNOS

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It is well documented that skeletal muscle vascular conductance and endothelium-dependent vasodilatation are reduced with ageing. Studies utilizing isolated skeletal muscle arterioles reveal that age-related deficits involve, at least in part, alterations in the NO signalling pathway (Muller-Delp *et al.* 2002; Delp *et al.* 2008). Although manifold factors could contribute to age-related declines in NO-mediated function, recent studies highlight the potential importance of tetrahydrobiopterin (BH_4), a nitric oxide synthase (NOS) cofactor. When BH_4 is limited, eNOS can become uncoupled and produce superoxide instead of NO. Thus, BH_4 is potentially of junctional importance in the link between NO, reactive oxygen species (ROS) and vascular function.

In humans, a single oral dose of BH_4 restored endothelium-dependent dilatation in the conduit brachial artery of sedentary older men (Eskurza *et al.* 2005). Furthermore, Delp *et al.* (2008) demonstrated that age-related declines in flow-mediated dilatation of rat soleus muscle arterioles resulted, at least in part, from limitations in availability of the eNOS cofactor tetrahydrobiopterin (BH_4), and that administration of the BH_4 precursor sepiapterin improved flow-induced dilatation in vessels from aged rats. These results collectively support the argument that interventions to preserve vascular BH_4 may be a therapeutic option for age-related vascular dysfunction (Pierce & LaRocca, 2008).

In the current issue of *The Journal of Physiology*, Sindler *et al.* proposed to determine if age-related declines in vascular BH_4 were associated with eNOS uncoupling and ROS production, and whether these age-related changes could be reversed by a therapeutic exercise training intervention. Aerobic exercise training has previously been shown to blunt or eliminate age-dependent declines in NO-mediated endothelium-dependent dilatation and to improve skeletal muscle blood flow in a variety of models (Seals *et al.* 2008), and the study of Sindler *et al.* proposes to add novel understanding of the underlying mechanisms related to BH_4. The approach is integrative, combining functional assessment of vasodilatation with biochemical measurements of BH_4, NO and ROS production, all in isolated arterioles from the soleus muscle under a number of controlled pharmacological conditions. As expected, flow-mediated dilatation was impaired with ageing and restored by exercise training; novel observations point to potential underlying mechanisms: age-related reduction in BH_4 occurred in conjunction with decline in flow-induced NO signalling, and increase in superoxide production (eNOS uncoupling); and exercise training prevented the age-related loss of BH_4 and improved NO bio-availability by balancing accelerated NO and ROS production. These results extend the appreciation of oxidative stress impacting NO bio-availability and vasomotor function beyond the traditional, but insufficient, concept of NO destruction by ROS (Rush *et al.* 2005).

The findings of the study of Sindler *et al.* are timely, reported roughly contemporaneously with identification of the need for pharmacological therapies directed toward a similar result, the restoration of BH_4 in aged blood vessels. This creates a discovery environment in which potential pharmacological- and exercise-oriented approaches to the problem will undoubtedly co-evolve. The findings of Sindler *et al.* additionally serve as a progenitor for many hypotheses regarding BH_4 in vascular adaptations to age and exercise. Fundamental questions raised include those related to identifying the cellular mechanisms accounting for age-related declines and exercise-related restoration of vascular BH_4. In this regard, emerging data suggest that H_2O_2 is a regulatory factor controlling BH_4 synthesis in vascular cells via induction of the enzyme GTP-cyclohydrolase I (GTPCH; Shimizu *et al.* 2003). The data of Sindler *et al.* indicate that H_2O_2 contributes to flow-induced dilatation of skeletal muscle arterioles of young sedentary animals and that, like the flow-induced dilatation functional response itself, this H_2O_2 signalling declines with age but is restored by exercise training. Is it possible that this same pattern of H_2O_2 signalling could contribute to maintenance of BH_4 in young arterioles, its loss with age, and its restoration with exercise in aged arterioles, consistent with the indicated effect of H_2O_2 on GTPCH expression and BH_4 synthesis? If so, H_2O_2 takes on another important role in the acute and chronic regulation of vascular function.

A recurring theme in vascular biology is heterogeneity in phenotype and function depending on vessel type (conduit *vs.* resistance), location (vascular bed), and physiological circumstances (e.g. other conditions *in vivo*). While these considerations are in part cautionary regarding the global interpretation of the results of Sindler *et al.*, they can more appropriately be seen as an invitation for additional studies designed to create a more integrative understanding of cellular mechanisms of vascular ageing and the therapeutic effects of exercise. The study of Sindler *et al.* creates a new paradigm justifying such an invitation with respect to BH_4, vasomotor function and the regulation of skeletal muscle blood flow.

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