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Aging and vascular endothelial function in humans

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Abstract

Advancing age is the major risk factor for the development of CVD (cardiovascular diseases). This is attributable, in part, to the development of vascular endothelial dysfunction, as indicated by reduced peripheral artery EDD (endothelium-dependent dilation) in response to chemical [typically ACh (acetylcholine)] or mechanical (intravascular shear) stimuli. Reduced bioavailability of the endothelium-synthesized dilating molecule NO (nitric oxide) as a result of oxidative stress is the key mechanism mediating reduced EDD with aging. Vascular oxidative stress increases with age as a consequence of greater production of reactive oxygen species (e.g. superoxide) without a compensatory increase in antioxidant defences. Sources of increased superoxide production include up-regulation of the oxidant enzyme NADPH oxidase, uncoupling of the normally NO-producing enzyme, eNOS (endothelial NO synthase) (due to reduced availability of the cofactor tetrahydrobiopterin) and increased mitochondrial synthesis during oxidative phosphorylation. Increased bioactivity of the potent endothelial-derived constricting factor ET-1 (endothelin-1), reduced endothelial production of/responsiveness to dilatory prostaglandins, the development of vascular inflammation, formation of AGEs (advanced glycation end-products), an increased rate of endothelial apoptosis and reduced expression of oestrogen receptor α (in postmenopausal females) also probably contribute to impaired EDD with aging. Several lifestyle and biological factors modulate vascular endothelial function with aging, including regular aerobic exercise, dietary factors (e.g. processed compared with non-processed foods), body weight/fatness, vitamin D status, menopause/oestrogen deficiency and a number of conventional and non-conventional risk factors for CVD. Given the number of older adults now and in the future, more information is needed on effective strategies for the prevention and treatment of vascular endothelial aging.

Keywords

aging; endothelium-dependent dilation; exercise; inflammation; nitric oxide; oxidative stress

INTRODUCTION

Despite reductions in death rates from CVD (cardiovascular diseases) over the last four decades, CVD remain the leading cause of morbidity and mortality in modern societies [1]. What is less appreciated, perhaps, is that the great majority of CVD are associated with dysfunction of arteries [1].

The effect of aging on CVD is illustrated simply, but powerfully, by the observation that the risk of CVD increases progressively with age [1]. As such, advancing age is the major risk factor for CVD, and in a broad sense, CVD are diseases of aging. Taken together, these facts

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lead to the conclusion that there is something about aging that causes dysfunction of arteries, which, in turn, increases the risk of developing CVD [2].

Given the current and projected increases in the number of older adults, we face the possibility of a 'new wave' of CVD in the near future with an associated increase in healthcare burden. As such, establishing a better understanding of the relationship between arterial aging and CVD represents one of our most important clinical challenges. Determining how arteries change with age to increase our risk of CVD, the mechanisms by which these changes are mediated and strategies for the prevention and treatment of arterial aging are, therefore, among our highest biomedical priorities.

VASCULAR ENDOTHELIAL DYSFUNCTION AND CVD RISK

Several changes to arteries probably contribute to the increase in CVD risk with aging. One of the most clinically important of these is the development of vascular endothelial dysfunction [2,3].

The vascular endothelium is a single layer of cells lining blood vessels that plays a key role in regulating the function and health of arteries [4,5]. Vascular endothelial cells synthesize and release a wide array of biologically active molecules that act in an autocrine or paracrine fashion to modulate arterial structure and vasodilatory, thrombolytic and vasoprotective functions. Arterial endothelial dysfunction refers to functional alterations in the normal endothelial phenotype of arteries that may contribute to the development and clinical expression of atherosclerosis and other vascular disorders [4–6]. These alterations include a shift to a vasoconstrictor, procoagulation, proliferative and pro-inflammatory state [7,8] (Figure 1).

Vascular endothelial dysfunction is observed in several forms of clinical CVD [4,5,9]. Endothelial dysfunction is also associated with major CVD risk factors including smoking, hypercholesterolaemia, hypertension, hyperglycaemia, diabetes, obesity, chronic inflammation and a family history of premature vascular occlusive diseases [4,5]. Vascular endothelial dysfunction is viewed as a key antecedent of clinical arterial diseases and serves as a marker of the inherent risk of developing CVD in an individual or group [5,10]. Given its central role in the development of clinical coronary, cerebrovascular and peripheral artery diseases, vascular endothelial dysfunction is considered an important therapeutic target for reducing the risk of CVD morbidity and mortality [5,11].

ASSESSMENT OF VASCULAR ENDOTHELIAL FUNCTION

Because of the range of biological effects of the vascular endothelium, arterial endothelial function can be assessed using several different approaches that include measuring fibrinolytic function, leucocyte adhesion and inflammatory markers [12–14]. However, the most common approach is to determine vasodilation in response to an endothelium-dependent stimulus, i.e. EDD (endothelium-dependent dilation) [5,8]. Although coronary EDD has been assessed in patients with heart disease and in subjects undergoing diagnosis for coronary disease [15–17], in general, EDD of peripheral arteries has been used to assess vascular endothelial function in humans [4,8]. There are limited data suggesting that peripheral EDD correlates with EDD measured in the coronary arteries [18,19] and, thus, may reflect disease processes in the coronary circulation.

In human subjects, peripheral artery EDD is assessed by two primary methods (Figure 2). These methods, including their respective strengths and limitations, have been described in detail elsewhere [5,8,21,22,26].

One approach is to use a chemical stimulus to evoke EDD [20]. A pharmacological agonist for NO (nitric oxide) synthesis and release from the vascular endothelium [most often ACh (acetylcholine)] is infused into an artery of a limb (usually the brachial artery), and the consequent increase in blood flow to the distal portion of the limb (usually the forearm) is measured using venous occlusion plethysmography [21,22]. A dose–response relationship is established, and group or condition differences are identified either by the slopes of the dose–response curves or the peak blood flows attained. The increase in blood flow reflects the dilation occurring in the resistance vessels (arterioles) of the distal limb. Thus this technique measures EDD of peripheral resistance vessels in response to a chemical stimulus.

The other method uses a mechanical stimulus to evoke an EDD [23]. This approach involves inflating a cuff on a limb (typically the upper forearm) to a suprasystolic external pressure for several minutes and measuring the dilation in a segment of an artery (typically the brachial artery) proximal to the occlusion in response to the acute increase in blood flow produced by rapid deflation of the cuff [24]. The ischaemia-evoked dilation of resistance vessels distal to the occlusion produces a marked temporary increase in blood flow (‘hyperaemic stimulus’) in the proximal conduit arteries that, in turn, causes a FMD (‘flow-mediated dilation’) of those arteries. Thus this procedure assesses the ability of peripheral conduit arteries to dilate in response to the physiological stimulus of an acute increase in intravascular shear produced by an increase in blood flow [25]. Because FMD is a function of the hyperaemic stimulus [25], for proper interpretation, hyperaemia should be assessed and used as a covariate if differences exits. [26–28].

Studies performed in experimental animals have established that the responses evoked by both approaches are ‘endothelium-dependent’ because they are abolished after removal of the vascular endothelium [20,29]. Moreover, the responses are primarily (although not completely) mediated by vascular endothelial production and release of NO because they are markedly attenuated by administration of agents that inhibit NO synthesis by eNOS (endothelial NO synthase), such as L-NMMA (N^G-monomethyl-L-arginine) [30,31]. Vasodilatory prostaglandins and endothelium-derived hyperpolarizing factors are considered to be the other endothelium-derived dilators that contribute to EDD [32,33].

In both experimental approaches, the possibility that group or condition differences observed are due to other (i.e. ‘endothelium-independent’) mechanisms is assessed by determining the vasodilatory responses to intra-arterial infusion of SNP (sodium nitroprusside) (i.a. infusion model) or sublingual administration of nitroglycerine (FMD model). These drugs serve as ‘NO donors’, thus providing a measure of the sensitivity of the vascular smooth muscle cells in the arterial wall to NO [29,34]. An absence of group or condition differences in response to endothelium-independent stimuli in the presence of clear differences in endothelium-dependent responses are interpreted as indicative of vascular endothelium-specific abnormalities in vasodilatory responsiveness.

Both ACh-induced increases in FBF_{ACh} [FBF (forearm blood flow) in response to ACh infusion] and brachial artery FMD provide important clinical insight into the overall health and functional integrity of the vascular endothelium. This conclusion is supported by the facts that both methods identify differences in vascular endothelial function in healthy adults in response to acute conditions that impair or augment EDD [35,36] or compared with adults with risk factors (e.g. chronic smoking, insulin resistance, etc.) and patients with CVD [4,30]. These techniques also predict future CV events, disease and/or prognosis in adults who are healthy at baseline [37–39] as well as in patients with CVD [40–42].

Despite these common features, however, the two methods appear to measure different properties of vascular endothelial vasodilatory capacity in that the responses are not

consistently related within the same individuals [43–45]. This may be due, in part, to the fact that arteriolar and conduit (large) artery function respectively is being assessed [5,43].

FOCUS OF THE PRESENT REVIEW

The present review will focus on the development of vascular endothelial dysfunction with human aging as reflected by impaired EDD. Our emphasis will be on findings available in healthy adults to provide as much insight as possible into the effects of aging as opposed to co-morbidities associated with aging. Observations made from experiments on animal models of arterial aging will be used to highlight cellular and molecular mechanisms. The latter portion of the review will emphasize modulating factors and strategies for the prevention and treatment of vascular endothelial dysfunction with aging.

VASCULAR ENDOTHELIAL DYSFUNCTION WITH AGING

Several lines of experimental evidence indicate that vascular endothelial dysfunction develops with aging in humans in the absence of clinical CVD and major risk factors for CVD. Impaired EDD, reduced fibrinolytic function, increased leucocyte adhesion and/or other markers of endothelial dysfunction have been observed in older compared with young adult humans, as well as rodents and non-human primates [3,46,47].

Chemical stimulation of peripheral artery EDD with aging

In humans, peak FBF_{ACh} decreases progressively with age, and this is observed in both sexes [48–52]. Based on available data, the slope of the decline appears to be less steep in women during the premenopausal years compared with men of similar age, whereas the rate of decrease with age in postmenopausal women is similar to men [48,49,51]. In humans, the impairment in chemically stimulated EDD appears to be agonist specific, as it is not observed with other endothelium-dependent dilators including bradykinin, substance P and isoproterenol [53]. Moreover, unlike FBF, responses to ACh are not obviously reduced with aging in the femoral artery of humans [52], although a ‘systemic’ arterial impairment of EDD has been established with age in mice [54]. These observations suggest that aging may have less of an effect on arteries in the leg compared with the arm, possibly as a result of differences in hydrostatic forces, activity patterns or other reasons. Although physiologically interesting, the clinical importance of such differences are less certain because the forearm, not the femoral, blood flow response to ACh is a predictor of future CVD risk [42,55,56].

FMD of peripheral arteries with aging

Brachial artery FMD is impaired in older compared with young healthy adults [57–63], although such observations can be affected by the methods used [28]. Brachial artery FMD may be preserved in men until approx. 40 years of age and in women until their early 50s [57]. Thereafter the rate of decline may be greater in men than women, although brachial artery FMD consistently is reduced even in healthy men and women by approx. 65 years of age [57]. Although data exist to the contrary [64], decreases in brachial artery FMD with aging appear to be independent of any reductions in the hyperaemic stimulus [60,63,65,66].

Impaired FMD with age also has been observed in the leg in humans [64]. In experimental animals, heterogeneity has been reported in the reductions of FMD in large arteries with age, and this is associated with artery-specific differences in enzymes involved in producing endothelium-modulating factors, including eNOS and SOD (superoxide dismutase) [67]. In rodents, age-related impairments in EDD differ between arterioles in the same tissue or organ (e.g. skeletal muscle) as a result of differences in oxidative capacity and basal blood flow patterns [31].

Coronary and renal artery EDD with aging

EDD in response to ACh is reduced with age in adult humans in both the large epicardial coronary arteries and in coronary resistance vessels [68–70]. In some cases, a paradoxical vasoconstriction of the coronary arteries is observed in older adults [17]. Unlike the large arteries, coronary resistance arteries do not develop atherosclerosis, suggesting that impaired EDD may precede this pathophysiological process. As observed in peripheral arteries, reductions in EDD with age in the coronary circulation are observed in adults without major risk factors or clinical disease [71], consistent with a primary effect of aging.

There is evidence that EDD in the renal circulation also is reduced with aging [72]. Moreover, recent findings indicate that EDD in response to ACh in peripheral arteries is a predictor of future decline in glomerular filtration rate in patients with essential hypertension [73]. However, presently, there is little information concerning the relationship between vascular endothelial and renal dysfunction with aging in the absence of clinical disease.

MECHANISMS OF IMPAIRED EDD WITH AGING

Vascular smooth muscle sensitivity to NO

In general, evidence from studies performed on healthy adults indicates that the vasodilatory responses to NO donors are unchanged with age [50,51,74]. Data from earlier investigations suggested a decrease in endothelium-independent dilation with age, albeit not as great as observed for EDD [48,49,52,75]; however, the subjects studied tended to have a more adverse CVD risk factor profile compared with those assessed in more recent investigations. Overall, it does not appear that reduced vascular smooth muscle sensitivity to NO contributes to reductions in EDD with aging in healthy adults.

NO bioavailability

Data in both humans and experimental animals indicate that impaired EDD with aging is mediated by a decrease in NO bioavailability [31,54,76,77]. This is supported by the facts that the reduction in EDD produced by pharmacological inhibition of NO production by eNOS is smaller with advancing age, and there no longer are significant age group differences in EDD in the absence of NO synthesis [31,54,76].

The mechanisms underlying reduced NO-mediated EDD with age could involve decreased stimulus (pharmacological or flow)-evoked NO production, increased NO removal (see below) or both. The exact contributions of altered NO production compared with removal during EDD are unknown. NO production is reduced in older compared with young adults under baseline resting conditions, as indicated by reduced vasoconstriction in response to infusion of L-NMMA [74]. Moreover, NO production in response to an increase in shear stress is reduced in old animals and contributes to impaired NO-mediated EDD [78].

Despite consistent observations of reduced NO bioavailability, analysis of arterial tissue in experimental animals indicates decreased, increased or unchanged eNOS expression and/or activation (i.e. phosphorylation at Ser^{1177}) with aging [76,79–81]. In healthy humans, eNOS protein expression tends to be greater in vascular endothelial cells obtained from the brachial artery of older compared with young adults [65], whereas eNOS phosphorylated at Ser^{1177} is significantly increased, suggesting a greater state of activation of the enzyme with aging [65] (Figure 3). If so, such activation with age in healthy adults may represent an attempt to compensate for low NO bioavailability.

BH_4 (tetrahydrobiopterin) bioactivity

BH_4 is an essential cofactor for NO synthesis by eNOS [82]. Inadequate availability of BH_4 results in 'uncoupling' of eNOS and synthesis of superoxide anion instead of NO [83–85]. Administration of BH_4 to young and older humans causes a selective improvement in EDD in older adults [60,86] (Figure 4). The EDD-enhancing effect of BH_4 administration in older adults is abolished by L-NMMA [86], suggesting that it is mediated by augmenting NO bioavailability. The mechanism responsible for the reduction in BH_4 bioactivity with aging is unclear. In rodents, BH_4 concentrations in arteries have been reported to be either reduced [87,88] or unchanged [89] with aging. However, consistent with observations in humans, augmenting vascular BH_4 bioavailability in skeletal muscle arterioles of old rats restores NO-mediated flow-induced dilation [87].

ADMA (asymmetric dimethylarginine) and arginase

ADMA, which reduces NO synthesis by competing with the substrate L-arginine for binding sites on eNOS, is increased in some CVD states. However, ADMA is not obviously increased with aging in the absence of disease [63]. Acute L-arginine administration, which should restore any deficit in L-arginine competitive binding to eNOS, does not increase brachial artery FMD in healthy older adults [63], although small increases in FMD have been reported in adults >70 years of age after 14 days of oral supplementation [90]. The lack of a clear role for ADMA in mediating impaired EDD with aging in humans may be explained, in part, by the fact that ADMA and the enzyme that controls its degradation, dimethylarginine dimethylaminohydrolase II, do not differ in vascular endothelial cells from older compared with young adults [63].

There is evidence in rats that the activity of arginase, an enzyme that competes with eNOS for L-arginine, is increased in arteries with aging and contributes to impaired EDD in aortic rings [91–94]. However, neither inhibition of arginase nor administration of L-arginine improves flow-mediated EDD in skeletal muscle arterioles of old rats, whereas increasing BH_4 restores function [87]. As emphasized elsewhere [92], it is possible that differences in the arteries studied contribute to such differences in mechanisms of vascular endothelial dysfunction with aging.

Oxidative stress

Based on markers of oxidant damage such as nitration of tyrosine residues on proteins (nitrotyrosine), oxidative stress is observed with aging in vascular endothelial cells of humans [59] and arteries of experimental animals [54,76,80,95,96]. Several lines of evidence suggest that development of oxidative stress contributes to vascular endothelial dysfunction with aging. In healthy adults varying in age, brachial artery FMD is inversely related to circulating markers of oxidative stress [61,97], as well as to nitrotyrosine staining in vascular endothelial cells [59] (Figure 5). Acute administration of antioxidants such as vitamin C selectively improves or restores EDD in older adults [58,74] (Figure 6).

The mechanisms contributing to arterial oxidative stress-associated vascular endothelial dysfunction with aging appear to involve increased production of reactive oxygen species in the face of unchanged or reduced antioxidant defences. The bioactivity of superoxide and other free radicals are increased with aging in skeletal muscle of humans [98] and in arteries of rodents [80,99,100]. Antioxidant enzyme expression in vascular endothelial cells is not different in young and older healthy adults [59], whereas expression and activity of these enzymes generally are unchanged or reduced with aging in arteries of experimental animals [76,80,101,102]. That SOD mimetics restore EDD in arteries of old rodents [54,80,103] supports a key role for increased superoxide in age-associated vascular endothelial dysfunction.

The sources of superoxide mediating impaired EDD with aging include up-regulation of the oxidant enzyme NADPH oxidase (Figure 7A), uncoupling of eNOS and increased mitochondrial production [59,76,104,105]. In contrast, the available evidence does not support a role for the oxidant enzymes xanthine oxidase or cytochrome *P*450 epoxygenase 2C9, at least in humans [61,106]. Increased superoxide could reduce NO bioavailability and impair EDD with aging by reacting with NO to form peroxynitrite. In turn, peroxynitrite oxidizes BH_4 to its inactive form, which both reduces NO production and increases superoxide production by eNOS [85].

ET-1 (endothelin-1)

ET-1 is the most potent vasoconstrictor molecule produced by the vascular endothelium and is implicated in various CVD states [107]. Plasma ET-1 concentrations increase with age in some adults [65,108], ET-1-mediated vasoconstriction is augmented in older adults [109,110] and synthesis of ET-1 is greater in cultured aortic endothelial cells obtained from older compared with young donors [111]. Recent evidence implicates ET-1 in vascular endothelial dysfunction and oxidative stress with aging. Expression of ET-1 is increased in vascular endothelial cells obtained from brachial arteries and antecubital veins of older compared with young adults (Figure 7B), is inversely related to EDD and is positively related to endothelial cell staining for nitrotyrosine, a marker of oxidant stress [65]. Inhibition of ET-1 signalling with an ET_A receptor antagonist improves EDD in arteries from old mice, while not affecting dilation in young controls [65], and new work indicates that this is mediated, in part, by increased endothelial production and exocytotic release of ET-1 [112].

Inflammation

Inflammation is believed to play an essential role in the aetiology of many CVD [113,114], and evidence is accumulating for similar involvement in vascular aging. Plasma concentrations of inflammatory proteins can increase with age even in healthy adults [59,62,115]. In vascular endothelial cells obtained from the brachial artery and/or antecubital veins of humans, expression of the pro-inflammatory nuclear transcription factor NF-κB (nuclear factor κB; total and nuclear) and pro-inflammatory cytokines IL-6 (interleukin-6), TNF-α (tumour necrosis factor-α) and MCP-1 (monocyte chemoattractant protein-1) are increased in older adults [59,62] (Figure 8). Expression of MCP-1 and matrix metalloproteinases are greater in the thickened arterial intima of older compared with young adult donors obtained during autopsy [116]. Similar observations have been made in experimental animals [117–120].

Among middle-aged and older adults in the Framingham Heart Study [121], brachial FMD is inversely related to plasma markers of inflammation, including CRP (C-reactive protein), IL-6 and ICAM-1 (intercellular adhesion molecule-1). These relationships were no longer significant after correcting for conventional risk factors, suggesting that the latter may be an important stimulus for inflammation with aging. In otherwise healthy overweight and obese middle-aged and older adults, inhibition of NF-κB signalling improves brachial artery FMD to near-normal young control levels by reducing oxidative stress [122], whereas inhibition of TNF-α restores EDD in old rodents [118]. Taken together, these observations suggest that inflammation contributes to the tonic suppression of EDD with aging, perhaps by inducing oxidative stress (and vice versa).

Prostaglandins

EDD in response to prostacyclin is impaired in older compared with young adults, and this is NO-dependent, as the difference is abolished by L-NMMA [123]. Basal (tonic) prostanoid vasodilation also is reduced in older adults [124]. These observations in humans are

consistent with data obtained from animal models, which suggest that the mechanisms involved may include increased expression of prostanoid vasoconstrictor proteins, and altered COX (cyclo-oxygenase) and prostaglandin H synthase activities [125–128].

Other mechanisms of vascular endothelial dysfunction with aging

Renin–angiotensin system activity—Increased renin–angiotensin system activity is implicated in several vascular disorders, and there is evidence for increased vascular expression of AngII (angiotensin II) and ACE (angiotensin-converting enzyme) with aging [129,130]. However, losartan, an AT_1R (AngII type 1 receptor) antagonist, has no effect on brachial FMD in older adults, despite reducing blood pressure and circulating inflammatory markers [131].

AGEs (advanced glycation end-products)—AGEs accumulate in arteries with aging and are believed to contribute to vascular dysfunction, perhaps via fibrosis and remodelling [132,133]. Consistent with this, the AGE cross-link breaker, alagebrium, improves brachial artery FMD, independent of changes in intravascular shear, in older adults with isolated systolic hypertension [134]. Expression of RAGE (receptor for AGEs) is not obviously increased with age in vascular endothelial cells from healthy adults [62]. However, it is possible that increased bioavailability of agonists with aging stimulates signalling with an unchanged expression of the receptor. In any case, increased concentrations of AGEs may be an important mechanism in age-associated endothelial dysfunction.

Increased apoptosis—Apoptosis, i.e. programmed cell death, is thought to be accelerated in the endothelium of several CVD states associated with vascular dysfunction [135]. Non-human primates demonstrate increased apoptosis and reduced density of endothelial cells with aging, and this is associated with impaired systemic EDD [136]. Thus an increased rate of endothelial apoptosis may decrease the number of healthy, normally functioning, vascular endothelial cells with aging and contribute to vascular endothelial dysfunction.

Reduced ERα (oestrogen receptor α) signalling—Recent findings indicate that expression of ERα, the primary receptor involved in oestrogen modulation of vascular function [137], is lower in vascular endothelial cells obtained from oestrogen-deficient postmenopausal women compared with premenopausal women [138]. In the overall group, ERα expression was positively related to brachial artery FMD, as well as to vascular endothelial cell expression of eNOS and eNOS phosphorylated at Ser^{1177} [138] (Figure 9). These observations are consistent with the idea that circulating oestrogen deficiency may lead to down-regulation of ERα and impaired EDD in postmenopausal women, in part as a result of reduced eNOS expression and activation.

MODULATING FACTORS AND STRATEGIES FOR PREVENTION AND TREATMENT

Several factors appear to modulate EDD with aging, and strategies aimed at modifying these factors may have efficacy in the prevention and treatment of age-related vascular endothelial dysfunction.

Regular exercise

This topic has been reviewed in detail recently [139,140]. The results of both cross-section comparisons of exercise-trained and sedentary adults and intervention studies clearly demonstrate that regular aerobic exercise is associated with enhanced EDD (both brachial

FMD and FBF_{ACh}) compared with the sedentary state in middle-aged and older men [50,58,60,75] (Figure 10). A similarly consistent effect of aerobic exercise on EDD has not been established in postmenopausal women [66,141,142]. Indeed, recent evidence indicates that brachial artery FMD is not influenced by moderate or vigorous aerobic exercise in many/most healthy postmenopausal women [141]. The mechanisms underlying possible sex-specific effects are not presently understood. The minimal available evidence suggests that regular resistance exercise has no influence on EDD, at least in postmenopausal women [142].

In middle-aged and older men, regular aerobic exercise enhances EDD by increasing NO bioavailability [75], as a consequence of reduced oxidative stress [58,75], perhaps mediated, in part, via preservation of BH_4 bioactivity [60]. Basal NO production also is maintained with aging in men who exercise [139]. Results of studies on rodents indicate that the cellular and molecular mechanisms involved include increases in eNOS expression and activity, reduced expression and activity of NADPH oxidase and increased SOD activity [76,143], as well as enhanced prostacyclin activity [144]. The stimulus for these changes may be intravascular shear, as experimental increases in flow induce eNOS and improve NO bioavailability and EDD in arteries from old rats [145].

Regular exercise also may preserve EDD with aging by protecting arteries against the deleterious effects of potentially ‘adverse’ factors, including conventional CVD risk factors such as LDL (low-density lipoprotein)-cholesterol [140,146].

Dietary factors

Several dietary factors may influence vascular endothelial function with aging. Low-sodium intake is associated with enhanced brachial artery FMD in middle-aged and older adults with elevated systolic blood pressure [147] (Figure 11), and dietary sodium restriction improves brachial FMD in overweight and obese adults [148]. Increasing servings of fruits and vegetables improves FBF_{ACh} in middle-aged and older adults with systolic hypertension [149], and a DASH (Dietary Approaches to Stop Hypertension) diet emphasizing reduced total and saturated fat and cholesterol and increased dietary fibre, potassium, magnesium and calcium improves brachial FMD in non-medicated middle-aged and older adults with modestly elevated systolic blood pressure [150].

Weight loss/caloric restriction

Energy-intake-restriction-induced body weight loss alone improves both NO-mediated FBF_{ACh} and brachial FMD in young, middle-aged and older overweight and obese adults [44] (Figure 12), with improvements related, in part, to reductions in body fatness [44]. Consistent with this, short-term caloric restriction in old mice improves NO-mediated dilation to ACh by increasing eNOS protein, reducing oxidative stress (via reductions in NADPH oxidase-mediated superoxide production and stimulation of SOD antioxidant activity) and restoring expression of the anti-aging enzyme sirtuin-1 [80]. Lifelong caloric restriction also preserves EDD in rodents by what appears to be similar mechanisms of action [151,152].

Vitamin D status

Brachial artery FMD is lower in healthy middle-aged and older adults with insufficient or deficient circulating vitamin D (serum 25-hydroxyvitamin D) compared with their vitamin D-sufficient peers, and overall, FMD is positively related to vitamin D status in this group [153] (Figure 13). The lower FMD in vitamin D-insufficient and -deficient middle-aged/older adults is mediated, in part, by increased NF-κB-related pro-inflammatory signalling

and is associated with reduced endothelial cell vitamin D receptor expression [153] (Figure 13).

Menopause and oestrogen deficiency

EDD is reduced in oestrogen-deficient postmenopausal compared with premenopausal women [49,57,138], even early in menopause [49,57], and is related to severity of hot flushes [154,155]. This impairment in EDD appears to be dependent on circulating sex hormone status, particularly oestrogen. The primary evidence is that ovariectomy results in reduced EDD [156,157] and administration of oestrogen increases EDD (both brachial FMD and FBF_{ACh}) in some postmenopausal women [156–159] as a result of enhanced NO bioavailability [157,160,161] and reduced COX activity [157]. However, oestrogen replacement does not always improve vascular endothelial function in this group [158,162–164]. The age of the postmenopausal women [165,166], the number of years postmenopausal (i.e. oestrogen-deficient) [166,167], the presence/absence of CVD [165,168] and the inclusion/exclusion of progestin [169–171] probably contribute to the surprisingly inconsistent findings on this topic.

Risk factors for CVD

Several conventional risk factors for CVD that are independently associated with EDD change with advancing age in a manner that could contribute to age-related vascular endothelial dysfunction.

Hypertension—Hypertension is associated with vascular endothelial dysfunction as indicated by impaired EDD [48,172]. Arterial blood pressure, particularly systolic pressure, increases with age [2], and middle-aged and older patients with essential hypertension have lower EDD than age-and sex-matched adults with normal blood pressure [173,174] (Figure 14). Therefore, maintaining arterial blood pressure within the normal range may act to preserve endothelial function during aging.

Hypercholesterolaemia—Hypercholesterolaemia also is associated with impaired EDD [175]. Plasma LDL-cholesterol increases with age [176], and middle-aged and older adults with even borderline elevations in LDL-cholesterol demonstrate impaired brachial artery FMD compared with their peers with optimal/near-optimal concentrations [146]. This modulatory influence is age-dependent in that it is not observed in young adults [51] (Figure 15). Lipid-lowering agents that activate selective energy-sensing pathways, such as fenofibrate and niacin, improve vascular function in middle-aged and older patients with Type 2 diabetes [177,178]. These and other agents may prove effective for improving age-associated vascular endothelial dysfunction, as recently shown for large elastic artery stiffness [179].

Overweight/obesity—Increased body fatness, particularly abdominal accumulation of fat, and obesity generally are associated with reduced EDD [180,181]. Because total and abdominal fatness and the prevalence of overweight/obesity increase with age, it is possible that changes in body fat or its distribution contribute to reductions in EDD with aging. Cross-sectional findings of inverse relationships between measures of EDD and body fat in small samples of adults varying with age are consistent with this possibility [50,182], as are the aforementioned results from weight loss intervention in humans and short-term calorie restriction in old mice [44]. However, recent evidence suggests that peripheral body fatness may actually be protective against the development of CVD [183–185]. Given that vascular endothelial dysfunction is a risk factor for CVD, more definitive insight as to the exact relationships between body fat, its distribution and EDD is needed in a larger cohort of subjects.

Non-conventional risk factors—Finally, non-conventional risk factors also may modulate vascular endothelial function with aging. For example, among middle-aged and older adults, FBF_{ACh} is inversely related to white blood cell count (neutrophil, eosinophil and monocyte counts), even within the clinically normal range [186] (Figure 16). Impaired EDD in individuals with higher white blood cell count is associated with lower NO bioavailability linked to reduced BH_4 bioactivity and with increased circulating concentrations of the oxidant enzyme myeloperoxidase. Data from experimental animals suggest that circadian dysregulation also may contribute to vascular endothelial dysfunction with aging by reducing eNOS-related NO production [187].

Other interventions

Given the need to identify strategies that can be used to prevent and treat age-associated vascular endothelial dysfunction, more clinical research studies are needed to establish the efficacy of novel interventions. Among these, new approaches that are effective in reversing oxidative stress and inflammation in the vascular endothelium with aging would seem particularly compelling, as standard antioxidant treatments have proven ineffective [58,188]. Lifestyle-based interventions should continue to be investigated as first-line therapeutic options and the potential beneficial effects on vascular endothelial function in middle-aged and older adults established. However, given the limitations with adoption of and long-term adherence to lifestyle interventions, in general, and for older adults specifically (e.g. because of limited access to resources or disability), it is likely that innovative, low-cost pharmaceutical and ‘nutraceutical’ treatment options also will be needed. The efficacy of some oral agents will need to be established first using preclinical models, but several promising ‘anti-aging’ pharmacological agents, nutraceutical compounds and dietary interventions are ready for translational vascular health studies in middle-aged and older adults.

SUMMARY AND CONCLUSIONS

Aging is the primary risk factor for CVD, and the development of vascular endothelial dysfunction is a key mechanism linking older age to increased risk of clinical CVD. Age-associated endothelial dysfunction, as indicated most commonly by impaired EDD, is mediated by reduced NO bioavailability and also possibly by decreased responsiveness to endothelial-released vasodilatory prostaglandins. Oxidative stress and inflammation are major ‘macromechanisms’ by which aging leads to reduced NO bioavailability and EDD. Vascular oxidative stress develops with aging as a result of increased production of reactive oxygen species, such as superoxide anion, in the face of unchanged or reduced antioxidant defences. Oxidative stress may reduce NO bioavailability and EDD with aging, in part, via oxidation of BH_4, which leads to uncoupling of eNOS. Increases in the endothelial vasoconstrictor molecule, endothelin-1, also appear to contribute to impaired EDD with aging. Several factors influence or may influence vascular endothelial function with aging, including regular aerobic exercise, dietary factors, body fatness, vitamin D status, menopause/oestrogen deficiency and conventional and non-conventional risk factors for CVD. Given the increasing numbers of older adults and associated health care burden, effective strategies are needed for the prevention and treatment of age-related vascular endothelial dysfunction.

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Abbreviations

ACh	acetylcholine
ADMA	asymmetric dimethylarginine
AGE	advanced glycation end-product
AngII	angiotensin II
BH_4	tetrahydrobiopterin
COX	cyclo-oxygenase
CVD	cardiovascular disease(s)
EDD	endothelium-dependent dilation
eNOS	endothelial NO synthase
ERα	oestrogen receptor α
ET-1	endothelin-1
FBF	forearm blood flow
FBF_{Ach}	FBF in response to ACh
FMD	flow-mediated dilation
IL-6	interleukin-6
LDL	low-density lipoprotein
L-NMMA	N^G-monomethyl-L-arginine
MCP-1	monocyte chemoattractant protein-1
NF-κB	nuclear factor-κB
SOD	superoxide dismutase
TNF-α	tumour necrosis factor-α

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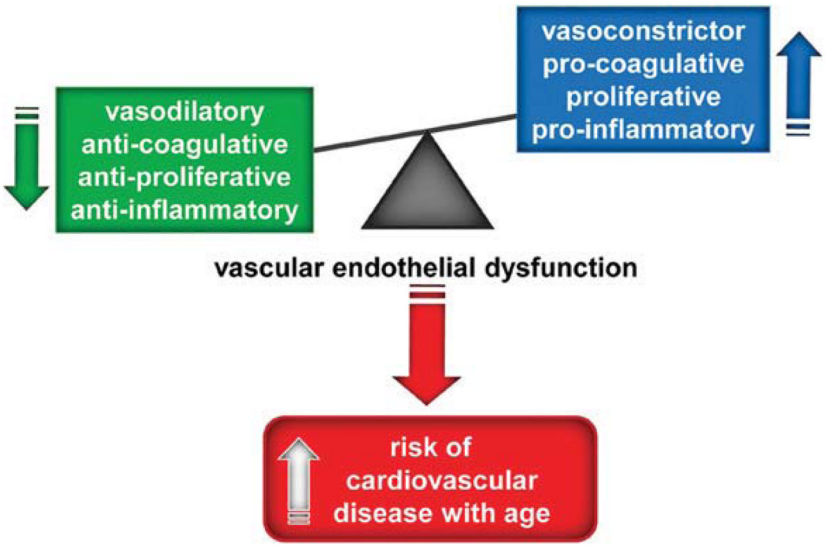
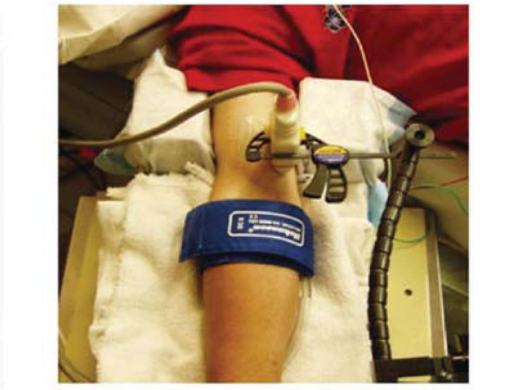
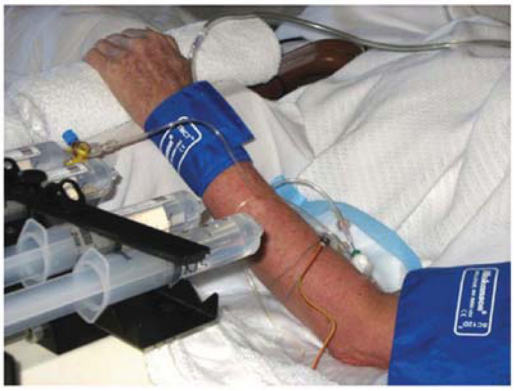


Figure 1. Vascular endothelial dysfunction and risk of CVD with aging

Vascular endothelial dysfunction is characterized by a shift from a vasodilatory, anti-coagulative, anti-proliferative and anti-inflammatory state to a vasoconstrictor, pro-coagulative, pro-proliferative and pro-inflammatory state, leading to an increased risk of cardiovascular disease with aging.



Brachial FMD Model



Forearm Blood Flow Model

Figure 2. Models to assess EDD in humans

Brachial artery FMD and FBF models for assessing EDD.

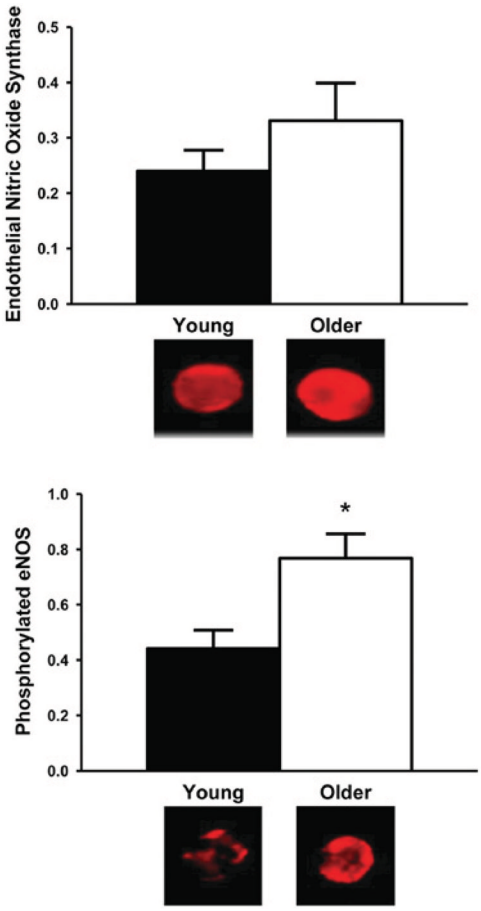


Figure 3. Endothelial eNOS protein expression and activation with aging in men

In vascular endothelial cells collected from the brachial artery of healthy human subjects, eNOS protein expression tends to be greater in older compared with young subjects, whereas eNOS phosphorylated at Ser^{1177} is significantly increased, suggesting a greater state of activation of the enzyme with aging. $*P < 0.05$ compared with young subjects; values are ratios to HUVEC control; representative images are shown below the histograms. Reproduced from [65], with permission. © (2009) The American Physiological Society.

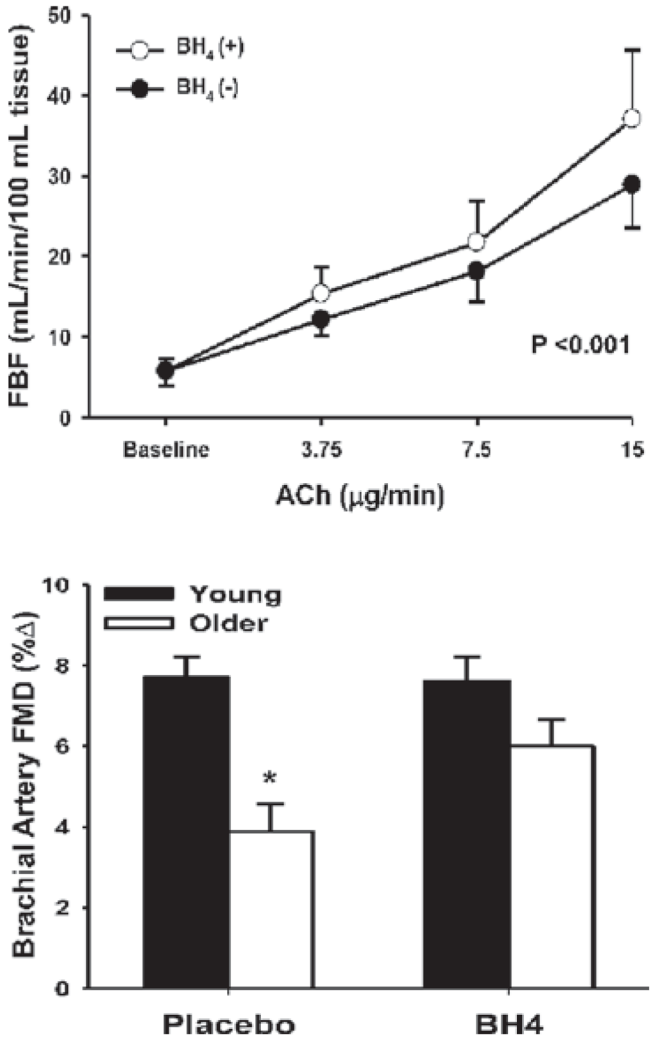


Figure 4. BH_4 and impaired EDD with aging in men

Administration of BH_4 improves endothelium-dependent dilation in middle-aged/older adults, as measured by FBF in response to ACh (upper panel) and brachial artery FMD (lower panel). *$P < 0.05$ compared with young adults. The upper panel was reprinted from *Atherosclerosis*, volume 186, Higashi, Y., Sasaki, S., Nakagawa, K., Kimura, M., Noma, K., Hara, K., Jitsuiki, D., Goto, C., Oshima, T., Chayama, K. and Yoshizumi, M., Tetrahydrobiopterin improves aging-related impairment of endothelium-dependent vasodilation through increase in nitric oxide production, pp. 390–395, copyright (2006), with permission from Elsevier (http://www.sciencedirect.com/science/journal/00219150). The lower panel was re-drawn from data in [60].

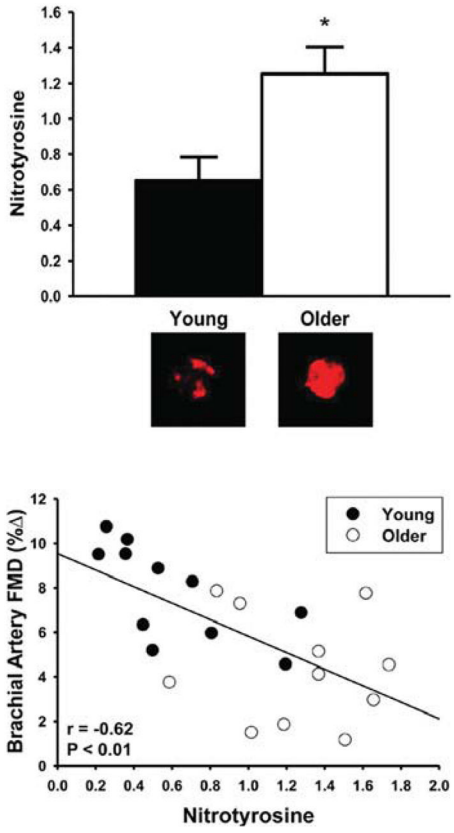


Figure 5. Endothelial nitrotyrosine and EDD with aging in men

Brachial artery endothelial cell staining for nitrotyrosine is greater in older compared with young healthy subjects (upper panel) and is inversely related to brachial artery FMD in the overall group (lower panel). **P* = 0.01 compared with young adults. Values are ratios to HUVEC control and representative images are shown below the histogram. Reproduced with permission from Donato, A.J., Eskurza, I., Silver, A.E., Levy, A.S., Pierce, G.L., Gates, P.E. and Seals, D.R., Direct evidence of endothelial oxidative stress with aging in humans: relation to impaired endothelium-dependent dilation and upregulation of nuclear factor-κB, Circ. Res. 100 (11), pp. 1659–1666. © (2007) Wolters Kluwer Health.

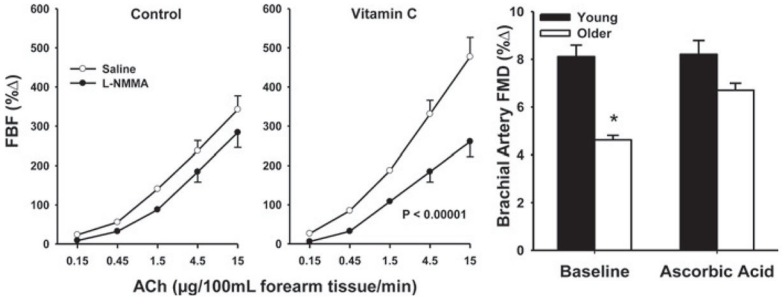


Figure 6. Oxidative-stress-related impairment of EDD with aging in humans

Acute administration of ascorbic acid (vitamin C) improves EDD in older adults, as measured by FBF to ACh (left-hand and middle panels) and brachial artery FMD (right-hand panel). $*P < 0.05$ compared with young adults. The left-hand and middle panels were reproduced with permission from Taddei, S., Galetta, F., Virdis, A., Ghiadoni, L., Salvetti, G., Franzoni, F., Giusti, C. and Salvetti, A., Physical activity prevents age-related impairment in nitric oxide availability in elderly athletes, Circulation 101 (25), pp. 2896–2901. © (2000) Wolters Kluwer Health. The right-hand panel was re-drawn from data in [58].

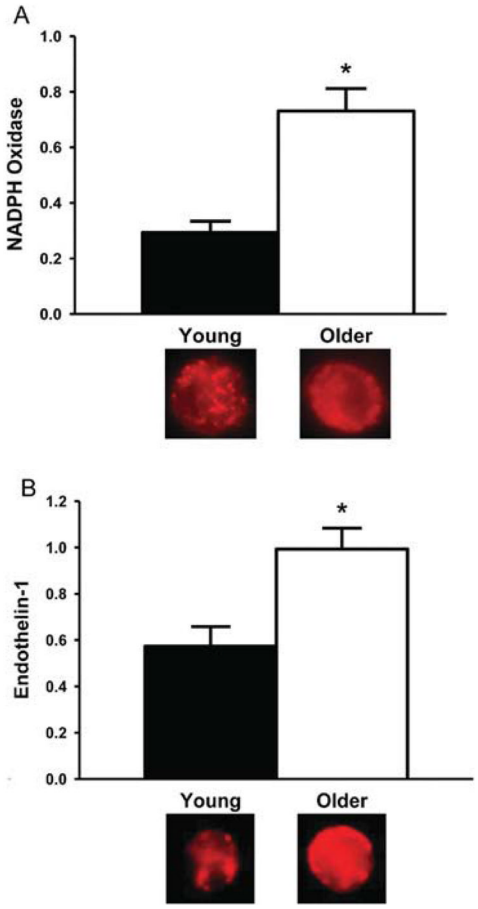


Figure 7. Endothelial NADPH oxidase and ET-1 with aging in humans

Brachial artery endothelial cell protein expression of (A) NADPH oxidase and (B) ET-1 are greater in older and young healthy adults. $*P < 0.05$ compared with young adults. Values are ratios to HUVEC control, and representative images are shown below the histograms. The results in (A) are from A.J. Donato, G.L. Pierce and D.R. Seals, unpublished work. Panel (B) was reproduced from [65], with permission. © (2009) The American Physiological Society.

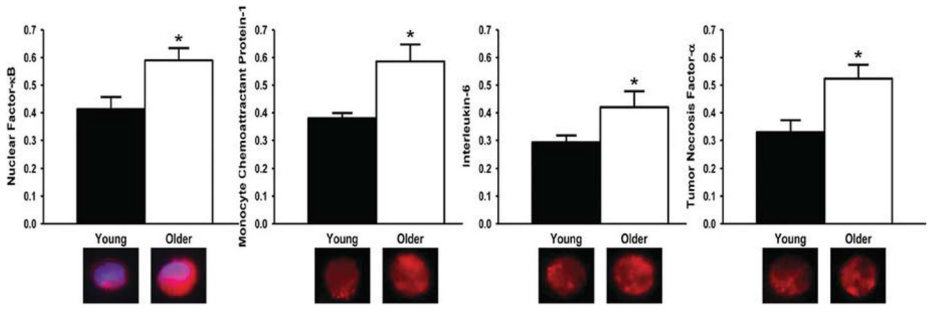


Figure 8. Endothelial pro-inflammatory proteins and aging in humans

In vascular endothelial cells obtained from an antecubital vein, expression of NF-κB, MCP-1, IL-6 and TNF-α are greater in older compared with young adults. *$P < 0.05$ compared with young adults. Values are ratios to HUVEC control, and representative images are shown below the histograms. Reproduced from Donato, A.J., Black, A.D., Jablonski, K.L., Gano, L.B. and Seals, D.R., Aging Cell, with permission. © (2008) Blackwell Publishing Ltd/Anatomical Society of Great Britain and Ireland (http://onlinelibrary.wiley.com/journal/10.1111/%28ISSN%291474-9726).

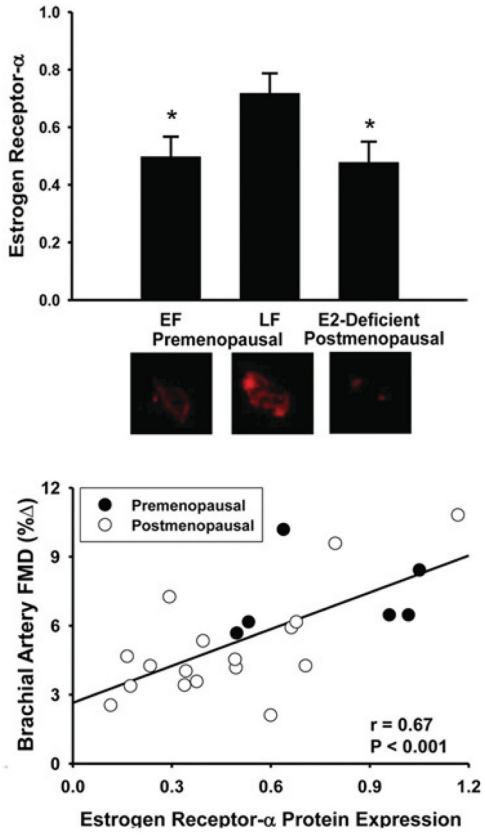


Figure 9. ERα and EDD with aging in women

In vascular endothelial cells obtained from an antecubital vein, ERα expression is lower in oestrogen-deficient postmenopausal women compared with premenopausal women in the late follicular (LF; high oestrogen), but not early follicular (EF; low oestrogen) phase (upper panel) and is related to brachial artery FMD in the overall group (lower panel). *$P < 0.001$ compared with LF. Values are ratios to HUVEC controls, and representative images are shown below the histograms. Reproduced with permission from Gavin, K.M., Seals, D.R., Silver, A.E. and Moreau, K.L., J. Clin. Endocrinol. Metab., Vascular endothelial estrogen receptor α is modulated by estrogen status and related to endothelial function and endothelial nitric oxide synthase in healthy women, volume 94 (9), September 2009, pp. 3513–3520. *Copyright 2009, The Endocrine Society.*

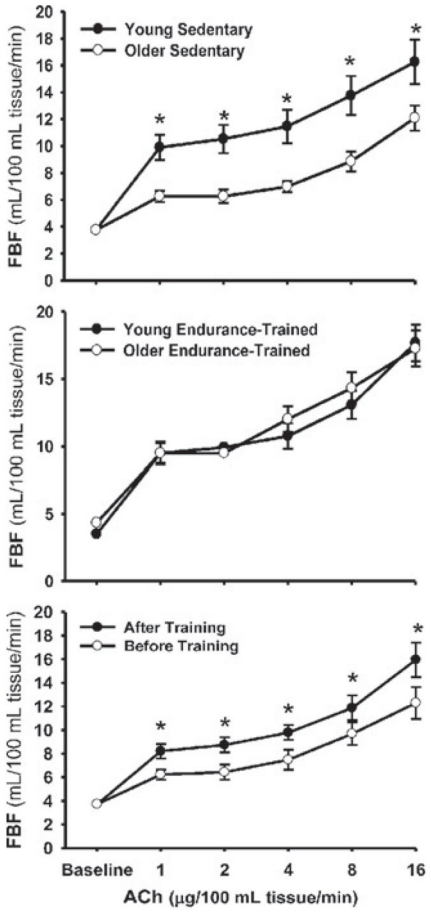


Figure 10. Regular aerobic exercise and EDD with aging in men

FBF responses to ACh in sedentary (top panel) and endurance-exercise-trained (middle panel) healthy young and middle-aged/older men and before and after an aerobic exercise intervention in older men (bottom panel). $*P < 0.05$. Reproduced from DeSouza, C.A., Shapiro, L.F., Clevenger, C.M., Dinenno, F.A., Monahan, K.D., Tanaka, H. and Seals, D.R., Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men, Circulation, volume 102 (12), pp. 1351–1357. © (2000) Wolters Kluwer Health.

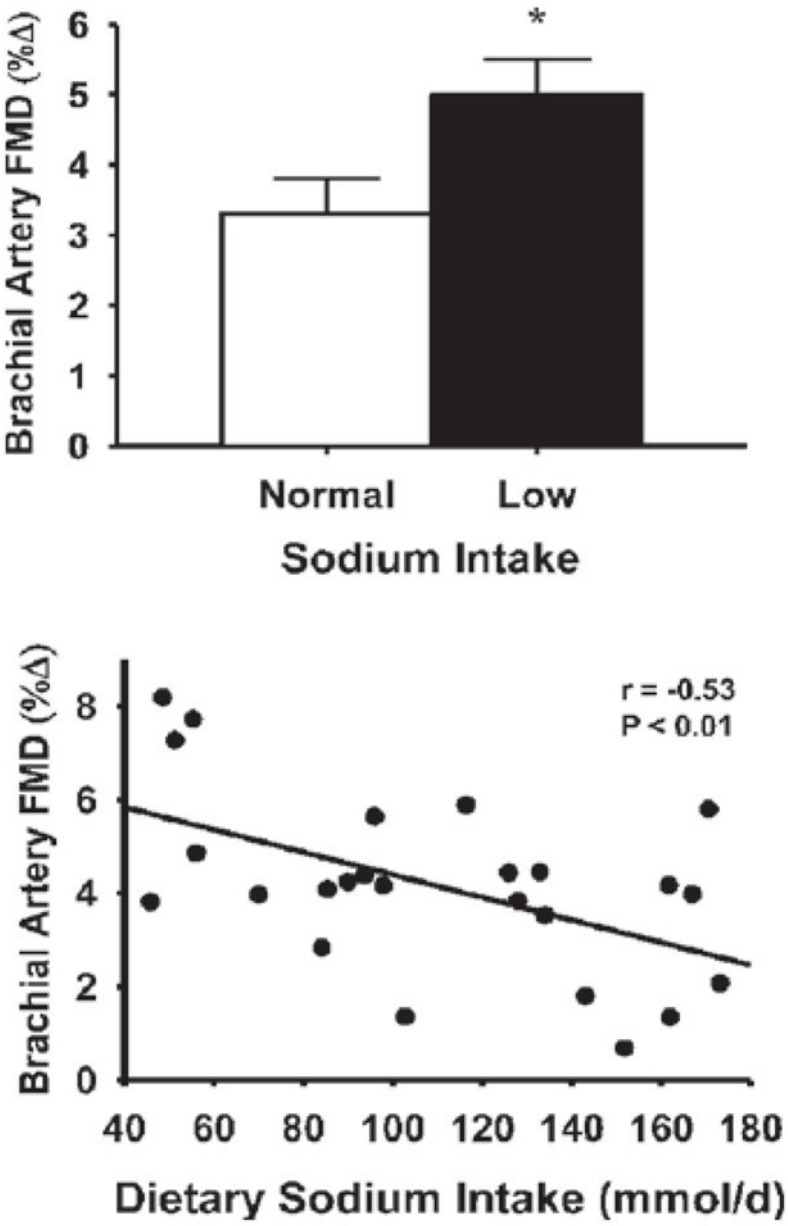


Figure 11. Dietary sodium intake and EDD in middle-aged and older adults

Brachial artery FMD is greater in middle-aged/older adults consuming low (73 ± 6 mmol/day) compared with normal (144 ± 6 mmol/day) sodium in their diet (upper panel) and is inversely related to dietary sodium intake among individuals (lower panel). *$P < 0.05$. Reproduced with permission from Jablonski, K.L., Gates, P.E., Pierce, G.L. and Seals, D.R., Low dietary sodium intake is associated with enhanced vascular endothelial function in middle-aged and older adults with elevated systolic blood pressure, Ther. Adv. Cardiovasc. Dis., volume 3, pp. 347–356. Copyright © (2009) by SAGE Publications (http://online.sagepub.com).

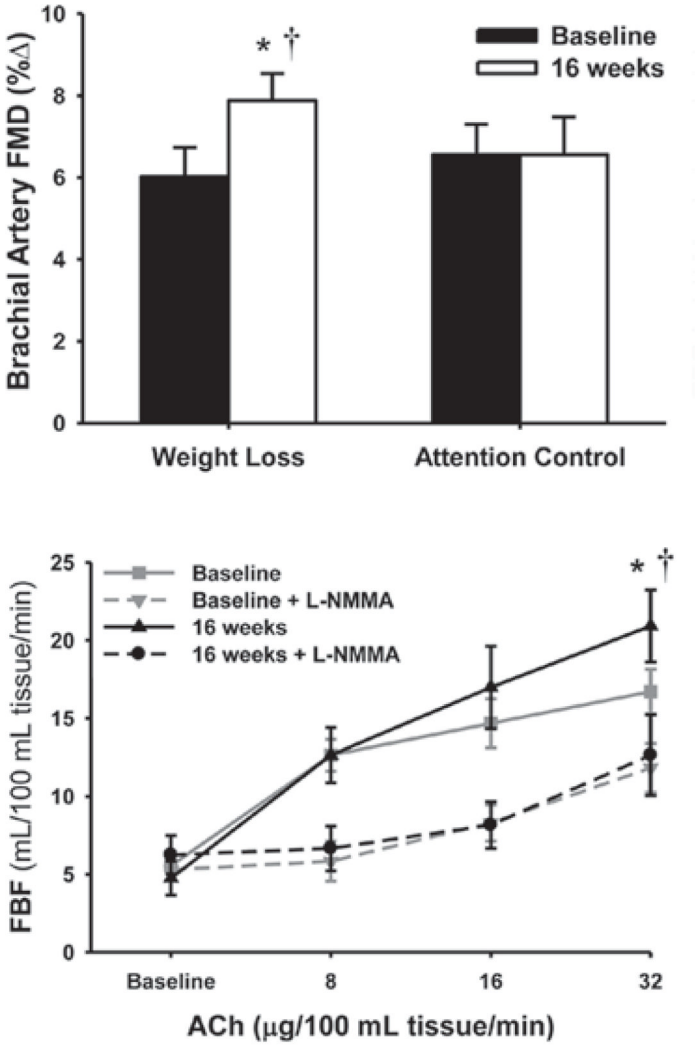


Figure 12. Weight loss and NO-mediated EDD in young, middle-aged and older adults

Energy-intake-restriction-induced body weight loss alone improves both brachial artery FMD (upper panel) and NO-mediated FBF_{ACh} (lower panel) in young and middle-aged/older overweight and obese adults. $*P < 0.05$ compared with baseline; $\dagger P < 0.05$ for the dose–time interaction. Reproduced with permission from Pierce, G.L., Beske, S.D., Lawson, B.R., Southall, K.L., Benay, F.J., Donato, A.J. and Seals, D.R., Weight loss alone improves conduit and resistance artery endothelial function in young and older overweight/obese adults, Hypertension, volume 52 (1), pp. 1–8. © (2008) Wolters Kluwer Health.

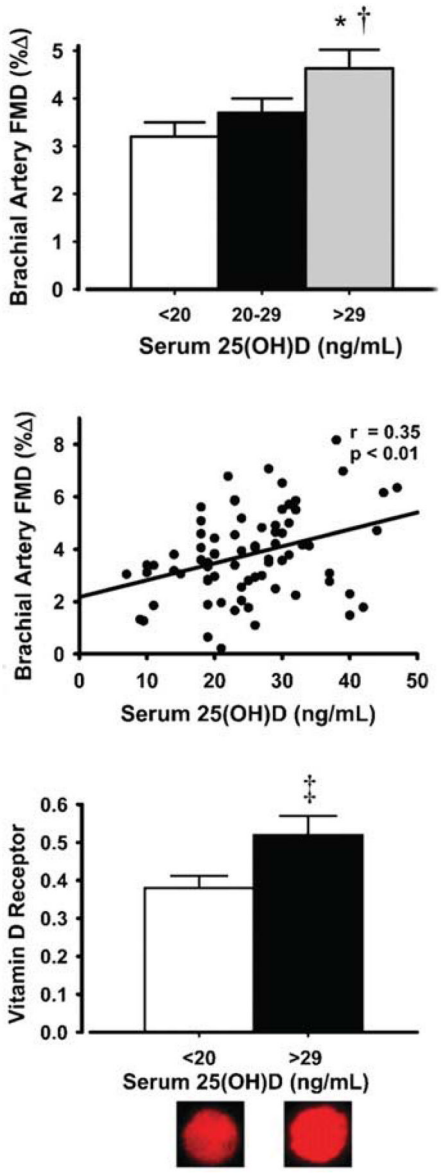


Figure 13. Vitamin D status, receptor expression and EDD in middle-aged and older adults

Brachial artery FMD is lower in vitamin D-deficient/-insufficient adults compared with sufficient middle-aged/older adults (top panel) and is positively related to serum 25-hydroxyvitamin D [25[OH]D] among individuals (middle panel). Vitamin D-deficient/-insufficient adults have lower endothelial cell vitamin D receptor expression (bottom panel). Values are means ± S.E.M. *$P < 0.01$ compared with deficient adults; †$P < 0.05$ compared with insufficient adults; ‡$P < 0.05$ compared with deficient adults. Values are ratios to HUVEC control, and representative images are shown below the histogram. Reproduced with permission from Jablonski, K.L., Chonchol, M., Pierce, G.L. Walker, A.E. and Seals, D.R., 25-Hydroxyvitamin D deficiency is associated with inflammation-linked vascular endothelial dysfunction in middle-aged and older adults, Hypertension, volume 57 (1), pp. 63–69. © (2011) Wolters Kluwer Health.

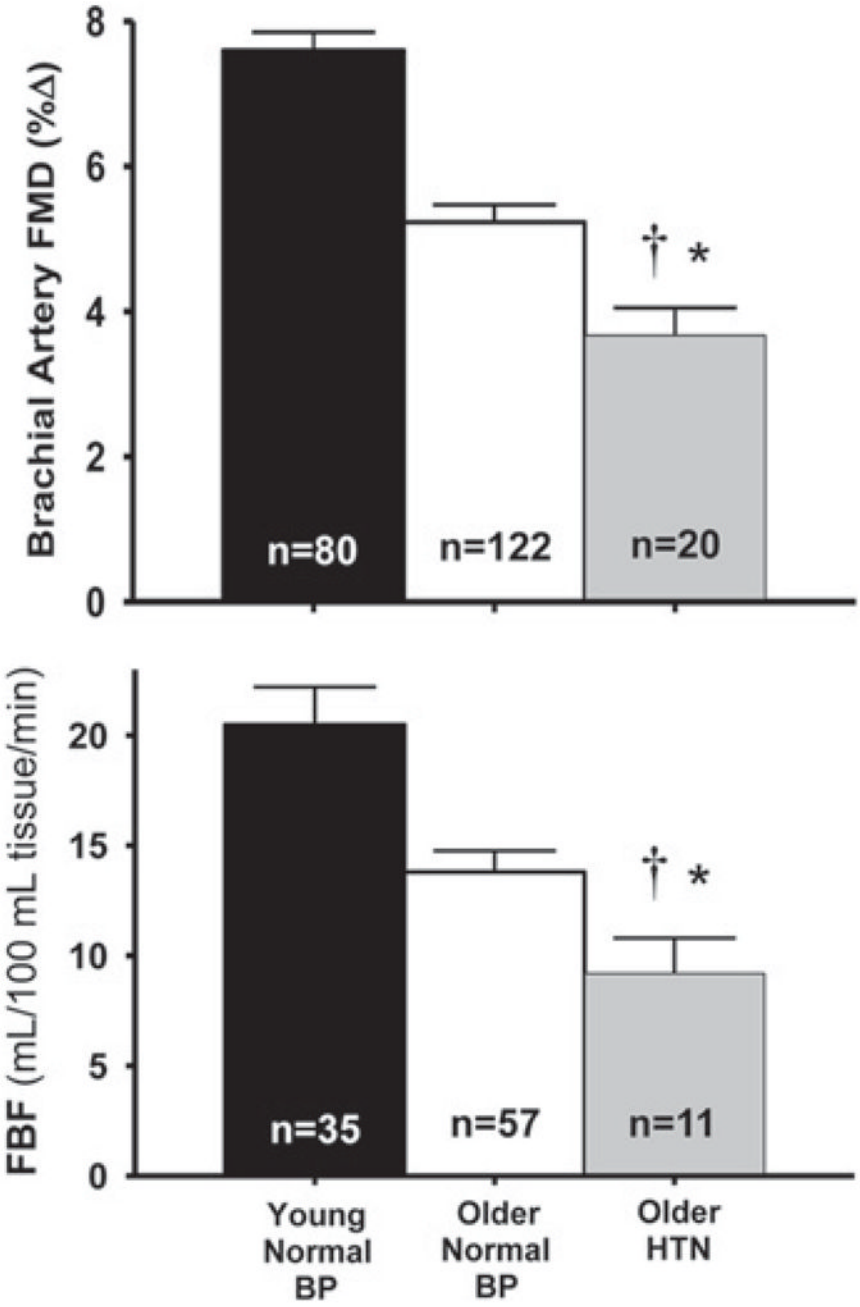


Figure 14. Blood pressure and EDD with aging in humans

Older patients with essential hypertension (HTN) have lower brachial artery FMD (upper panel) and peak FBF to ACh (lower panel) compared with older adults with normal blood pressure. *$P < 0.05$ compared with older normal adults; †$P < 0.01$ compared with young adults. BP, arterial blood pressure. These results are from K.L. Jablonski, G.L. Pierce, A.J. Donato, A.E. Walker and D.R. Seals, unpublished work.

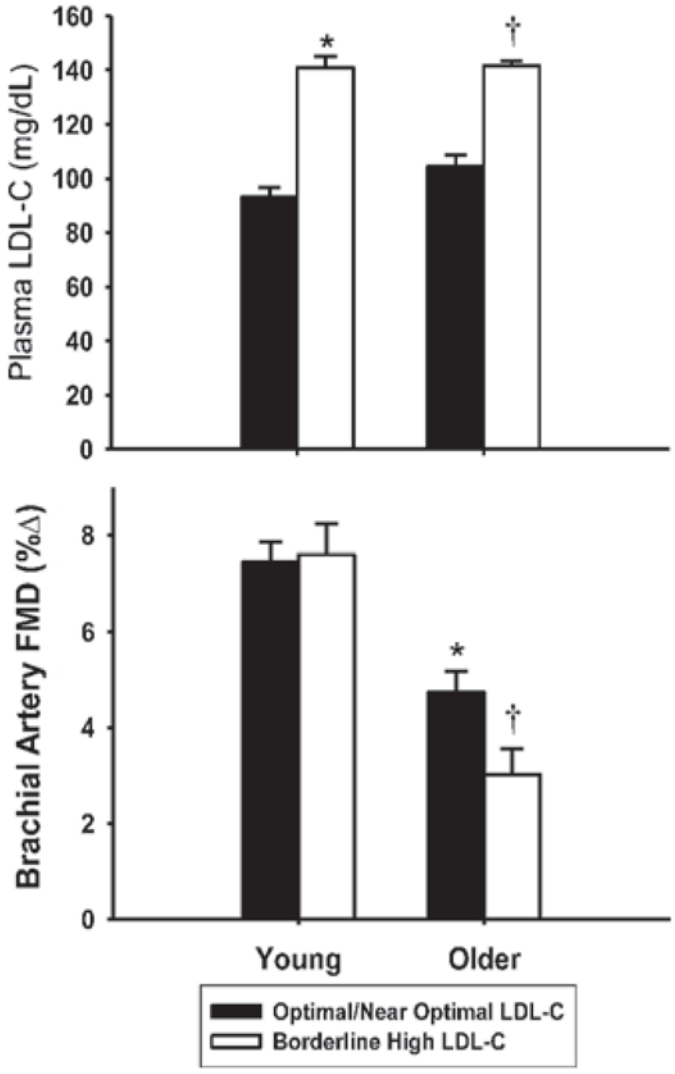


Figure 15. LDL-cholesterol and EDD with aging in men

In contrast with young controls, middle-aged/older adults with borderline high LDL-cholesterol have impaired brachial artery FMD compared with peers with optimal/near-optimal LDL-cholesterol. $*P < 0.05$ compared with young adults with optimal/near-optimal LDL-cholesterol; $\dagger P < 0.05$ compared with older with optimal/near-optimal LDL-cholesterol. Reprinted by permission from Macmillan Publishers Ltd: *American Journal of Hypertension* (Walker, A.E., Eskurza, I., Pierce, G.L., Gates, P.E. and Seals, D.R. (2009) Modulation of vascular endothelial function by low-density lipoprotein cholesterol with aging: influence of habitual exercise, volume 22, pp. 250–256), copyright (2009) (http://www.nature.com/ajh/index.html).

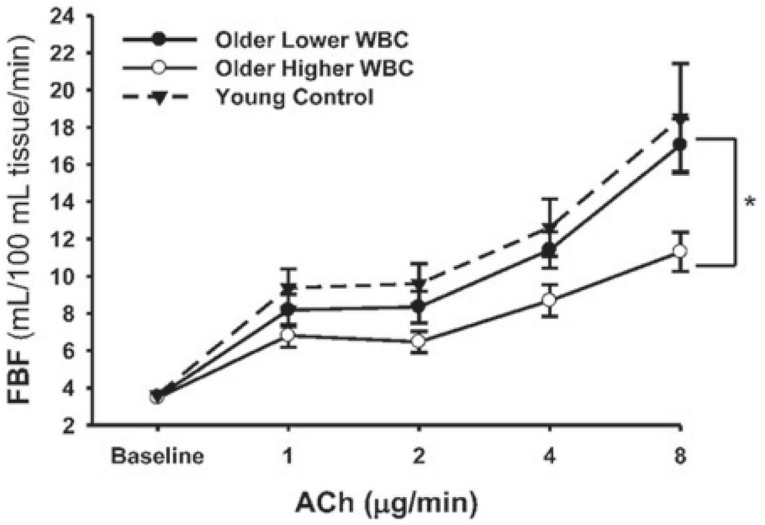


Figure 16. White blood cell count and EDD with aging in humans

Older adults with a higher white blood cell (WBC) count have impaired FBF_{ACh} compared with older adults with lower WBC (*$P < 0.05$) and young adults. Reproduced with permission from Walker, A.E., Seibert, S.M., Donato, A.J., Pierce, G.L. and Seals, D.R., Vascular endothelial function is related to white blood cell count and myeloperoxidase among healthy middle-aged and older adults, Hypertension, volume 55 (2), pp. 363–369. © (2010) Wolters Kluwer Health.