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The ageing endothelium, cardiovascular risk and disease in man

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Ageing is a major risk factor for cardiovascular disease, not only because there is a process of vascular ageing *per se* but also because ageing increases the time of exposure to other cardiovascular risk factors. Endothelial dysfunction is now considered an early and important mechanism that predisposes to atherothrombotic damage and thus contributes to the occurrence of cardiovascular events. The normal endothelium exerts a major vascular-protecting role by secreting substances, the most important of which is nitric oxide (NO). In disease conditions (such as the presence of cardiovascular risk factors), activation of endothelial cells can lead to the production and release of contracting factors, which counteract the beneficial effects of NO, and reactive oxygen species (ROS), which cause NO breakdown. Besides the opposite effects on vascular tone, NO and endothelium-derived contracting factors also respectively inhibit and activate several other mechanisms that are involved in the pathogenesis of atherothrombosis. Moreover, endothelial dysfunction is associated with vascular subclinical damage and, importantly, an increasing body of evidence strongly suggests that it might be an independent predictor for the risk of future cardiovascular events. Like the other traditional risk factors, ageing has been demonstrated to be associated with progressive impairment of endothelial function, in both conduit arteries and resistance vessels, mainly because of an increased production of ROS. Therefore, it is conceivable that endothelial dysfunction plays a major role in predisposing to age-related increased cardiovascular risk in the elderly.

Epidemiological data clearly show that ageing is a major risk factor for cardiovascular (CV) disease, since CV morbidity and mortality increase steeply with advancing age (Lakatta & Levy, 2003). This negative effect of ageing has an increasing impact on both public health and health expenses with the progressive ageing of worldwide populations.

The mechanisms which can explain this phenomenon are various and not mutually exclusive. Ageing *per se*, independent from other CV risk factors, is able to cause a progressive impairment of the CV system in apparently healthy subjects, as a consequence of the natural involvement of cellular processes of the inveterating cells (Lakatta & Levy, 2003; Fig. 1). However, the passing years also increase the time of exposure of the CV structures to other major CV risk factors (Fig. 1).

Among biological structures that are progressively affected by ageing, endothelium is of crucial importance, given its fundamental role in the regulation of vascular structure and functions. Indeed, the endothelium exerts a major autocrine/paracrine regulatory role by secreting substances that control both vascular tone and structure, the most important of which is nitric oxide (NO), derived from the metabolism of L-arginine by the constitutive enzyme, endothelial NO synthase (eNOS; Luscher & Vanhoutte, 1990). Nitric oxide is produced and released under the influence of endothelial agonists (e.g. acetylcholine, bradykinin) acting on specific endothelial receptors, and by mechanical forces, such as shear stress. Experimental evidence has demonstrated that in disease conditions (such as the presence of CV risk factors), endothelial cells can turn from protective to pro-atherosclerotic elements, producing several vascular-contracting, pro-aggregating and pro-inflammatory factors, including cyclo-oxygenase-derived products, endothelin-1 (ET-1) and reactive oxygen species (ROS), which in turn cause NO breakdown (Luscher & Vanhoutte, 1990; Ross, 1993).

Thus, endothelial dysfunction, caused by impaired NO availability, is now considered an early and major promoter for atherosclerosis and thrombosis. The clinical relevance of such a hypothesis is supported by the finding that endothelial dysfunction is detectable in the presence of most CV risk factors, is associated with vascular structural changes (Taddei & Salvetti, 2002) and is an independent predictor of CV events (Lerman & Zeiher, 2005). It is noteworthy that it is not practically feasible to assess NO availability directly at the vascular levels, and therefore in clinical and experimental research vascular reactivity studies are implemented to estimate NO availability indirectly in a given vascular bed.

Effect of ageing on endothelial function: evidence and possible mechanisms

Several studies have evaluated the impact of ageing on endothelial function, in both the coronary and the peripheral circulation. In patients without angiographically evident coronary atherosclerosis, ageing was associated with a progressive impairment of endothelial function in large epicardial arteries as well as in the coronary microcirculation (Yasue *et al.* 1990; Egashira *et al.* 1993). However, in these studies the negative confounding effect of other CV risk factors or early coronary atherosclerosis cannot be excluded. A more direct line of evidence for the detrimental effect of age derives from studies performed peripherally. In particular, this issue has been investigated in the peripheral microcirculation by measuring the vasodilatation in the forearm by strain-gauge venous plethysmography, in

response to endothelium-dependent vasodilators, such as acetylcholine (Taddei *et al.* 1995) or methacholine (Gerhard *et al.* 1996), infused into the brachial artery. Indeed, in healthy adult humans, advancing age was found to alter the maximal forearm vasodilatory response to acetylcholine progressively, with a strong significant inverse correlation between the two parameters. In contrast, the response to sodium nitroprusside, an endothelium-independent vasodilator, was minimally affected by ageing (Taddei *et al.* 1995). The results of this study, considering the absence of confounding factors, suggest that ageing is an independent factor that predisposes to the progressive impairment of endothelial function in the peripheral microcirculation. This phenomenon was also investigated in the peripheral macrocirculation. In particular, in healthy subjects, endothelium-dependent vasodilatation in the brachial artery, measured as flow-mediated dilatation (FMD), was demonstrated to decrease progressively with advancing age in men, with a 10 year delay in women (Celermajer *et al.* 1994). More recently, the large cohort of the Framingham Heart Study confirmed these results (Benjamin *et al.* 2004).

The biochemical and pathophysiological processes involved in age-related endothelial dysfunction are not clearly known yet (Fig. 2). However, the modifications in the L-arginine–NO pathway and NO availability associated with ageing have been investigated by the forearm blood flow technique with brachial artery infusion of L-arginine and N^{G}-monomethyl-L-arginine (L-NMMA), respectively. It was found that in young adults (<30 years old), L-arginine has no effect and L-NMMA almost completely inhibited the response to
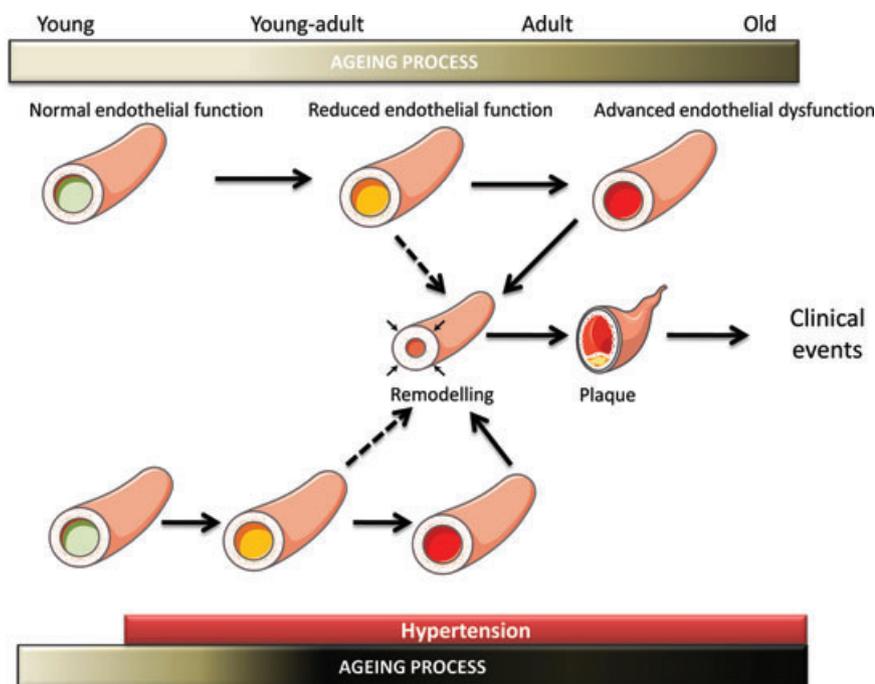

Figure 1. Schematic representation of the effect of the ageing process on vascular function
From youth, through adult life and into late maturity, vascular endothelium goes through a process of progressive reduction in nitric oxide availability and development of endothelial dysfunction. This condition increases the onset of structural alteration of the vessel wall (remodelling and atherosclerotic plaque formation), eventually leading to clinical events (e.g. myocardial infarction, stroke). The lower part of the figure shows how the superimposition of a cardiovascular risk factor, such as hypertension, on the natural ageing process, accelerates vascular inveteration and anticipates the onset of endothelial dysfunction, vascular remodelling and plaque formation.

acetylcholine, indicating a preserved and NO-mediated endothelial function (Taddei *et al.* 1997, 2001). In adults, L-arginine normalized the blunted response to acetylcholine, and the degree of inhibition exerted by L-NMMA progressively declined with age (Taddei *et al.* 1997, 2001). In older subjects (>60 years old), L-arginine still potentiated, but did not normalize, the response to acetylcholine, while L-NMMA was almost ineffective on the response to acetylcholine (Taddei *et al.* 1997, 2001). Therefore, it seems that in healthy subjects, ageing-related endothelial dysfunction is characterized by a progressive impairment in the L-arginine–NO pathway, which is reversible with L-arginine administration, at least until the age of 60 years. After this age, NO availability seems to be completely compromised, because the response to acetylcholine is completely resistant to L-NMMA and can only be minimally improved by L-arginine administration.

A major determinant of reduced NO bioavailability with ageing is the excessive generation of ROS, which rapidly inactivate NO. Indeed, the intrabrachial administration of the potent antioxidant vitamin C, while not affecting endothelial function in healthy young subjects, is able to restore the impaired vasodilatory response to acetylcholine and the response to L-NMMA (i.e. NO bioavailability) in older subjects (>60 years old; Taddei *et al.* 2001). These results indicate that in healthy subjects, ROS production participates in causing dysfunction of endothelium-dependent vasodilatation only in subjects aged 60 years or older; additional experiments identified cyclo-oxygenase (Taddei *et al.*

1998) and NADPH-oxidase (Donato *et al.* 2007) as crucial enzymes in the process of ROS production.

As far as the age-related disruption of the L-arginine–NO pathway is concerned, available data are not consistent. It was shown that L-arginine transport is not impaired with ageing in the forearm microcirculation of healthy subjects (Ahlers *et al.* 2004), nor is an increased L-arginine breakdown by arginase (Durante *et al.* 2007), suggesting that a substrate deficiency is not involved. However, recently Eskurza *et al.* (2005) demonstrated that the administration of tetrahydrobiopterin (BH$_4$), the eNOS cofactor, is able to augment brachial artery FMD in older adults, suggesting BH$_4$ deficiency as an additional mechanism that contributes to the age-related decrease in NO production. In contrast, a decreased expression of eNOS was also suggested to contribute to this process (Minamino *et al.* 2002). Additionally, it is intriguing to hypothesize that a competitive inhibition of eNOS might occur in aged subjects, by a putative increase in the circulating levels of the endogenous eNOS inhibitor, asymmetric dimethyl arginine (ADMA). However, studies attempting to evaluate whether ageing is associated with accumulation of ADMA have provided inconsistent results (Schulze *et al.* 2005; Marliss *et al.* 2006). Another aspect of endothelial physiology is represented by the production of contracting and mitogenic factors, in particular ET-1. Recent findings indicate that ET-1 signalling is increased in healthy older adults and contributes to tonic vasoconstriction in peripheral arteries (Van Guilder *et al.* 2007). Accordingly, *in vitro* production of ET-1 is greater in aortic endothelial cells obtained from

Figure 2. Mechanisms involved in development of age-related impairment of nitric oxide availability
Data from the literature suggest that the ageing process is associated with a reduced production of NO, putatively following a downregulation of endothelial nitric oxide synthase (eNOS expression), reduced availability of its substrate, L-arginine, and cofactor, tetrahydrobiopterin (BH$_4$), and an increased level of its endogenous inhibitor, asymmetric dimethylarginine (ADMA). Moreover, increased breakdown of the newly formed NO also occurs with ageing, as a consequence of the enhanced production of superoxide anions (O_2^-) by cyclo-oxygenase (COX) and NADPH-oxidase. Downwards and upwards arrows indicate decreased and increased content, production or expression, respectively. Dashed and continuous arrows indicate inhibition and stimulation, respectively.
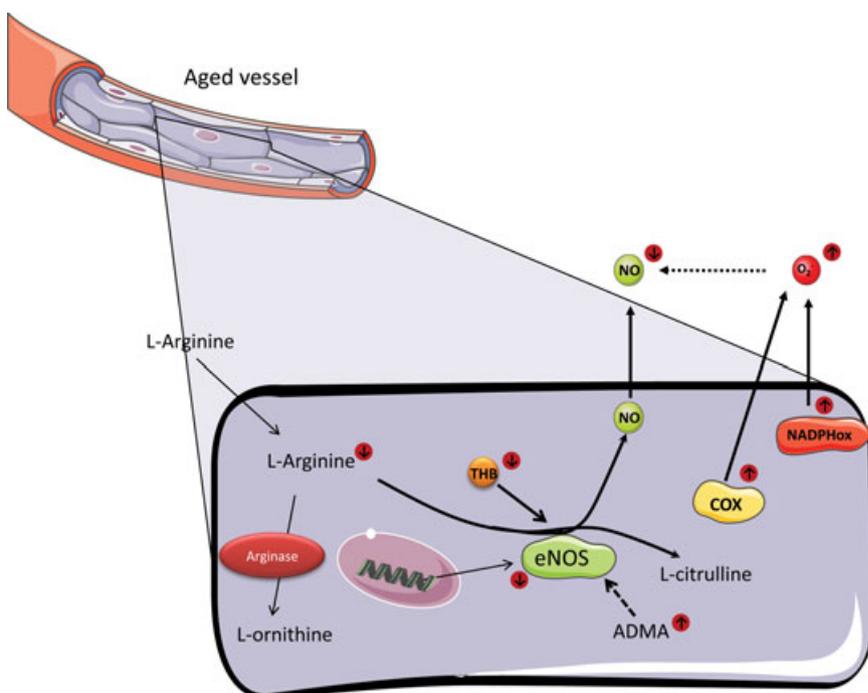
older compared with younger adults (Tokunaga *et al.* 1992).

Clinical significance of endothelial dysfunction

Endothelial dysfunction, identified as the presence of a reduced vasodilatory response to endothelium-dependent stimuli, is associated with major CV risk factors, including, as commented, the ageing process (Brunner *et al.* 2005). Moreover, it is known that the presence of multiple risk factors, each contributing to impair NO bioavailability by partly different mechanisms, is able to worsen endothelial function proportionally (Vita *et al.* 1990; Benjamin *et al.* 2004). Besides being a hallmark of CV risk, endothelial dysfunction is thought to contribute to the progression of atherosclerotic disease. This is supported by the finding that the presence of subclinical organ damage, represented by carotid intima–media thickening, left ventricular hypertrophy and renal damage, or coronary atherosclerosis is linked to the extent of endothelial dysfunction (Ghiadoni *et al.* 1998; Verdecchia *et al.* 2003; Juonala *et al.* 2004; Brunner *et al.* 2005; Rundek *et al.* 2006). In addition to extensive carotid damage, especially in the elderly, intracerebral microcirculatory endothelial dysfunction, through impairment of the blood–brain barrier, might also play a role in the genesis of lacunar infarcts, putatively implicated in the development of cognitive impairment and dementia. However, at the moment, no study has evaluated the association between peripheral endothelial function and brain lesions, although some data support this concept (Hassan *et al.* 2003).

On the basis of the aforementioned experimental, pathophysiological and clinical evidence, several studies have been conducted to evaluate whether the presence of endothelial dysfunction might be independently associated with CV prognosis (Taddei & Salvetti, 2002; Lerman & Zeiher, 2005). The available results indicate that endothelial dysfunction, in whichever vascular bed detected, is an independent predictor of clinical events. Indeed, this prognostic role has been demonstrated in the peripheral and central circulation, in the microcirculation and large arteries, and independently from the endothelial stimulus used (Taddei & Salvetti, 2002; Brunner *et al.* 2005; Lerman & Zeiher, 2005). However, given the small overall number of clinical events evaluated and the lack of a blinded study, it is premature to define the presence of endothelial dysfunction as an independent risk factor for the risk of CV events. A crucial aspect, in order to support this hypothesis, would be represented by demonstration that the correction of endothelial dysfunction is associated with an improvement in the CV prognosis. However, given the fact that no drug is available to target endothelial dysfunction specifically, so far we have only indirect hints. Modena *et al.* (2002)

evaluated the effect of indirect correction of endothelial dysfunction in terms of risk CV events. They treated a group of postmenopausal hypertensive women with endothelial dysfunction (assessed by brachial artery FMD) with various antihypertensive drugs. After 6 months, they divided the population into those women who had experienced an amelioration of FMD and those who did not. After a 10 year follow-up, a significant lower rate of CV events was found in the former subgroup, despite similar reduction in blood pressure between groups (Modena *et al.* 2002). These data support the concept that improving endothelial dysfunction is a potentially useful tool to reduce CV risk, independent from the benefits of correcting other CV risk factors.

Conclusions

The worldwide general increased life duration renders the ageing process a key element in the determination of CV risk. This phenomenon is explainable by various mechanisms, and endothelial dysfunction could be a the major mechanism. Since the ageing process is not reversible, the therapeutical approach to target age-related endothelial dysfunction might represent a major tool to improve the CV outcome of elderly patients without other CV risk factors. In this view, in the treatment of global CV risk, particularly in older subjects, it is desirable to implement those drug classes which show the ancillary property of improving endothelial function.

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