

## SYMPOSIUM REVIEW

# Habitual exercise and vascular ageing

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Age is the major risk factor for cardiovascular diseases (CVD) and this is attributable in part to stiffening of large elastic arteries and development of vascular endothelial dysfunction (e.g. impaired endothelium-dependent dilatation, EDD). In contrast, regular aerobic exercise is associated with reduced risk of CVD. Endurance exercise-trained middle-aged/older adults demonstrate lower large elastic artery stiffness and greater EDD than their sedentary peers. With daily brisk walking, previously sedentary middle-aged/older adults show reduced stiffness and improved EDD. The mechanisms underlying the effects of regular aerobic exercise on large elastic artery stiffness with ageing are largely unknown, but are likely to include changes to the composition of the arterial wall. Enhanced EDD in older adults who exercise is mediated by increased nitric oxide (NO) bioavailability associated with reduced oxidative stress. Arteries from old rodents that perform regular aerobic exercise demonstrate increased expression and activity of endothelial NO synthase, reduced oxidative damage associated with reduced expression and activity of the oxidant enzyme NADPH oxidase, and increased activity of the antioxidant enzyme superoxide dismutase. Aerobic exercise also may protect arteries with ageing by increasing resistance to the effects of other CVD risk factors like LDL-cholesterol. Habitual aerobic exercise is an effective strategy to combat arterial ageing.

(Received 20 July 2009; accepted after revision 24 August 2009; first published online 1 September 2009)

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**Abbreviations** BH<sub>4</sub>, tetrahydrobiopterin; CVD, cardiovascular diseases; EDD, endothelium-dependent dilatation; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; NO, nitric oxide; SOD, superoxide dismutase.

Cardiovascular diseases (CVD) remain the leading cause of mortality in modern societies. Approximately 80% of all CVD deaths are associated with dysfunction and disorders of arteries (Thom *et al.* 2006). That advancing age is the major risk factor for CVD (Lakatta & Levy, 2003) strongly suggests there is something about ageing that causes changes in arteries that lead to an increased risk of CVD.

It is likely that a wide array of adverse changes to arteries contribute to the increased risk of CVD with ageing. Two changes that are believed to be among the most important, however, are increased stiffness of large elastic arteries and dysfunction of the vascular endothelium (Lakatta & Levy, 2003). As such, factors that can delay, slow and/or prevent

these changes hold promise for reducing age-associated CVD.

Regular aerobic exercise is associated with a reduced risk of CVD in middle-aged and older adults (Blair *et al.* 1989; Manson *et al.* 2002; Mora *et al.* 2007), although the mechanisms of action are incompletely understood. Here we review experimental evidence supporting the idea that this effect of habitual aerobic exercise may be mediated, at least in part, by reducing age-related increases in large elastic artery stiffness and the development of vascular endothelial dysfunction. We also discuss the integrative physiological mechanisms by which regular exercise may protect against these arterial changes with ageing. A broader treatment of the topic of habitual exercise and arterial ageing can be found elsewhere (Seals *et al.* 2008).

## Large elastic artery stiffness

The large elastic arteries are responsible for 'buffering' the haemodynamic impact of the increase in left ventricular

This review was presented at *The Journal of Physiology* Symposium on *Physiological regulation linked with physical activity and health*, which took place at the 36th International Congress of Physiological Sciences in Kyoto, Japan on 31 July 2009. It was commissioned by the Editorial Board and reflects the views of the authors.

stroke volume that occurs with each contraction of the heart. These arteries receive the stroke volume by expanding and then recoiling, providing the energy to drive blood downstream and ensuring constant flow to the distal organs and tissues. Thus, their role is different from that of peripheral arteries, which are charged with the control of vascular conductance, distribution of blood flow, delivery of oxygen and nutrients, etc.

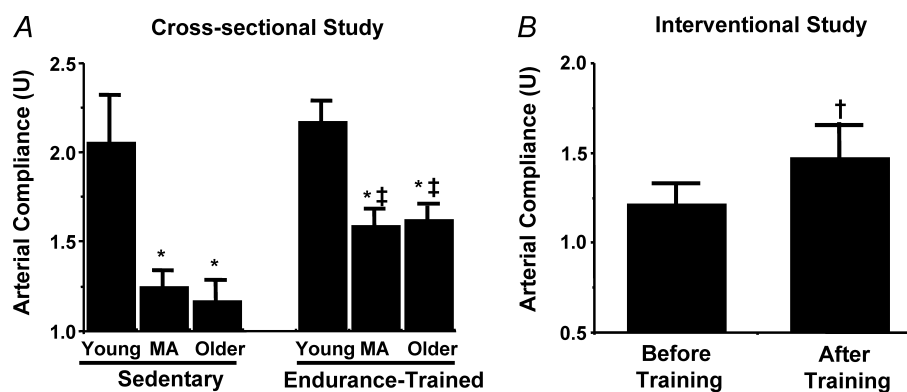
**Changes with age and habitual exercise.** Ageing, in the absence of disease, results in stiffening of large elastic arteries such as the aorta and carotid arteries in both humans and experimental animals (Avolio *et al.* 1985; Reddy *et al.* 2003). Aortic pulse wave velocity, the most common and, perhaps, clinically important measure of large elastic artery stiffness, increases progressively with age in adults without clinical CVD (Avolio *et al.* 1985; Mitchell *et al.* 2004). In turn, directly measured carotid artery compliance (assessed by simultaneous B-mode ultrasound imaging and applanation tonometry) decreases with age in healthy adults without major risk factors for CVD (Tanaka *et al.* 2000; Moreau *et al.* 2003) (Fig. 1). In contrast, peripheral arteries do not stiffen with ageing (Tanaka *et al.* 1998), indicating that increases in stiffness are specific to the large elastic arteries.

Regular aerobic exercise is associated with less stiffening of large elastic arteries with ageing in healthy adults. Aortic pulse wave velocity is lower in middle-aged and older men (Vaitkevicius *et al.* 1993) and postmenopausal women (Tanaka *et al.* 1998) who regularly perform aerobic exercise compared with their healthy sedentary peers and, in some cases, is not different from young controls (Tanaka *et al.* 1998). Consistent with these observations, age-associated reductions in carotid artery compliance in endurance exercise-trained men (Tanaka *et al.* 2000) (Fig. 1) and women (Moreau *et al.* 2003) are only ~50% as great as those observed in healthy non-exercising

adults. The intensity, duration and frequency of aerobic exercise required for improving large elastic artery stiffness and other vascular functions (see below) have not been established. However, in previously sedentary, healthy middle-aged and older men (Tanaka *et al.* 2000) (Fig. 1) and estrogen-replaced postmenopausal women (Moreau *et al.* 2003), 3 months of daily brisk walking restores carotid artery compliance to levels observed in endurance exercise-trained adults of the same age and, in some cases, to levels of young adults (Moreau *et al.* 2003). It is unclear if aerobic exercise is as effective in adults with chronic elevations in systolic blood pressure (Seals *et al.* 2001).

**Mechanisms.** There is surprisingly little direct evidence as to the cellular and molecular mechanisms responsible for age-associated increases in large elastic artery stiffness, and even less information concerning the effects of habitual exercise. A major limitation in this regard is that these arteries are inaccessible in humans, both physically and with respect to experimental manipulation of potential signalling pathways.

It is generally believed that changes in the expression, architecture and/or bioactivity of structural proteins in the walls of large elastic arteries are major contributors to their stiffening with ageing. This is part of an overall remodelling of the extracellular matrix that includes increases in collagen and its cross-linking, fragmentation of elastin, and increases in fibronectin among other changes (Lakatta & Levy, 2003). Although the medial layer of arteries is thought to be the primary site for these changes, the potential role of the adventitia remains largely unexplored. Inflammation has been identified as a possible trigger for initiating these events (Vlachopoulos *et al.* 2005), but direct cause and effect evidence with ageing is lacking. In addition to such structural changes, increases in vascular smooth muscle tone and endothelial dysfunction (McEniery *et al.* 2006; Soucy *et al.* 2006)



**Figure 1. Carotid artery compliance, aging and habitual aerobic exercise**

*A*, carotid artery compliance in sedentary and endurance exercise-trained healthy young, middle-aged (MA) and older men. \* $P < 0.05$  vs. young, ‡ $P < 0.05$  vs. sedentary of same age. *B*, carotid artery compliance before and after 3 months of moderate intensity aerobic exercise in healthy MA and older men. † $P < 0.05$  vs. before training. Values are means  $\pm$  S.E.M. From Tanaka *et al.* (2000). Reproduced with permission from *Journal of Applied Physiology*.

also may contribute to increases in large elastic artery stiffness with ageing. Oxidative stress could be involved, but evidence is inconsistent (Eskurza *et al.* 2004a; Moreau *et al.* 2005).

Little is known about the mechanisms by which regular aerobic exercise suppresses large elastic artery stiffening with ageing. Reduced vascular oxidative stress may explain the enhanced carotid artery compliance in exercising compared with sedentary postmenopausal women (Moreau *et al.* 2006). Increases in carotid artery compliance in response to aerobic exercise intervention in middle-aged and older men (Tanaka *et al.* 2000) and postmenopausal women (Moreau *et al.* 2003) are not related to improvements in conventional risk factors for CVD. Limited data from experimental animals do not show an association between habitual exercise-related reductions in large elastic artery stiffness and changes in total expression of major structural proteins (Nosaka *et al.* 2003).

### Vascular endothelial dysfunction

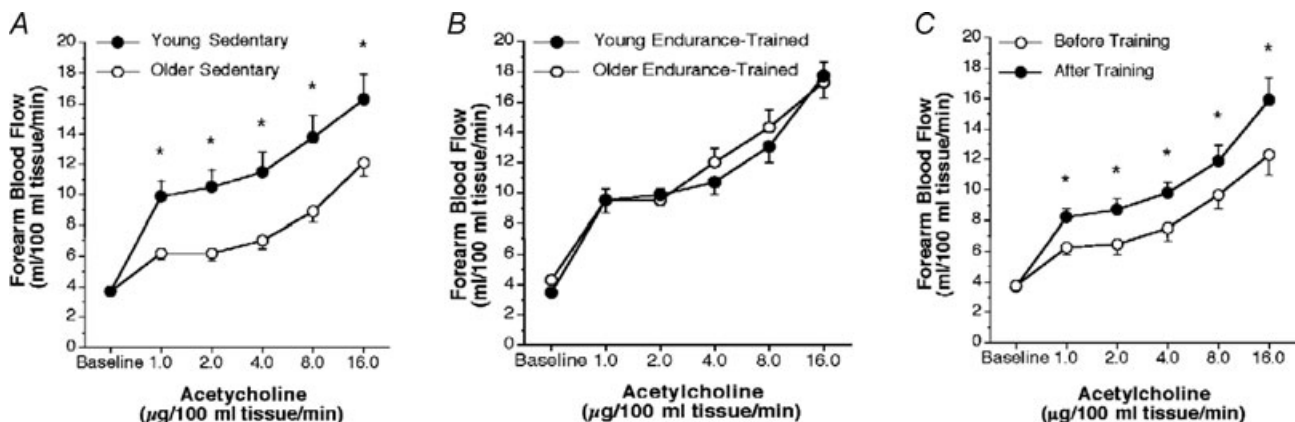
Several expressions of arterial endothelial dysfunction develop with ageing, including impaired endothelium-dependent dilatation (EDD). EDD is evoked by both pharmacological (e.g. acetylcholine) and physiological (increases in blood flow or shear stress-mediated dilatation) stimuli that cause endothelial synthesis and release of dilators including nitric oxide (NO), vasodilatory prostaglandins and endothelial hyperpolarizing factors, with consequent relaxation of vascular smooth muscle.

**Changes with age and habitual exercise.** Ageing is associated with impaired EDD in response to both acetylcholine and increased shear in humans and experimental

animals. In humans, the peak forearm blood flow response to acetylcholine is progressively lower in healthy men and women of increasing age (Taddei *et al.* 1995; DeSouza *et al.* 2000) (Fig. 2). Brachial artery flow-mediated dilatation becomes progressively lower after the ages of approximately 40 and 50 years, respectively, in men and women without CVD (Celermajer *et al.* 1994). EDD is reduced systemically, as well as in isolated conduit arteries and arterioles of old compared with young animals in the absence of atherosclerosis (Muller-Delp *et al.* 2002; Lesniewski *et al.* 2009).

Habitual aerobic exercise is associated with enhanced EDD during ageing, at least in men. Middle-aged and older endurance exercise-trained men demonstrate greater EDD than their healthy, but less active peers (DeSouza *et al.* 2000; Taddei *et al.* 2000; Eskurza *et al.* 2004a) and, in some cases, similar to EDD observed in young men (DeSouza *et al.* 2000; Eskurza *et al.* 2004a) (Fig. 2). In previously sedentary middle-aged and older men, 3 months of daily moderate-intensity walking restores the forearm blood flow responses to acetylcholine to levels seen in age-matched endurance exercise-trained and in young men (DeSouza *et al.* 2000) (Fig. 2). Similar improvements have been observed with an exercise intervention in middle-aged adult males with the metabolic syndrome (Lavrencic *et al.* 2000). It is unknown if habitual aerobic exercise improves EDD in postmenopausal women and, if so, if this effect is similar to that observed in men.

Motorized treadmill exercise training improves EDD in isolated skeletal muscle arterioles from old rats (Spier *et al.* 2004, 2007). Recent work shows that in old mice, even modest volumes of spontaneous wheel running, designed to more closely mimic voluntary aerobic exercise in older humans, are sufficient to restore acetylcholine-mediated dilatation both systemically and in isolated conduit arteries to levels observed in young mice (Durrant *et al.* 2009).



**Figure 2. Endothelium-dependent dilation, aging and habitual aerobic exercise**

Forearm blood flow responses to brachial artery infusion of acetylcholine in healthy men. A, young and older sedentary; B, young and older endurance exercise-trained; C, before and after 3 months of daily walking in older men; \* $P < 0.05$ ; values are mean  $\pm$  s.e.m. From DeSouza *et al.* (2000). Reproduced with permission from *Journal of Applied Physiology*.

**Mechanisms.** Evidence in both humans and experimental animals implicate increased NO bioavailability and reduced oxidative stress in the effects of regular exercise on EDD with aging (Taddei *et al.* 2000; Eskurza *et al.* 2004b; Spier *et al.* 2004; Durrant *et al.* 2009).

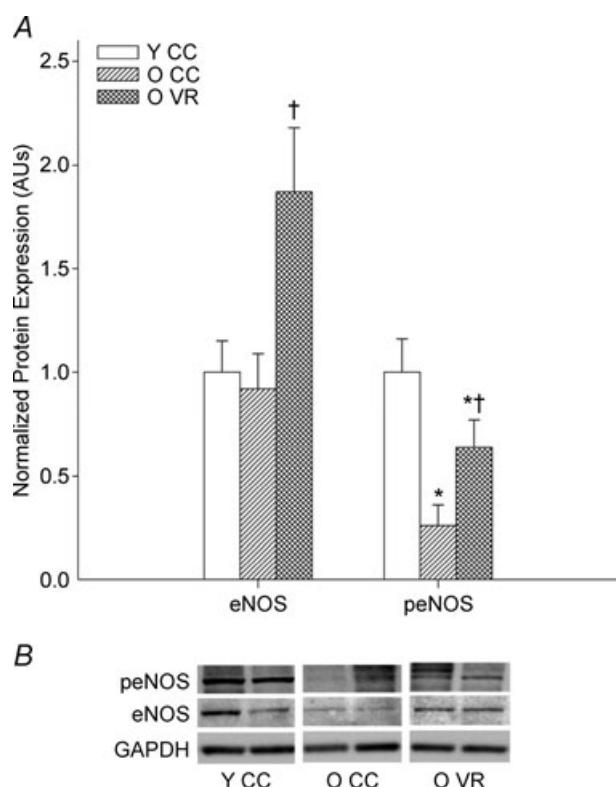
NO. Inhibitors of endothelial NO synthase (eNOS), the enzyme responsible for NO production in the vascular endothelium, have a much greater effect in suppressing resting blood flow (Seals *et al.* 2008) and EDD (Taddei *et al.* 2000; Spier *et al.* 2004; Durrant *et al.* 2009) in exercising older humans and animals than in sedentary controls of similar age, indicating augmented basal and stimulated NO bioavailability. Indeed, differences in EDD between sedentary and aerobically exercise-trained older humans and rodents are largely or completely explained by differences in NO bioavailability (Taddei *et al.* 2000; Spier *et al.* 2004; Durrant *et al.* 2009). Moreover, in previously sedentary healthy middle-aged and older men, daily brisk walking restores basal NO production to levels observed in young men (Seals *et al.* 2008).

eNOS gene and protein expression (Spier *et al.* 2004; Durrant *et al.* 2009) and activation (Durrant *et al.*

2009) are increased in arteries of old exercising rodents compared with sedentary cage controls (Fig. 3), and in mammary arteries of patients with coronary artery disease who perform exercise training compared with untrained controls (Hambrecht *et al.* 2003). No information is currently available on the effects of habitual exercise on eNOS expression or activation in middle-aged and older men and women without clinical CVD.

**Oxidative stress.** Acute administration of antioxidants to middle-aged and older humans (Taddei *et al.* 2000; Eskurza *et al.* 2004b) and isolated conduit arteries of old mice (Durrant *et al.* 2009) improve EDD in the sedentary, but not in the exercise-trained state, consistent with the idea that exercise exerts its effects by reducing oxidative stress-related suppression of EDD. Direct evidence that voluntary aerobic exercise reduces vascular oxidative stress with ageing comes from recent work showing that in old mice given access to running wheels, aortic staining for nitrotyrosine, a cellular marker of oxidative modification of proteins, is markedly lower than in old cage controls and similar to young mice (Durrant *et al.* 2009) (Fig. 4). Nitrotyrosine staining increases with ageing in vascular endothelial cells obtained from healthy sedentary humans (Donato *et al.* 2007), but it is not known if older adults who exercise regularly demonstrate less endothelial cell oxidative stress than their sedentary peers.

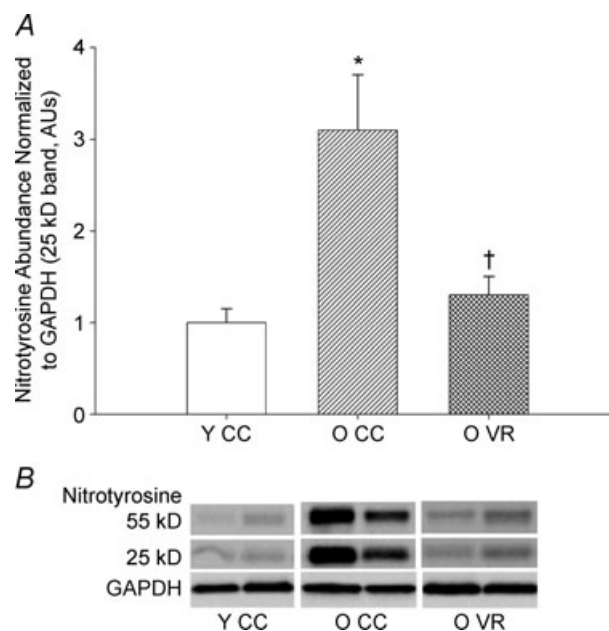
The mechanisms by which habitual aerobic exercise may suppress the development of vascular oxidative



**Figure 3. Endothelial nitric oxide synthase (eNOS), aging and habitual aerobic exercise**

Aortic expression of total and phosphorylated (p-) eNOS in young (Y) and old (O) cage control (CC) and voluntary wheel running (VR) mice. A, summary graph (protein expression normalized to GAPDH).

\* $P < 0.05$  vs. Y CC, † $P < 0.05$  vs. O CC; values are mean  $\pm$  s.e.m. B, representative blot. From Durrant *et al.* (2009).

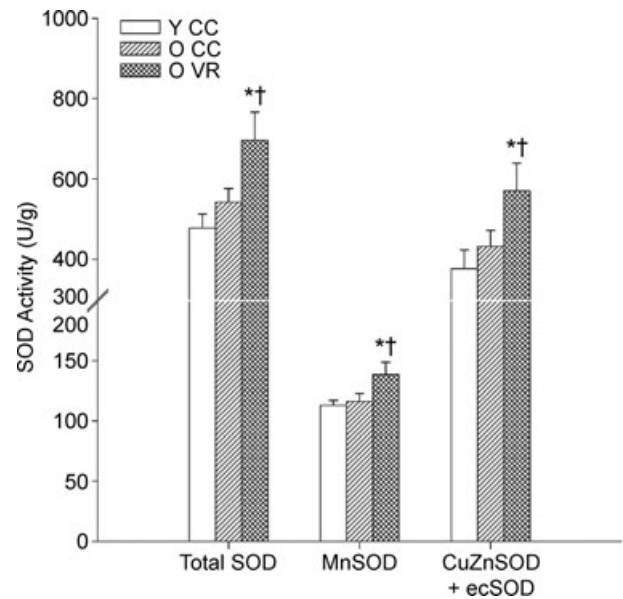


**Figure 4. Nitrotyrosine, aging and habitual aerobic exercise**

Aortic nitrotyrosine abundance in young (Y) and old (O) cage control (CC) and voluntary wheel running (VR) mice. A, summary graphs (protein expression normalized to GAPDH). \* $P < 0.05$  vs. Y CC, † $P < 0.05$  vs. O CC; values are mean  $\pm$  s.e.m. B, representative blot. From Durrant *et al.* (2009).

stress with ageing are incompletely understood. Aerobic exercise-trained patients with coronary disease have lower mammary artery production of reactive oxygen species and expression of the oxidant enzyme NADPH oxidase than patients who do not undergo training (Adams *et al.* 2005). With regard to ageing, recent findings in mice demonstrate that voluntary wheel running in old animals is associated with reduced protein expression of the p67phox subunit of NADPH oxidase and reduced NADPH oxidase activity in the absence of changes in the other major oxidant enzyme found in arteries, xanthine oxidase (Durrant *et al.* 2009) (Fig. 5). Consistent with this, inhibition of NADPH oxidase activity with apocynin improves isolated conduit artery dilatation in response to acetylcholine in old sedentary mice by increasing NO bioavailability, but has no effect on old mice after voluntary wheel running (Durrant *et al.* 2009). Although NADPH oxidase expression increases with age in healthy sedentary adults (Donato *et al.* 2007), it is unknown if older humans who exercise have reduced vascular expression of NADPH oxidase compared with their less active peers.

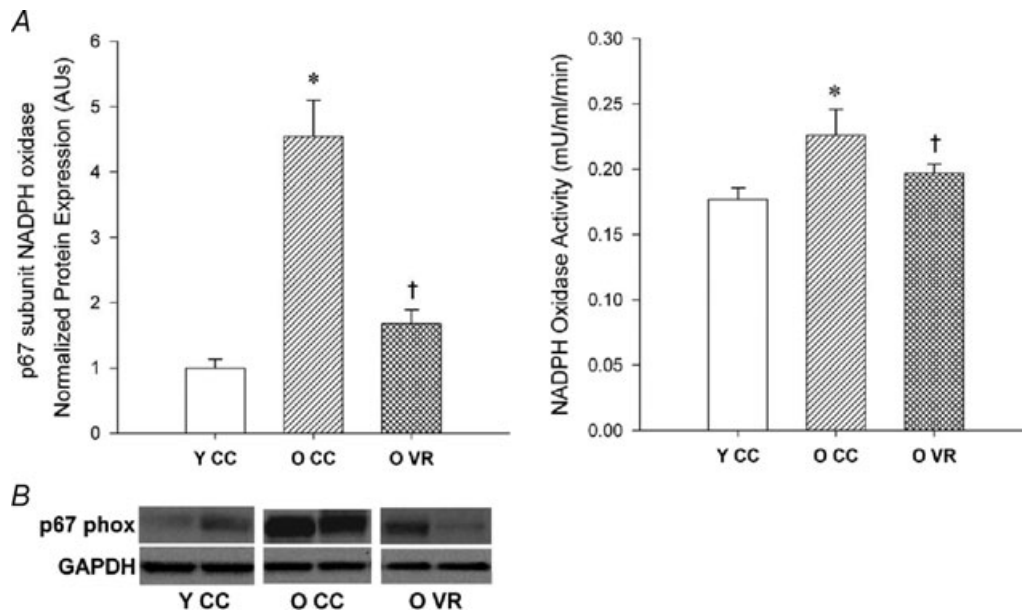
Recent data in mice indicate that the antioxidant enzyme, superoxide dismutase (SOD), may be a key mechanism by which regular aerobic exercise suppresses the development of vascular oxidative stress with ageing. Total SOD, manganese (mitochondrial) SOD and copper–zinc (cytosolic) + extracellular SOD activities are greater in aorta of old mice that perform voluntary wheel running compared with old sedentary animals (Durrant *et al.* 2009). Arterial expression of extracellular



**Figure 6. Superoxide dismutase (SOD) activity, aging and habitual aerobic exercise**

Aortic SOD activity in young (Y) and old (O) cage control (CC) and voluntary wheel running (VR) mice. \* $P < 0.05$  vs. Y CC, † $P < 0.05$  vs. O CC; values are mean  $\pm$  s.e.m. Mn: manganese, CuZn: copper zinc, ec: extracellular; From Durrant *et al.* (2009).

SOD also tends to be greater in old rodents that exercise (Durrant *et al.* 2009; Trott *et al.* 2009) (Fig. 6). Consistent with these findings, treatment of isolated conduit arteries with Tempol, a SOD mimetic, restores dilatation to



**Figure 5. NADPH oxidase, aging and habitual aerobic exercise**

Aortic NADPH oxidase (p67 subunit) in young (Y) and old (O) cage control (CC) and voluntary wheel running (VR) mice. *A*, summary graphs for protein expression (left panel) and enzyme activity (right panel) (protein expression normalized to GAPDH). \* $P < 0.05$  vs. Y CC, † $P < 0.05$  vs. O CC; values are mean  $\pm$  s.e.m. *B*, representative blots. From Durrant *et al.* (2009).

acetylcholine in sedentary old mice by restoring the NO component of EDD, but has no effect in old exercising mice (Durrant *et al.* 2009). It is unknown if older adult humans who exercise have greater vascular expression or circulating activity of SOD compared with sedentary older adults.

**Tetrahydrobiopterin.** Tetrahydrobiopterin (BH<sub>4</sub>) is an essential co-factor for eNOS production of NO. BH<sub>4</sub> is readily oxidized to its biologically inactive form, BH<sub>2</sub>, by peroxynitrite formed by the reaction of NO with the reactive oxygen species molecule superoxide (Milstien & Katusic, 1999). Findings in both humans (Higashi *et al.* 2006) and rodents (Delp *et al.* 2008) implicate reduced BH<sub>4</sub> concentrations or bioactivity in impaired NO-mediated EDD with sedentary ageing. Acute administration of BH<sub>4</sub> improves EDD in healthy older sedentary adults, but has no effect on EDD in young controls or in endurance exercise-trained older adults (Eskurza *et al.* 2005). The latter findings support the idea that preserved bioactivity of BH<sub>4</sub> may be an important mechanism by which habitual exercise enhances EDD in older adults. This could be mediated via reduced oxidative stress, which would preserve BH<sub>4</sub> bioactivity, thus maintaining NO production and EDD.

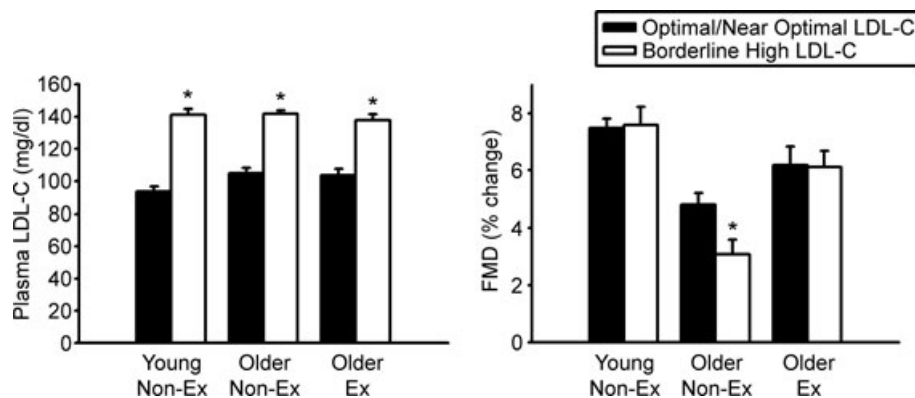
**Endothelin-1.** Endothelin-1 (ET-1) is the most potent vasoconstrictor molecule produced by the vascular endothelium and opposes NO-mediated vasodilatation (Van Gulder *et al.* 2007; Donato *et al.* 2009). Expression of ET-1 in vascular endothelial cells increases with age in sedentary healthy men (Donato *et al.* 2009). Sedentary older adults demonstrate tonic ET<sub>A</sub> receptor-mediated vasoconstriction, which is abolished by aerobic exercise training (Van Gulder *et al.* 2007). In old mice, carotid artery EDD is improved during ET<sub>A</sub> receptor blockade, indicating that ET-1 signalling tonically suppresses EDD with ageing (Donato *et al.* 2009). However, it is unknown

if lower vascular endothelial ET-1 expression and/or bioactivity contribute to the enhanced EDD of older adults who exercise compared with their sedentary peers.

### Protection from adverse effects of risk factors

Up to 60% of the CVD risk-lowering influence of regular aerobic exercise can be explained by its favourable effects on conventional (e.g. body fatness, plasma LDL cholesterol, blood pressure and blood glucose) and non-conventional (e.g. inflammatory/haemostatic markers) risk factors (Mora *et al.* 2007). A novel, complementary hypothesis is that habitual exercise not only improves CVD risk factors, but also protects arteries from the levels of risk factors to which they are exposed. In a broader context, it is possible that sedentary ageing increases our risk of developing CVD by lessening our 'resistance' to these factors, whereas physically active ageing preserves such resistance.

Recent evidence in humans provides support for this hypothesis (Walker *et al.* 2009). Among older sedentary healthy men, those with borderline high plasma LDL-cholesterol demonstrated impaired EDD compared with men who had optimal/near optimal LDL-cholesterol (Fig. 7). In contrast, similar differences in plasma LDL-cholesterol were not associated with differences in EDD among older endurance exercise-trained men (or young untrained men). Thus, older men who regularly performed aerobic exercise appeared to be 'protected' from the adverse effects of plasma LDL-cholesterol on EDD. It is possible that habitual exercise protects arteries against the effects of other risk factors with ageing and/or atherogenic physiological states (e.g. high fat diets, postprandial lipaemia), and that this is an important mechanism by which vascular function is preserved and the risk of CVD is reduced in middle-aged and older adults who exercise.



**Figure 7. Plasma LDL-cholesterol, aging, endothelium-dependent dilation and habitual aerobic exercise**

Plasma low-density lipoprotein cholesterol (LDL-C) (left) and brachial artery flow-mediated dilation (FMD) (right) in young and older non-exercising (Non-Ex) and exercising (Ex) healthy men with either optimal/near optimal (filled bars) or borderline high (open bars) LDL-C; \* $P < 0.05$  vs. optimal/near optimal group of same age and exercise status; values are mean  $\pm$  s.e.m. Reprinted by permission from MacMillan Publishers Ltd from *American Journal of Hypertension* (Walker *et al.* 2009).

## Summary and conclusions

Ageing is the major risk factor for CVD. Stiffening of large elastic arteries and the development of vascular endothelial dysfunction, most commonly measured as impaired EDD, are key contributors to the increased risk of CVD with ageing. Adults who regularly perform aerobic exercise have less large elastic artery stiffness and largely preserved vascular endothelial function with ageing. A moderate-intensity aerobic exercise intervention can improve large elastic artery stiffness in middle-aged men and women and improve/restore EDD in men. It is unknown if regular exercise improves EDD in postmenopausal women.

The mechanisms by which habitual aerobic exercise favourably influences large elastic artery stiffness are unknown, but do not obviously involve modification of conventional CVD risk factors. Current evidence indicates that regular aerobic exercise preserves/restores EDD with ageing by increasing NO bioavailability as a result of increasing the expression and activation of endothelial NO synthase, reducing vascular oxidative stress and increasing the bioactivity of BH<sub>4</sub>. Habitual aerobic exercise lowers oxidative stress in old mice by reducing NADPH oxidase expression/activity-associated superoxide production and increasing SOD activity. Regular exercise also may act to preserve vascular function with advancing age by protecting arteries from the adverse effects of conventional risk factors for CVD.

Taken together, these effects may explain, at least in part, why middle-aged and older adults who exercise demonstrate a higher functional capacity and a lower prevalence of CVD compared with their sedentary peers. Given the increased numbers of older adults today and predicted in the future, regular aerobic exercise can play a major role in the prevention of premature CVD-related morbidity and mortality and the promotion of healthy vascular ageing.

## Future directions

Much remains to be understood about habitual exercise and these arterial changes with ageing. A major void is the absence of information on the mechanisms by which regular aerobic exercise lessens/improves large elastic artery stiffness with ageing. Given the inherent limitations in human investigations, such mechanisms will need to be identified by *in vivo* and *ex vivo* investigations using animal models, and perhaps, cell culture. Studies aimed at identifying the cellular and molecular events involved and the sites of those events (e.g. medial vs. adventitial layers of arteries) are needed.

In this context, the possible role of reduced oxidative stress in habitual exercise-associated attenuation of large elastic stiffening with ageing is of interest (Moreau

*et al.* 2006). The potential involvement of vascular inflammation in the development of arterial stiffness and endothelial dysfunction with ageing, and the intriguing possibility that the anti-vascular ageing effects of regular aerobic exercise may be mediated in part by suppressing the development of inflammation in arteries also merit investigation.

To complement and extend observations from animal models and cell culture, more work is needed on the effects of ageing and habitual exercise on vascular cells and tissue obtained from humans, particularly for studies of endothelial function in which sampling from peripheral sites is both feasible and useful (because dysfunction is observed in peripheral arteries). Novel translational techniques (Rizzoni *et al.* 2001; Colombo *et al.* 2002) are available to pursue such investigations.

Finally, clinical studies focused on optimal types and 'doses' (intensity, duration and frequency) of exercise for preventing and treating these and other expressions of arterial ageing are needed. At present, we have no definitive information as to whether habitual aerobic exercise improves EDD in postmenopausal women, a population at increased risk of CVD as a result of the combination of age and oestrogen deficiency. Investigations aimed at determining the efficacy of regular aerobic exercise in treating large elastic artery stiffness and vascular endothelial dysfunction in middle-aged and older adults with CVD risk factors and/or clinical CVD also are warranted.

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### Author contributions

All authors contributed to drafting and revision of the article and provided final approval of the version to be published.

### Acknowledgements

This work was supported by NIH AG006537, AG013038, AG022241, AG031141, AG015897, AG000279, AG031617 and RR00051 and AHA 0715735Z.