Ageing and endothelial dysfunction

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Although the available information is limited, a survey of the literature concerning the effect of ageing on endothelium-dependent responses in animal blood vessels suggests that the release of endothelium-derived relaxing factors (nitric oxide and endothelium-derived hyperpolarizing factor) is reduced, whereas that of endothelium-derived vasoconstrictor prostanoids is augmented. The very few in vitro data on isolated human blood vessels and a number of studies conducted in intact people concur with the animal data. Taken in conjunction, these findings suggest that ageing is accompanied by progressive endothelial dysfunction, which sets the scene for development of atherosclerosis.

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**Introduction**

Little doubt remains that the endothelium contributes to local control of tone and propensity to grow of underlying vascular smooth muscle. In order to accomplish this, the endothelial cells generate both relaxing and growth-inhibiting factors (nitric oxide [NO], prostacyclin, endothelium-derived hyperpolarizing factor [EDHF]), and vasoconstrictor and growth-promoting substances (superoxide anions, endoperoxides, thromboxane A2, endothelin [ET]-1, angiotensin II; Fig. 1)*[1–18]*. The contribution of each of those signals varies from blood vessel to blood vessel.

A number of variables can upregulate or downregulate the ability of the endothelial cells to release ‘good’ (relaxing and growth-inhibiting) and ‘bad’ (contracting and growth-promoting) factors. Thus, appropriate intake of antioxidants, red wine, green tea and omega-3 unsaturated fatty acids improves endothelial function, as does regular exercise. Conversely, smoking, excessive cholesterol intake, hyperglycaemia and hypertension all cause considerable impairment of the ability of endothelial cells to release ‘good’ messengers (in particular NO) and augment their propensity to release ‘bad’ factors. Impaired release of NO is of major importance because this endothelial mediator not only relaxes vascular smooth muscle, but also inhibits several processes that ultimately facilitate development of atherosclerosis, rendering endothelial dysfunction the key initial step in the formation of the atherosclerotic plaque (Fig. 2)*[8,11,12,19–25]*.

The present review discusses the negative impact of ageing on endothelial function, which sets the scene for development of vascular disease*[^26–35]*.

**Animal studies**

Assessment of alterations in endothelium-dependent responses of the ageing blood vessel wall must take into account the changes in responsiveness that occur in the vascular smooth muscle. Differences in the effect of ageing depend on the vasodilator or vasoconstrictor used. Species differences, particularly with regard to normal duration of life, further confound the issue.

**Endothelium-dependent relaxations**

In several species relaxations in response to various endothelium-dependent stimuli decrease with age in isolated or perfused arteries (Fig. 3)*[^35–60]*. However, a reduction in response to endothelium-dependent dilators is not observed in all species*[^61]*, in all strains of a given species*[^62–64]*, or even in all blood vessels of a given strain*[^35,51,65,66]*. For a given blood vessel the impact of ageing is observed more in preparations from male than from female animals*[^59,67]*.
Nitric oxide

As a rule, relaxations that occur in response to exogenous nitrovasodilators are unchanged or decrease less with age as compared with those in response to endothelium-dependent vasodilators, in particular that component of relaxation that can be prevented by inhibitors of nitric oxide synthases (NOSs)\[35,37,39–41,44,60\]. These observations strongly suggest that the ability of the ageing endothelium to release NO is reduced. This conclusion is particularly relevant to arteries such as the rat aorta, in which NO is the major, if not the only mediator of the endothelium-dependent response to acetylcholine\[68\]. However, that conclusion should be tempered by the consideration that ageing endothelial cells release more scavengers of NO (see Endothelium-dependent contractions, below). In vivo, vasodilator or depressor responses to endothelium-dependent agonists are reduced with age, whereas the response to exogenous nitrovasodilators is unchanged in old animals\[69,70\]. Furthermore, the vasoconstrictor responses to ET-1 that are observed in young (lesser response) become similar following administration of an inhibitor of NOSs\[71\]. These observations in whole animals support the view that release of endothelial NO becomes less with age.

This interpretation is reinforced by findings that the expression of endothelial NOS or activity of this enzyme is reduced in older animals; the basal and stimulated release of NO and expression of soluble guanylate cyclase in vascular smooth muscle are also reduced, as is the ability of vascular smooth muscle to produce cyclic guanosine monophosphate. It should be noted, however, that such changes have not been observed by all investigators\[51,69,72–75\].

Endothelium-derived hyperpolarizing factor

Endothelium-dependent hyperpolarizations in response to several agonists are reduced in arteries from old rats as compared with those from young animals (Fig. 4)\[76–78\]. These findings suggest that the ageing endothelium releases less EDHF. Such an interpretation would explain observations that the component of relaxation that occurs in

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*Figure 1* Multiplicity of endothelium-derived relaxing and contracting factors. α=alpha-adrenergic; AA=arachidonic acid; ACh=acetylcholine; ATII=angiotensin II; BK=bradykinin; COX=cyclo-oxygenase; ECE=endothelin-converting enzyme; EDHF=endothelium-derived hyperpolarizing factor; ET=endothelin-1; 5-HT=5-hydroxytryptamine (serotonin); O₂=superoxide anions; P=purines; PGI₂=prostacyclin; NO=nitric oxide; NOS=nitric oxide synthase; T=thrombin; TA₂/Endo=TP-receptor; TXA₂=thromboxane A₂; VP=vasopressin. From Vanhoutte\[9\], with permission.
Figure 2  Postulated signal transduction processes in a normal endothelial cell. Activation of the cell causes the release of endothelium-derived relaxing factor (EDRF)/nitric oxide (NO), which has important protective effects in the vascular wall. α=alpha-adrenergic; B=bradykinin; cAMP=cyclic adenosine monophosphate; ET=endothelin receptors; G=coupling proteins; 5-HT=5-hydroxytryptamine (serotonin); LDL=low-density lipoproteins; P=purinoceptor. From Vanhoutte [20], with permission.

Figure 3  Relaxations in response to increasing concentrations of noradrenaline (norepinephrine) in rings with (left) and without (right) endothelium of young (4–6 months old) and aged (3–4 years) pigs. The experiments were performed in the presence of propranolol, in order to block beta-adrenoceptors. From Murohara et al. [40], with permission.
response to acetylcholine, which is not prevented by inhibitors of NOS but is abolished by potassium channel blockers, is reduced in blood vessels of ageing animals [47,54,64].

Prostacyclin

Although expression of prostacyclin messenger RNA does not decrease with age in the rat aorta, the ability of the endothelial cells to produce the vasodilator prostaglandin is reduced. This finding, taken in conjunction with the diminished expression of prostacyclin receptor messenger RNA in the vascular smooth muscle of old animals, implies that the contribution of prostacyclin to endothelium-dependent relaxations may decrease with age [79,80].

Endothelial-dependent contractions

Endothelium-dependent contracting factor

The endothelium-dependent contractions caused by acetylcholine and other vasoactive agents in rat blood vessels are augmented with age [38,44,49,81,82]. Indeed, production of an endothelium-derived contracting factor (EDCF) that scavenges NO and leads to activation of TP receptors on the vascular smooth muscle may well be the primary reason for the impairment of endothelium-dependent relaxations (and of the relaxations that occur in response to nitrovasodilators) in the ageing blood vessel wall (Fig. 5) [10,20,35,37,38,46,57,82–89]. The most obvious candidates as scavengers of NO are superoxide anions, production of which is augmented in ageing endothelial cells [48,60]. Irrespective of the role of oxygen-derived free radicals in superoxide anions,
radicals, the augmented endothelium-dependent contractions are due to greater production of vasoconstrictor prostanoids, which results from the overexpression and the overactivity of cyclo-oxygenases that accompany the ageing process\[35,37,80,91\]. Augmented sensitivity of the vascular smooth muscle to the vasoconstrictor prostanoids may contribute\[49,90,91\].

**Endothelin**

The activity of endothelin-converting enzyme is augmented in blood vessels of the ageing rat\[51\]. In the same species age increases the messenger RNA of prepro-ET-1, as well as the content of the vasoconstrictor peptide\[75\]. In various rat strains, the density of ET-1-binding sites increases with age\[92\]. Also in rats, age may unmask the presence of ET\(^B\) receptors that mediate contractions\[93\]. These observations all imply that ET may play an important role in control of vascular function in old age. This, of cause, is in accord with the reduced production of NO, which not only inhibits the release of ET-1 but also potently antagonizes its action(s) on vascular smooth muscle (Fig. 6)\[9,15,20\].

**Studies conducted in humans**

**In vitro**

Few comparisons have been made of the responsiveness of isolated arteries from young and old people. In the isolated basilar artery, the endothelium-dependent relaxation that occurs in response to thrombin is reduced progressively with advancing age, whereas the responses to the calcium ionophore A23187 and bradykinin are independent of age (Fig. 7)\[94\]. In the coronary artery, endothelium-dependent hyperpolarizations are more prominent in tissues from young than in those from old donors (Fig. 8)\[95\].

**In vivo**

Several studies comparing the vascular responsiveness of young and old people strongly suggest that endothelium-dependent vasodilatation decreases progressively with advancing age, whereas responses to exogenous nitrovasodilators are affected little. This is the case for increases in blood flow or arterial diameter caused by acetylcholine, as well as for flow-dependent dilatations both in the forearm and in the coronary circulation (Fig. 9)\[96–112\]. On the basis of the increases in renal plasma flow caused by L-arginine, a similar blunting of endothelium-dependent vasodilatation occurs in the kidney\[113\]. Typically, the reduction occurs steadily with age in males, whereas in females the age-dependent reduction in endothelium-dependent responsiveness becomes obvious only after the menopause (Fig. 9)\[100,102,107,110\]. The decrease in endothelium-dependent vasodilatation with age in humans can be prevented by inhibitors of cyclo-oxygenase and vitamin C, suggesting that exaggerated oxidative stress is responsible, presumably caused by augmented production of EDCF\[103,104\]. However, the blunting of endothelium-dependent dilatations could also be explained by an age-dependent increase in the levels of asymmetric dimethylarginine, an endogenous inhibitor of endothelial NOS\[114\].

**Underlying mechanism**

It is tempting to assume that the endothelial dysfunction observed with ageing reflects normal turnover of endothelial cells. These cells, after maturation of the vasculature, are programmed to live for a finite number of years, after which they undergo apoptosis. They detach from the blood vessel wall that they line and are carried away by the

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This turnover, which can be accelerated by risk factors such as high cholesterol, hypertension, diabetes and smoking, creates areas of denudation along the vasculature, and thus interrupts the tight contact between neighbouring endothelial cells. In the absence of contact inhibition, the remaining cells can multiply to cover the denuded area. Whether circulating stem cells participate in the relining process is unknown. Although the regenerated cells are truly endothelial in nature, they are dysfunctional in that they exhibit a selective loss in the responses that

Figure 7  Scatterplots of correlations between percentage human basilar artery relaxation induced by (left) 0·5 unit.ml⁻¹ thrombin or (right) 10⁻⁵ mol.l⁻¹ bradykinin and age. Each point represents an individual experiment. Best-fit line for thrombin is \( y = 84·5 - 0·97x \). NS=not significant. From Hatake et al.⁹⁴, with permission.

Figure 8  In a human coronary artery from a very young donor (left), bradykinin at 10⁻⁸ mol.l⁻¹ causes an endothelium-dependent hyperpolarization, as measured using a glass microelectrode placed in a subintimal vascular smooth muscle cell. In the artery from an older patient (right), the response is not seen. Data from Nakashima et al.⁹⁵.


not surprisingly, manoeuvres that upregulate the production of NO (and possibly of EDHF) delay or reverse the occurrence of the endothelial dysfunction that is observed with advancing age. These include the following: administration of $\text{L}$-arginine (providing the precursor for NO); regular physical exercise (upregulating the production of NO and EDHF); and treatment with antioxidants (scavenging of superoxide anions, which shorten the half-life of NO) and converting enzyme inhibitors (protecting bradykinin from breakdown, thus permitting prolonged activation of NOS through the $G_q$ pathway).

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**References**


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**Figure 9** Relation between age and maximal forearm blood flow (FBF) response to acetylcholine (15 µg/100 ml forearm tissue per minute) in male (top) and female (bottom) normotensive persons. In women, the break in the line indicates the change point of the age-related decline in endothelium-dependent vasodilation. Data are absolute values. From Taddei *et al.*[102], with permission.
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