

How Old are Your Arteries? Exercise-Mediated Protection From Age-Associated Vascular Stiffness

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As we age, the blood vessel becomes more susceptible to pathologies including hypertension and atherosclerosis. A famous saying of Thomas Sydenham, English physician, states: “man is as old as his arteries.” However, changes in the function and structure of the arteries with aging, such as arterial stiffening and thickening, do not occur to the same extent in all people. Studies by Seals et al strongly suggest that habitual physical activity/aerobic exercise can slow or prevent the aging of the blood vessels, as measured by arterial compliance, even in previously sedentary middle-aged and older adults.^{1–3} Exercise training is known to induce several adaptations in the cardiovascular system, in part, by increases in laminar blood flow.⁴ This, in turn, leads to increased production of endothelial-derived nitric oxide (NO) that is critical to the regulation of vascular responses, including vascular tone and permeability, platelet adhesion and aggregation, endothelial-leukocyte interactions, and vascular smooth muscle cell proliferation.⁵ Therefore, it is not surprising that NO has been touted as having an important role in the architecture of the blood vessel. In this issue of *JAHA*, Stepan and colleagues demonstrate that exercise suppresses an age-associated increase in vascular transglutaminase activity and propose that this NO-mediated effect contributes to exercise-induced arterial remodeling and specifically the attenuation of age-associated vascular stiffness.⁶

A major advance in the field of vascular biology research was the development of methodologies to assess the elastic properties of arteries in humans. The vasodilatory response to acetylcholine has been a gold-standard method for evaluating

vascular relaxation with aging and exercise training. However, the invasive nature of this measurement prevents it from being widely used as a clinical diagnostic. Rather, the determination of the pulse wave velocity (PWV) has been used in the clinical setting as a noninvasive measurement of the wave reflections occurring in the walls of the aorta. The pressure waves move more rapidly in the stiffer aorta, and therefore, high aortic PWV is an independent predictor of arterial stiffness (compliance) as well as cardiovascular morbidity and mortality.⁷ Although exercise is well known to have beneficial effects on overall vascular health in aging through enhancing local endothelial nitric oxide synthase (eNOS) and NO release, less clear has been the molecular mechanism linking exercise and enhanced NO production with chronic improvements in vascular compliance.

Nitric oxide donors and endogenously produced NO exert a variety of effects through the second messenger guanylate cyclase, which in turn activates cyclic GMP.^{7,8} The effect of NO, however, is not completely blocked by methylene blue, an inhibitor of guanylate cyclase, suggesting that NO mediates some benefits independent of cGMP.^{8,9} In fact, many of the known effects of NO appear to be mediated by a chemical modification of cysteine residues, termed S-nitrosylation, that affects the function of many proteins.¹⁰ One of the proteins regulated and nitrosylated by NO is tissue transglutaminase (TG2). TG2 may represent an important link between endothelial dysfunction, characterized by decreased NO bioavailability, and long-term remodeling of the vascular wall, especially of the large elastic arteries that influence pulse wave velocity. Tissue transglutaminase has been demonstrated previously to be produced by most cells of the vascular wall including smooth muscle, endothelia, and fibroblasts.^{11,12} Although TG2 has been demonstrated to play a role intracellularly as a GTPase, it is the secretion of TG2 to the extracellular matrix that appears to promote vascular stiffness.¹³ An increase in TG2 activity promotes post-translational modification of proteins through production of an epsilon (gamma-glutamyl) lysine crosslink within the adventitial and subendothelial layers of the vessel wall.¹⁴ A recent study by Santhanam et al demonstrated a clear increase in TG2 activity with age that was linked to increased arterial stiffness.¹⁵ In addition, they demonstrated that

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treatment of rats with a TG inhibitor reduced the age-associated increase in vascular stiffness. Collectively, these observations suggest that TG2 may play an important role in the process of vascular stiffening with aging.

The current study by Steppan et al in this edition of *JAHA* added more insight regarding the regulatory role of TG2.⁶ They provide evidence that exercise-mediated NO suppresses tissue TG2 activity to potentially prevent vascular aging/stiffness. For these studies, they first evaluated the impact of 4 weeks of treadmill exercise in a small cohort of 20-month old rats and found a modest attenuation of the TG2 activity in the exercise-trained rats compared with the age-matched sedentary control rats. However, despite this influence on TG2 activity, there was no improvement in the PWV, suggesting that compliance was unchanged in these older animals. This prompted the authors to speculate that the prevention of the age-associated PWV changes may require a longer intervention (14 weeks) in younger animals. Using 11-month-old rats with age-matched and younger (6 months) sedentary rats as controls, they observed restoration of phosphorylation at the eNOS activating site (Ser1177), in the old exercise rats. Moreover, increased phosphorylation of vasodilator-stimulated phosphoprotein (VASP), a protein known to be regulated in a cGMP-dependent manner, confirmed an increase in NO production and NO/cGMP signaling by exercise in the old rats. In addition, the TG crosslinking activity was elevated only in the old sedentary group compared with the young controls. The researchers attribute the increase in TG crosslinking activity to suppression of NO bioavailability with aging. Consistent with this, the examination of the aorta of the old-trained, old-untrained, and young rats revealed that TG2 S-nitrosylation in the old exercise cohort was significantly increased compared with the age-matched sedentary counterparts and was comparable to the young sedentary controls. The authors explain that altered TG2 function through S-nitrosylation is due to an augmentation of NO production by exercise. Mechanistic aortic tensile tests demonstrated that aortic samples from the exercise-trained rats were statistically more elastic compared with their sedentary controls, despite the fact that the tensile properties of the aorta in the older trained rats resemble that of the older untrained when compared with the young rats.

These studies provide an exciting potential mechanistic link between endothelial function and vascular remodeling that may help explain observations made in those that are regularly physically active. However, based on recent work by this group, the suppression of vascular TG2 crosslinking activity would be hypothesized to improve vascular compliance following this prolonged period (14 weeks) of exercise training. In this recent study, Santhanam and colleagues reported significantly lower PWV following a 3-month treatment of cystamin, a TG inhibitor, in older rats (22 to

24 months of age) compared with age-matched control rats using single-point PWV.¹⁵ Nevertheless, although TG2 activity was decreased by exercise in the current study, vascular stiffness remained unchanged compared with their sedentary counterparts. Moreover, in the current study, the authors employed a more thorough evaluation of the vascular stiffness by determining the PWV across a dynamic range of blood pressures (from ≈ 50 to 200 mm Hg of systolic pressure) through in vivo pharmacologic alterations with phenylephrine and sodium nitroprusside. Their rationale for this more invasive approach was that wall stiffness depends on blood pressure, which, in turn, affects PWV. Therefore, by evaluating the PWV across this dynamic blood pressure range they can provide a more complete evaluation of vascular stiffness. However, despite the collection of this thorough PWV profile, no differences in vascular stiffness were evident between the old-sedentary and old-trained cohorts. The authors suggest that the accumulation of cross-linked collagen prior to the initiation of the exercise regimen (at 11 months) may have overshadowed the benefits of TG2 S-nitrosylation and suppressed protein cross-linking during the 14-week exercise training period.

Although Steppan and colleagues nicely characterize the effect of moderate aerobic exercise on TG2 activity in the rat model of aging, this interesting study prompts several additional questions. A primary question that requires attention is whether the suppression of extracellular TG2 activity is necessary for exercise-mediated improvements in age-associated vascular stiffness. The authors have demonstrated exercise to suppress vascular TG2 activity and to have a modest improvement in elasticity in rats >1 year old. However, the invasive and sophisticated measure of pulse-wave velocity did not provide evidence of an exercise-mediated improvement in the vascular stiffness of these older animals. Does this result portend that exercise is incapable of inducing a benefit in the setting of advanced vascular stiffness? As the authors described in the limitation section of their paper, an earlier initiation of exercise might have suppressed the TG2-mediated cross-linking of adventitial proteins. However, does this suggest that exercise can only suppress further increases in stiffness rather than the reversal of this cross-linking process? The evidence of improved arterial compliance from human studies is rather limited. Steppan et al caution that observed differences in the traditional non-invasive measure of pulse wave velocity may be due to improvements in blood pressure following exercise training and not represent true improvements in vascular stiffness.⁶ However, previous exercise studies by Tanaka et al, using a more direct measure of arterial compliance, resulted in significant reductions in arterial stiffness without alterations in arterial blood pressure. These studies were performed in middle-aged men and improvements were

evident following only 3 months of aerobic exercise.¹ Therefore, there is some discrepancy between the animal and human exercise studies regarding improvements in arterial compliance. As stated by Steppan et al, future studies investigating the importance of exercise intensity and the impact of an earlier intervention are required.

Collectively, Steppan et al provide a novel mechanism to explain how exercise-induced improvements in endothelial function can directly lead to structural remodeling in the vascular adventitia. The suppressed S-nitrosylation of TG2 in older sedentary animals promotes an increase in tissue TG activity and, with time, an increase in vascular stiffness. However, the induction of NO with exercise promotes S-nitrosylation of TG2 and thereby suppresses tissue TG activity. These events can block the crosslinking of extracellular matrix proteins and may therefore attenuate the rigidity of the vascular adventitia. Future studies will be needed to determine what dose of exercise is optimal for maintaining healthy “young” arteries and to ascertain if these molecular events regarding TG2 are necessary to prevent age-associated vascular stiffness.

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Disclosures

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