You’re Only as Old as Your Arteries: Translational Strategies for Preserving Vascular Endothelial Function with Aging

Endothelial dysfunction develops with age and increases the risk of age-associated vascular disorders. Nitric oxide insufficiency, oxidative stress, and chronic low-grade inflammation, induced by upregulation of adverse cellular signaling processes and imbalances in stress resistance pathways, mediate endothelial dysfunction with aging. Healthy lifestyle behaviors preserve endothelial function with aging by inhibiting these mechanisms, and novel nutraceutical compounds that favorably modulate these pathways hold promise as a complementary approach for preserving endothelial health.

Populations are aging worldwide. The number of older adults is projected to double over the next few decades, and we are ill-equipped to handle the attendant health care burden. Physiological dysfunction develops with advancing age, which increases the risk of clinical disease and leads to functional limitations. As such, we are facing a new epidemic of chronic degenerative diseases, disability, and associated health care costs driven by population aging (27, 136). An essential strategy for addressing this challenge is to identify and implement approaches that prevent or delay these events until an older age (“compression of dysfunction, morbidity, and disability”) (64, 92, 116). This would extend “healthspan”, i.e., the portion of the lifespan during which function is sufficient to maintain autonomy, control, independence, productivity, and well being (91, 101). The key to achieving this objective is establishing evidence-based approaches that preserve physiological function with aging.

Cardiovascular Disease and Endothelial Dysfunction

Despite declines in prevalence over recent decades, cardiovascular diseases (CVD) remain the leading cause of morbidity, disability, and death in modern societies. Atherosclerosis, characterized by recruitment of white blood cells to the arterial wall, a subsequent accumulation of lipids, and eventual lesion formation, is the main pathophysiological process underlying clinical CVD, and arterial endothelial dysfunction is the primary antecedent for atherosclerotic diseases (69, 77, 98).

The vascular endothelium is a single cell layer that forms the inner lining of arteries and represents the interface between the flow of blood in the lumen and the medial and adventitial layers of the arterial wall (FIGURE 1). The endothelium secretes a broad array of biologically active molecules that act in autocrine and/or paracrine fashion to modulate the function and health of the artery and surrounding tissue (28, 63, 117). In healthy arteries, the balance of the molecules synthesized and released by the endothelium favors vasodilation and inhibition of coagulation, proliferation, and inflammation, whereas endothelial dysfunction can be defined as any phenotype in which this normal functional state is altered (i.e., toward a vasoconstrictor, pro-coagulative, proliferative, and pro-inflammatory profile) (3, 55, 166). Nitric oxide (NO) exerts a vascular-protective influence on all of these processes and is therefore considered to be the most important endothelium-derived molecule. Indeed, sufficient bioavailability of NO is essential for optimal arterial function and health (63, 196).

The most commonly used method of assessing endothelial function is to measure the dilation produced by a mechanical (increase in blood flow or shear rate) or chemical (typically acetylcholine) stimulus that activates endothelial NO synthase (eNOS) and increases the production of NO, resulting in cyclic guanosine monophosphate-induced vascular smooth muscle relaxation, a set of processes collectively termed NO-mediated “endothelium-dependent dilation” (EDD) (FIGURE 1). EDD can also be modulated by other endothelium-derived molecules and processes, including endothelin-1, vasodilatory and vasoconstrictor prostaglandins, and endothelium-dependent hyperpolarization (EDH), induced by an incompletely defined group of endothelium-derived hyperpolarizing factors (EDHF) and/or direct electrical coupling of vascular endothelial and smooth muscle cells (4, 50, 59, 192). As indicated by impaired EDD, vascular endothelial dysfunction
is observed in most forms of clinical CVD, as well as in individuals with major risk factors for CVD (smoking, diabetes, dyslipidemia, hypertension, etc.) (12). Consistent with this, reduced EDD is a predictor of future CVD and CV events (141, 197, 214, 215) and is now viewed as a systemic circulatory disorder affecting multiple domains of organ and tissue function (102, 183).

Endothelial Dysfunction With Aging

Advancing age is the main risk factor for clinical CVD, and this relation is driven largely by the development of arterial dysfunction and disease (98, 113). Importantly for the present discussion, aging is associated with endothelial dysfunction characterized by reduced acetylcholine- and flow-mediated EDD (13, 25, 166, 182, 186) (FIGURE 1). Impaired EDD with aging is observed even in healthy adult humans and experimental animals free of other risk factors or clinical CVD, suggesting a primary effect of age (25, 65, 96, 104, 180, 182, 186). In addition to CVD, endothelial dysfunction is believed to play a major role in several other disorders of aging, including cognitive impairments (brain aging), Alzheimer’s disease, motor dysfunction, insulin resistance, and sarcopenia (6, 76, 138, 174, 184, 205). This age-related endothelial dysfunction is mediated largely by reduced NO bioavailability (13, 55, 181, 186), although a shift to more vasoconstrictor prostaglandin production and increases in endothelin-1 also appear to contribute (46, 125). The impairment in EDD with aging also may be modulated by changes in EDH (122, 216).

Primary Mechanisms

The primary pathophysiological process responsible for reduced NO bioavailability and endothelial dysfunction with aging is oxidative stress, as indicated by increased levels of cellular signatures of oxidative modification of biomolecules such as nitrotyrosine (3, 166). Vascular oxidative stress develops with aging as a result of excessive superoxide production and insufficient compensatory upregulation of antioxidant enzyme defenses (78, 104). Oxidative stress in arteries is bi-directionally associated with chronic low-grade inflammation; increases in the expression of pro-inflammatory factors, including cytokines and adhesion molecules, accompany increases in markers of vascular oxidative stress with age (54, 78). Thus insufficient NO bioavailability, superoxide-driven oxidative stress, and inflammation are the primary mechanisms of endothelial dysfunction with aging (FIGURE 2A).

Vascular NO insufficiency with aging is mediated in part by decreased NO production by eNOS as a consequence of inadequate bioavailability of the essential co-factor, tetrahydrobiopterin (BH₄) (37, 57, 80), and possibly by increased activity of arginase, an enzyme that competes with eNOS for the critical substrate involved in NO production, L-arginine (8, 206, 213). Increased reaction of superoxide with NO due to excessive superoxide also contributes to reductions in NO with vascular aging (3, 79, 166). This reaction leads to accelerated formation of peroxynitrite, a highly reactive nitrogen species that oxidizes BH₄ to its inactive form (BH₃), reducing the bioavailability of this co-factor required for NO synthesis (193), while also causing nitration of tyrosine residues on proteins (nitrotyrosine) (3).

Excessive vascular superoxide production with aging is mediated by increased expression and activity of the potent oxidant-forming enzyme NADPH oxidase, as well as by eNOS uncoupling and increased superoxide formation by the electron transport chain associated with mitochondrial dysfunction (45, 53, 188, 212). Although the relative contributions of these mechanisms are unknown, inhibiting NADPH oxidase (53), inducing eNOS recoupling by increasing BH₄ bioavailability (57, 130, 168), and scavenging of mitochondrial superoxide with mitochondrial-specific antioxidants (67) all improve/restore NO-mediated EDD in older adults and/or rodents.

Vascular inflammation with aging is mediated in part by activation of the master pro-inflammatory transcription factor nuclear factor κB (NF-κB) (44,
peroxide (O$_2^-$) availability and increased oxidative stress/inflammation characterized by increased superoxide dismutase (SOD2) and heme oxygenase (86, 109). Recent work also indicates an important role of sirtuin-1 (SIRT-1), an NAD$^+$-dependent protein deacetylase and ADP-ribosyltransferase with potent energy sensing/mobilizing and anti-aging effects (29, 35, 73), is reduced in aorta of mice and vascular endothelial cells of humans with aging (47, 48, 154). The reduction in arterial SIRT-1 activity accounts for the age-related impairment in EDD in mice (47), and decreases in SIRT-1 expression in arterial endothelial cells with aging in humans are related to corresponding reductions in EDD (47). The mechanisms linking AMPK and SIRT-1 signaling to endothelial function are incompletely understood. These pathways may directly influence eNOS activity (22, 139, 151), although modulation of prostaglandin and/or EDHF regulation of EDD also is a possibility (107).

Finally, cellular stress resistance pathways, including important vascular antioxidant defense systems, are either unchanged or reduced in arteries of rodents (38, 53, 154, 175, 185) and arterial endothelial cells of healthy humans (45, 143) with advancing age, despite marked increases in oxidative stress. Many of these endogenous antioxidant pathways are controlled by the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), which binds to the antioxidant response elements (ARE) in the promoter regions of genes to induce upregulation of antioxidant enzymes such as glutathione, manganese (mitochondrial) superoxide dismutase (SOD2), and heme oxygenase (86, 109). Recent work also indicates an important role for the activated protein-1 transcription factor JunD in regulating vascular superoxide, antioxidant pathways, NO bioavailability, and endothelial function with aging in mice (137).

Taken together, these observations suggest that strategies that act to suppress vascular oxidative stress and chronic low-grade inflammation and boost NO bioavailability and/or other endothelium-dependent vasodilatory signaling pathways should be effective for maintaining healthy endothelial function with aging.

**Strategies for Optimizing Endothelial Function with Aging**

Possible strategies for maintaining optimal endothelial function with aging in humans may have one or more of the following features: 1) evidence...
of vascular-protective effects based on previous preclinical, clinical, or even epidemiological observations; 2) favorably influence the primary mechanisms and/or modulating processes of endothelial dysfunction (FIGURE 2); 3) hold translational promise (feasibility) for use in human populations; 4) show potential to both prevent age-related declines in endothelial function as well as improve/restore endothelial function in middle-aged and older adults with existing dysfunction.

Several translational approaches are available to test candidate interventions (47, 100, 164). For example, stimulus-induced NO release in vitro in endothelial cell culture (18, 103), acetylcholine-evoked dilation of isolated arteries from rodents or humans studied ex vivo (2, 36, 156, 161), and dilation to intra-arterial infusions of acetylcholine in vivo in humans all are well established methods of assessing endothelial function (58, 88, 182, 207) (FIGURE 3). Similarly, “flow-induced” NO release/dilation can be studied in endothelial cell culture (26, 152), in arteries of rodents (97, 133, 173), and in vivo in conduit arteries of humans (24, 74). “Pharmacodissection” of mechanisms influencing EDD also can be explored using these models. Moreover, the same molecular markers of processes of interest (nitrotyrosine, oxidant enzyme expression, inflammatory proteins) can be studied in primary or secondary endothelial cell culture, arteries of rodents, and vascular endothelial cells obtained from human subjects (43, 45, 47, 100, 187). Finally, promising prevention and therapeutic strategies, including lifestyle interventions, can be modeled and studied from cell culture to populations of humans (164).

**Lifestyle-Based Strategies**

**Aerobic exercise.** As reviewed in detail previously (165, 167), regular aerobic exercise has a strongly favorable effect on vascular endothelial function with aging. In men, aerobic exercise appears to largely prevent the decline in EDD with age and to restore EDD in middle-aged and older, previously sedentary subjects to levels observed in young subjects.

![FIGURE 3](http://physiologyonline.physiology.org/) Example of translational assessment of endothelial function

Age-related differences in endothelial function can be examined in cell and animal models, and in human subjects. A: acetylcholine (ACh)-mediated NO production can be assessed in isolated and/or cultured endothelial cells (EC) using NO-specific fluorescent probes and microscopy. B: endothelium-dependent dilation (EDD) can be examined in arteries isolated from young and old rodents (or humans) by measuring increases in arterial diameter to cumulative addition of ACh. C: differences in EDD with aging can also be assessed in human subjects by monitoring changes in forearm blood flow in response to brachial artery infusions of ACh and/or other pharmacological agents. Another example would be flow/shear shear-stress stimulation of endothelial cells in culture, flow-mediated dilation (FMD) of arteries studied ex vivo, and in vivo assessments of FMD in humans (e.g., brachial artery FMD) (see text).
young adults (40, 144, 167). The preservation of endothelial function with aging by aerobic exercise in men is mediated by improved NO bioavailability secondary to reduced vascular oxidative stress (56, 179). Observations in old male rodents are consistent with these findings and show an exercise-associated increase in eNOS expression and activation (53, 172). Restored NO-mediated dilation with voluntary aerobic exercise in old mice also is associated with reduced arterial nitrotyrosine and NADPH oxidase expression/activity, and an increase in superoxide dismutase activity (53). In support of these preclinical results, older sedentary men show an endothelial oxidative stress profile featuring increased endothelial cell nitrotyrosine and NADPH oxidase, and reduced circulating endothelium-derived (extracellular) superoxide dismutase activity compared with young controls, whereas older exercising men do not demonstrate these changes (143). Regular aerobic exercise also has potent anti-inflammatory effects on aging arteries (106) and vascular endothelial cells (143), and this may be mediated in part by suppression of NF-κB signaling (105, 106, 143, 145, 200).

The effects of regular aerobic exercise on endothelial function have been less well studied in women, and the results are not as consistent as those reported in men. Some studies in postmenopausal women have found significant increases in brachial artery FMD in response to an aerobic exercise program (9, 176, 217). In contrast, other recent work indicates that regular aerobic exercise is not associated with enhanced EDD in estrogen-deficient postmenopausal women (23, 144) but that estrogen replacement restores the ability to improve EDD with aerobic exercise training in this group by ameliorating oxidative stress (131).

Little is known about the intensity, frequency, and duration of exercise required to improve endothelial function in middle-aged/older adults. A single bout of aerobic exercise has been reported to improve brachial FMD in postmenopausal women (75). Moreover, low- to moderate-intensity aerobic exercise performed ≥3 days/wk appears to be a sufficient stimulus to improve EDD in the absence of changes in conventional risk factors for CVD (40, 131, 144, 176).

Last, recent work suggests that, in middle-aged and older adults, habitual aerobic exercise may exert direct endothelium-protective effects from adverse circulating factors such as elevated low-density lipoprotein cholesterol (198) and impaired fasting glucose levels (41), probably by increasing resistance to oxidative stress. Indeed, endothelial cell manganese SOD expression and circulating extracellular SOD activity, key antioxidant defense pathways involved in endothelial function (14, 89), are reduced with aging in sedentary healthy men but are fully preserved in older men who exercise (143). Consistent with this notion, aerobic exercise protects against Western diet-induced impairment in EDD in old mice by increasing resistance to superoxide-associated oxidative stress and preserving NO bioavailability (108). Although little information is available on vascular tissue per se, it is likely that some of the beneficial effects of aerobic exercise on arterial function with aging are mediated by activation of the homeostatic/stress resistance pathways mentioned above (17, 60, 111, 134, 153) (FIGURE 4A).

Energy restriction and weight loss. Energy restriction in the absence of malnutrition is associated with numerous beneficial effects on physiological function with aging (120, 203) and, therefore, would appear to be a candidate lifestyle strategy for preserving endothelial function. Consistent with this notion, in mice, lifelong restriction of energy intake preserves EDD with aging by maintaining NO bioavailability secondary to reduced vascular superoxide production and oxidative stress (31, 48, 189). The reduction in oxidative stress is associated with inhibition of NADPH oxidase isoform 2, increases in antioxidant defenses (total SOD and catalase activities), and normalization of stress resistance-nutrient sensing pathways (e.g., SIRT-1), all of which likely contribute to the favorable effects of energy restriction on EDD (48). These vascular endothelial-protective effects of lifelong energy restriction and related mechanisms of action can be recapitulated by short-term (8 wk) caloric restriction in mice begun late in life (154). Similarly, short-term energy restriction-based weight loss largely restores EDD in middle-aged and older overweight/obese adults by increasing NO bioavailability (142). Thus short- or long-term energy restriction appears to enhance NO-mediated endothelial function with aging, and this is associated with reduced oxidative stress and modulation of energy-sensing stress resistance pathways (FIGURE 4A).

Diet composition. The composition of the diet also has an important modulatory influence on endothelial aging. Among middle-aged and older adults who are overweight/obese or have modestly elevated systolic blood pressure, lower sodium intake is associated with greater EDD (84), and dietary sodium restriction improves EDD (42, 85) by increasing NO secondary to reduced superoxide-associated oxidative stress, enhanced BH4 bioavailability, and increased circulating SOD activity (85) (FIGURE 4B). In addition to low-sodium content, diets that are high in fruits, vegetables, fiber, and specific electrolytes (potassium, magnesium, and calcium), and low in total and...
 saturated fat and cholesterol (e.g., Mediterranean and DASH diets) improve EDD in these same groups (11, 93, 123) (FIGURE 4C). Acute ingestion of whey-derived protein extract (5) and cocoa (128) also are reported to enhance EDD in middle-aged or older adults. In general, evidence is mounting for the CVD risk-lowering effects of consumption of seeds, including whole grains, nuts, legumes, cocoa products, and coffee (158), and this is likely to be mediated in part via preservation of endothelial function. Although current evidence is limited, many of these healthy dietary approaches may exert their beneficial endothelial actions in part via activation of cell homeostasis/stress resistance pathways that inhibit oxidative stress and inflammation (118, 119, 163) (FIGURE 4C).

**Alternative Strategies**

Healthy lifestyle behaviors should be considered the “first-line” strategies for prevention and treatment of endothelial dysfunction with aging and are likely to be the most effective overall approach when practiced. However, despite aggressive and informed public advocacy efforts for adopting healthy lifestyle choices, many middle-aged and older adults do not meet minimal guidelines for exercise and do not consume a healthy diet. As a result, there is growing interest in the use of pharmacological agents that induce at least some of the effects of healthy lifestyle behaviors. For simplicity, these will be broadly categorized as either pharmaceutical drugs or nutraceuticals.
Pharmaceutical drugs. Prescription pharmaceutical drugs represent the category of pharmacological agents that have been most thoroughly tested for safety and efficacy in humans, usually in the setting of clinical trials on patients with existing clinical CVD or major risk factors for CVD. Several such agents, including angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, aldosterone receptor antagonists, calcium channel blockers, statins, thiazolidinediones, and more recently developed beta blockers (e.g., nebivolol, carvedilol), generally improve endothelial function in middle-aged and older patients with cardiometabolic diseases or risk factors (10, 195). However, these medications generally are used to slow or prevent progression of an existing disorder and reduce the occurrence of adverse clinical outcomes. They are not indicated per se for primary prevention or treatment of endothelial dysfunction with aging in the absence of clinical disease. Moreover, there is relatively little information on the use of these agents in healthy middle-aged and older adults. Recent work shows that fenofibrate (199), combined fluvastatin and valsartan (statin and angiotensin receptor blocker, ARB) (115), and prescription anti-inflammatory agents (discussed below) reduce oxidative stress and/or inflammation and improve EDD in healthy middle-aged and older subjects, independent of changes in lipids or blood pressure; in contrast, mineralcorticoid receptor blockade (82) and ARB treatment alone (148) had no effect in this population. Although these agents are not approved for treatment of endothelial dysfunction with primary aging, some may be considered for “repurposing” or “off-target” use in older adults without major risk factors/disease based either on currently available efficacy and safety data or on results from additional clinical testing in that population (132, 140). Thus, in the future, these FDA-approved medications may provide therapeutic options to prevent and treat age-associated endothelial dysfunction and its pathophysiological sequelae.

Nutraceutical compounds. A nutraceutical (nutriceutical) is a food or food supplement with naturally occurring ingredients purported to have beneficial effects on human health. Categories of nutraceuticals include dietary supplements, functional foods, and medical foods (211). From a consumer standpoint, this class of compounds is gaining interest in part because of their lower cost (i.e., compared with nongeneric prescribed drugs), access (no medical prescription is needed), and, in many cases, their “natural” (nonsynthetic) origin. From a manufacturing standpoint, these compounds can be attractive because they require a far less costly, risky, and time-consuming regulatory approval process (albeit with less financial reward than a highly profitable patented drug). Nutraceuticals also are attractive in meeting the growing consumer demand for “anti-aging” strategies, and, in response, many compounds now are being developed based on observations from basic biology of aging research (83, 90, 135). Some of these compounds may mimic certain effects of vascular-protective lifestyle strategies. Although a number of clinical trials are underway (clinicaltrials.gov), in general, more uniform evidence for efficacy based on well controlled trials in humans is needed for this category of agents, including clinical outcomes. Other concerns include inconsistency in the composition of nutraceuticals among commercially available formulations and the overall less stringent regulations for use. A brief summary of current information on selective compounds reported to influence age-related endothelial dysfunction and their mechanisms of action is presented below (FIGURE 4, D AND E).

Inorganic nitrite. Nitrite and nitrate, the metabolites of NO oxidation, are now viewed as potential precursor molecules for increasing NO bioavailability and preventing/treating physiological dysfunction in states of NO insufficiency, including settings of CV risk/disease such as essential hypertension (66, 114, 155, 169). Given the primary role of reduced NO bioavailability in endothelial aging, these molecules represent an intriguing strategy for improving NO-mediated endothelial function with advancing age. In agreement with this concept, short-term (3 wk) supplementation of inorganic nitrite (sodium nitrite in drinking water) delivered late in life rescues impaired EDD in mice by restoring NO bioavailability as a result of normalizing vascular oxidative stress (170). The latter is associated with reduced superoxide production via eNOS recoupling and inhibition of NADPH oxidase, as well as increases in antioxidant defenses and reversal of vascular inflammation (170). Results of a recent brief report suggest that increasing circulating nitrite levels via chronic supplementation with sodium nitrate modestly improves EDD in middle-aged/older adults without clinical CVD (149), and an initial clinical trial investigating the efficacy of sodium nitrite supplementation in this population is underway (clinicaltrials.gov). Boosting nitrite and NO bioavailability through increased dietary intake of foods high in nitrate (e.g., green leafy vegetables or beetroot) (15, 110, 218) may exert similar beneficial effects. Thus nitrite/nitrate supplementation is a promising area for preserving vascular health, in general, and endothelial function, in particular, with aging (169).

Antioxidants and other common supplements. Based on the central influence of oxidative stress in mediating endothelial dysfunction with aging, antioxidant administration would seem to hold
strong therapeutic potential. However, chronic supplementation of conventional oral antioxidants (vitamins C, E, etc.) generally has not shown efficacy in patients with CVD (52, 95), and their effectiveness for improving age-associated endothelial dysfunction is unclear. On the one hand, acute administration of supra-physiological concentrations of superoxide-scavenging agents (vitamin C, TEMPOL) essentially restores EDD in older humans and mice (56, 105, 181), as does single oral ingestion of an antioxidant "cocktail" containing vitamins C and E plus lipoic acid (209). Moreover, short-term (3 wk) oral treatment with TEMPOL, a SOD-mimetic, completely reverses endothelial dysfunction in old mice by restoring NO and mitigating vascular oxidative stress and inflammation (61), and 3 mo of vitamin C supplementation improves endothelial fibrinolytic function in overweight/obese middle-aged men (191). On the other hand, chronic (30 days) oral supplementation of vitamin C (500 mg/day) has no effect on EDD in older, otherwise healthy men with impaired baseline endothelial function (56). Given the emerging role of mitochondrial dysfunction and excessive mitochondrial superoxide production in vascular aging (34, 51, 94), mitochondrial-specific antioxidants (e.g., MitoQ), peptides, and/or other "mitochondrial health"-enhancing agents (67, 87, 171, 177, 204) represent a novel alternative approach for preserving endothelial function with aging. Consistent with this notion, short-term (4 wk) supplementation with MitoQ delivered in the drinking water completely restores endothelial function and NO bioavailability in old mice as a result of reduced vascular oxidative stress. These improvements are associated with normalization of vascular mitochondrial superoxide production and increased expression of markers of vascular mitochondrial health (67).

Finally, other dietary nutrients and vitamins such as omega 3 and 6 fatty acids, folate, and vitamin D, also exert protective effects on endothelial function in select populations. There is cross-sectional evidence that, in late middle-aged/older healthy adults, endothelial function is positively related to serum vitamin D [25(OH)D] concentrations and that impaired endothelial function in older adults with lower circulating vitamin D is mediated in part by NF-κB-dependent vascular inflammation (84a). However, the results from existing trials investigating these compounds in middle-aged/older adults without clinical disease are inconclusive (112, 121, 126, 190, 201, 220).

**Anti-inflammatory agents.** Anti-inflammatory compounds represent a complementary strategy to antioxidant supplementation for prevention/treatment of endothelial dysfunction with aging. Short- or longer-term treatment with the nonsteroidal drugs aspirin or closely related salicylates improves NO-mediated EDD in middle-aged and older mice and rats, and this is associated with reductions in oxidative stress (16, 39, 105). Similarly, salsalate, a salicylate-based prescription drug used to treat inflammatory diseases and, more recently, Type 2 diabetes (71), is a potent inhibitor of NF-κB signaling that improves EDD in obese/overweight middle-aged and older adults by reducing endothelial oxidative stress and inflammation (145), although no effects have been reported in patients with Type 2 diabetes or coronary disease (70, 178). Other natural and synthetic anti-inflammatory agents currently available or under development also may prove effective in older adults. In this context, acute administration of the TNF-α inhibitor etanercept recently was shown to improve EDD in healthy estrogen-deficient postmenopausal women (129).

**Tetrahydrobiopterin.** Given the role of reduced BH4 bioactivity in multiple settings of endothelial dysfunction, including older age (127, 162), BH4 is an attractive candidate to recouple eNOS and enhance enzymatic NO production with aging. In healthy middle-aged and older adults, a single dose of BH4 improves EDD by increasing NO bioavailability (57, 80). Chronic (4-8 wk) oral BH4 supplementation increases plasma BH4 concentrations, lowers blood pressure, improves EDD, and reduces circulating markers of oxidative stress in middle-aged and older patients with hypercholesterolemia or resistant hypertension (30, 146). No information is available on the effects of chronic BH4 supplementation on endothelial dysfunction in healthy older adults. Thus BH4-boosting interventions are presently an understudied area for prevention and treatment of endothelial dysfunction with primary aging.

**Autophagy activators.** Stimulating autophagy can enhance physiological function in several non-vascular tissues with aging (147, 219) and, therefore, may well have similar beneficial effects on the vasculature. Indeed, recent work in mice supports the concept that reversing age-related impairments in vascular autophagy enhances endothelial function. Oral supplementation of trehalose, a natural disaccharide that stimulates autophagy (157, 160), restores vascular autophagy and rescues NO-mediated EDD in old mice by normalizing superoxide-related oxidative stress, while also ameliorating vascular inflammation (100). Similarly, the autophagy enhancer spermidine normalizes vascular markers of autophagy and reverses the age-associated impairment in EDD in old mice by restoring NO bioavailability secondary to reduced arterial superoxide production (99). These preclinical observations provide an experimental basis for determining the effects of trehalose,
spermidine, and other autophagy-boosting compounds on endothelial dysfunction with aging in humans.

**Modulators of energy sensing pathways.** There is accumulating evidence that activation of cellular energy sensing pathways may be an effective strategy for preserving endothelial function with aging. These pathways are believed to be involved in mediating many of the benefits of energy restriction on longevity and physiological function (7, 19). Thus compounds that stimulate these cellular processes have the potential to exert similar effects. Daily administration of the AMPK-activator aminomimidazole carboxamide ribonucleotide (AICAR) for 2 wk restores arterial AMPK protein expression and state of activation, and reverses the age-associated impairment in EDD in old mice by ameliorating excessive superoxide suppression of endothelial function, independent of NO or prostaglandin signaling (107). These preclinical findings provide support for conducting translational studies in middle-aged and older humans targeting chronic AMPK activation.

Similarly, activation of SIRT-1 by the nonflavonoid polyphenol, resveratrol, or with red wine improves EDD with aging in rodents fed normal chow or high-fat diets (33, 139), an effect associated with increased eNOS and reduced vascular NADPH oxidase activity, oxidative stress, and inflammation (139). Resveratrol also improves EDD assessed ex vivo in surgically removed superior thyroid arteries of middle-aged and older patients with hypertension and dyslipidemia (22). The improvements were associated with modulation of NO metabolism via AMPK-activation of eNOS, increased bioavailability of BH4, and reduced vascular oxidative stress associated with a Nrf2-dependent increase in the expression of manganese SOD. However, no effects of resveratrol on EDD were observed in age-matched control subjects without these disorders. Acute resveratrol administration is reported to improve brachial artery EDD in middle-aged and older overweight and obese adults with elevated blood pressure (208), and chronic resveratrol supplementation enhances EDD in older obese adults (207a). Thus SIRT-1 activation with resveratrol and perhaps other activators of this energy-sensing molecular network (e.g., NAD+ -boosting agents) may improve endothelial function with aging, but more evidence is needed from trials in humans, including middle-aged and older adults without clinical disease or elevated disease risk.

**Endogenous antioxidant-enhancing compounds.** With the largely failed trials of oral antioxidant supplementation in patients with CVD (95), there has been growing interest in nutraceuticals that boost endogenous antioxidant stress resistance pathways. Several such nutraceuticals upregulate endogenous antioxidant enzymes through activation of the Nrf2-ARE pathway (20, 90).

One of the strongest nutraceutical activators of Nrf2 is curcumin, a nonflavonoid polyphenol found in the curry spice turmeric (21, 194). Ingestion of curcumin-supplemented chow for 4 wk restores EDD in old mice to levels of young controls by increasing NO bioavailability and normalizing superoxide production and oxidative stress (62). The latter were associated with reduced expression of the NADPH oxidase subunit p67 and restoration of manganese SOD (62). Consistent with these preclinical findings, oral supplementation of curcumin improves EDD and lowers circulating C-reactive protein in healthy postmenopausal women without changing plasma lipids or blood pressure (1).

Many other nutraceuticals are reported to have Nrf2-activating actions and may, therefore, enhance endothelial function, including epigallocatechin 3-gallate (EGCG) from green tea, the sulfur-containing nutraceuticals, allicin (garlic) and L-sulforaphane (broccoli), quercitin (apples), rosmarinic acid or carnosic acid (carnosol) (both from rosemary), coffee-derived diterpenes, lycopene (tomato products), shogaol (ginger), and capsaicin (90). Commercially available nutraceutical products, such as Protandim, containing a blend of herbal ingredients, also have purported Nrf2-activating properties and could be effective (49, 194). Establishing the safety and efficacy of Nrf2-ARE-activating nutraceutical compounds for promoting endothelial health with aging in humans will be important, particularly given the recent termination of a clinical trial on bardoxolone methyl, a synthetic triterpenoid Nrf2 activator that was withdrawn because of toxicity (150).

**Future Directions**

Given the broad impending societal effects of the changing demographics of aging, preservation of endothelial function with age is a highly clinically relevant area of physiological research with strong demand for additional investigation. Much more needs to be understood about the genetic/environmental interactions and biological mechanisms that modulate endothelial function with aging. One of the many open issues on this topic is how environmental factors to which we are exposed with aging (e.g., stress, diet, physical activity) interact with homeostatic/stress resistance pathways to modulate oxidative stress/inflammation and thereby influence endothelial function. Such research should provide insight into additional therapeutic targets.
Although there is good evidence that healthy lifestyle behaviors, when practiced, are the most potent “medicine” for preserving endothelial function with aging, many fundamental questions remain unanswered. What type (mode), intensity, frequency, and duration of exercise is necessary to optimize endothelial function across adulthood? What components of a healthy diet are most important, and how much do we need to consume, or is our overall diet the most influential factor rather than specific components (as some recent findings in nutrition research suggest)?

A better understanding of the safety and efficacy of already approved pharmaceutical agents known to improve endothelial function in patients with cardiometabolic risk factors and diseases is required to determine their potential application to middle-aged/older adults without clinical disorders. It has been suggested that aging and clinical CVD involve the same pathophysiological processes and that such clinical states are an accelerated form of aging (98). If so, one could reasonably postulate that established pharmacological therapies in CVD should be effective in primary aging, as this certainly seems to be true for lifestyle factors.

A major developing area of investigation is the use of established or novel nutraceutical compounds with potential “healthy lifestyle-mimicking” effects. The benefits, safety, formulaic consistency, and mechanisms of action of such compounds will need to be demonstrated in well controlled clinical trials. Many compounds show promise in preclinical models, but will they prove effective in aging humans? It is unlikely that such compounds will have the broad health-promoting benefits of healthy lifestyle practices, but selective beneficial effects certainly are possible. Moreover, understanding the interaction between lifestyle, pharmaceutical agents, and nutraceutical agents will be a vital part of future investigation in this area. Potentially negative interactions between statins, antioxidant treatments, and resveratrol with aerobic exercise have been reported (68, 72, 124, 210), suggesting the possibility of unfavorable interactions between certain pharmacological agents and healthy lifestyle strategies. Relatedly, if we successfully identify efficacious lifestyle and pharmaceutical and nutraceutical therapies, do we need to employ several of these approaches or a select few (or only one)? Understanding the pathways activated by these diverse stimuli will be necessary to guide investigation on this issue. Comparative effectiveness analyses and prospective trials likely will be required to address these issues.

Finally, strategies shown to be effective in the prevention and treatment of age-associated endothelial dysfunction might prove beneficial for other clinically important expressions of vascular aging, such as large elastic artery stiffening. For any approach demonstrating promise, it will be essential to establish efficacy (including dose-response) in both middle-aged/older men and postmenopausal women given the unique interactions of sex hormone changes and aging in the two groups. Fortunately, contemporary translational physiological research approaches are now available to pursue these and other important issues concerning arterial health with human aging.

Conclusions

As summarized in FIGURE 4, vascular endothelial dysfunction develops with advancing age and plays a key role in the increased risk of CVD and several other major disorders of aging. Age-associated endothelial dysfunction is mediated largely by NO insufficiency linked to vascular oxidative stress and chronic low-grade inflammation, as modulated by altered cellular homeostasis/stress resistance processes. Physical activity and diet exert a strong influence on endothelial function with aging, but lifestyle-based strategies are limited by low adherence in most human populations. Continued advocacy for broad engagement in these healthy behaviors is essential, because these approaches will have the greatest impact on endothelial function, as well as numerous other (nonvascular) physiological declines with aging. Selective use of established vascular-protective agents also needs to be explored. Last, the development of novel nutraceutical compounds that favorably modulate homeostatic stress resistance pathways and/or adverse aging processes to reduce oxidative stress and inflammation, and enhance bioavailability of NO, holds considerable promise as a complementary approach for maintaining optimal endothelial health with aging.

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