Ageing of the conduit arteries

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Abstract
Conduit arteries become stiffer with age due to alterations in their morphology and the composition of the major structural proteins, elastin and collagen. The elastic lamellae undergo fragmentation and thinning, leading to ectasia and a gradual transfer of mechanical load to collagen, which is 100--1000 times stiffer than elastin. Possible causes of this fragmentation are mechanical (fatigue failure) or enzymatic (driven by matrix metalloproteinases (MMP) activity), both of which may have genetic or environmental origins (fetal programming). Furthermore, the remaining elastin itself becomes stiffer, owing to calcification and the formation of cross-links due to advanced glycation end-products (AGEs), a process that affects collagen even more strongly. These changes are accelerated in the presence of disease such as hypertension, diabetes and uraemia and may be exacerbated locally by atherosclerosis. Raised MMP activity, calcification and impaired endothelial function are also associated with a high level of plasma homocysteine, which itself increases with age. Impaired endothelial function leads to increased resting vascular smooth muscle tone and further increases in vascular stiffness and mean and/or pulse pressure. The effect of increased stiffness, whatever its underlying causes, is to reduce the reservoir/buffering function of the conduit arteries near the heart and to increase pulse wave velocity, both of which increase systolic and pulse pressure. These determine the peak load on the heart and the vascular system as a whole, the breakdown of which, like that of any machine, depends more on the maximum loads they must bear than on their average. Reversing or stabilising the increased arterial stiffness associated with age and disease by targeting any or all of its causes provides a number of promising new approaches to the treatment of systolic hypertension and its sequelae, the main causes of mortality and morbidity in the developed world.

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As the arteries grow hard, the heart grows soft [H. L. Mencken].

Why are conduit arteries distensible?
As their name suggests, the function of the conduit arteries (aorta, carotid, iliac, femoral and brachial) is to provide a low resistance path for the blood supply to the visceral organs and the limbs. The aorta and carotid arteries in particular have an equally important buffering function achieved by the compliant nature of their walls, allowing them to expand to accommodate the blood ejected by the left ventricle during systole.

These arteries are compliant because the heart is a reciprocating pump [1]. As the left ventricle contracts, it ejects a bolus of blood into the aorta. The systolic pressure stretches the aortic wall to accommodate the bolus, forming an elastic reservoir and storing elastic tensile energy in the process. As the end of systole approaches, the left ventricle relaxes and can no longer oppose the tension in the aortic wall. Blood then begins to flow back into the heart until the aortic valve shuts. In diastole, having nowhere else to go, the blood begins its passage through the systemic circulation, driven largely by the elastic energy stored in the aortic wall, during which time the wall returns to its end-diastolic diameter. Thus, for a given stroke volume, the stiffer the aorta, the higher is the systolic pressure required to stretch its wall. It follows that a rigid aorta would require an infinitely high systolic pressure to produce flow in systole. Conversely, if the heart were able to maintain a steady flow in the manner of a rotary pump, there would be no need for an elastic reservoir and the arterial system could be rigid.

The bolus of blood ejected into the aorta during systole gives rise to a pressure pulse wave, manifesting itself as a ‘ripple’ in the vascular wall which travels along the wall at a velocity (pulse wave
velocity, PWV) dependent on its material stiffness, its thickness, the timing and magnitude of wave reflection (see below) and, to a lesser extent, the inertia of the blood and viscous losses in the wall material. The material stiffness of an artery is defined here in a general sense, as its ability to resist distension when a force is applied to it and is an intrinsic property of its materials. Functional stiffness is its effective or measured stiffness and depends on the material stiffness and the thickness of the wall relative to its lumen diameter. Thus, a thick-walled artery of a given material stiffness will distend less in response to a given increase in pressure than a thin-walled one with the same material properties. i.e. it is effectively stiffer.

‘Compliance’ is used here to mean the inverse of effective stiffness.

It is worth emphasizing that the pulse wave velocity differs from the velocity of the blood in much the same way that the speed of a breaker approaching a beach differs from that of the much slower moving tide. Pulse wave velocity, which is easy to measure non-invasively, can be used to estimate arterial stiffness (see eg [2,3]).

The relationship between pulse pressure and its dependence on the stiffness of the arterial wall is incorporated in the concept of impedance, a measure of all the factors which combine to limit the flow due to a given pulsatile pressure gradient [4]. Thus, for a given cardiac output, impedance determines pulse pressure and therefore the peak load on the heart, just as resistance defines the relationship between the steady components of pressure and flow, and therefore the mean load on the heart [5].

**Pulse wave reflection and the link between arterial stiffness and pulse pressure**

Reflections of the pressure and flow waves generated by the heart occur wherever they encounter a change in impedance. This can happen when there is a change in stiffness or lumen cross-sectional area, or a combination of both [6]. When the impedance increases, for instance at the entrance to a stiff vascular prosthesis, the reflection is said to be positive and pressure increases while flow decreases. Abrupt changes in characteristic impedance are found at many arterial bifurcations. It is also likely that reflections occur in and around most vascular lesions.

In addition to these localized reflection sites, which may lead to local fluctuations in pressure and flow and are thought to account in part for the preponderance of atheromatous lesions near bifurcations in the conduit arteries and the heart [7–9], as well as the confluence of the two vertebral arteries with the basilar, diffuse reflections occur throughout the vascular system, leading to wide variations in pulse pressure at different sites. There are two causes of these diffuse reflections. First, as mentioned above, all junctions are potential reflection sites, therefore the measured reflected wave in any conduit artery will result from the superposition of numerous reflections at various distances distal to the measurement site. Second, due to the progressive increase in the ratio of collagen to elastin (see below) and decrease in diameter with distance from the heart, impedance gradually increases along the aorta and its major branches, causing a gradual rise in pulse pressure and a corresponding fall in pulsatile flow [10]. It follows that the combined effect of local reflections in the proximal aorta and carotid arteries, plus the diffuse reflections due to regional variations in conduit artery elasticity, will have a strong influence on the mechanical pre- and after-load on the heart. Similarly, pulse pressure measured at distal sites is strongly influenced by peripheral and central reflections which, as explained in Figure 1, account for the age-related increase in peripheral and central pulse pressure [11].

The measured aortic pressure waveforms (heavy lines) are composed of a forward travelling wave generated by the heart (thin lines) and a combination of many reflected waves (dashed lines). In young subjects (left-hand side) the reflection of the systolic wave occurs late in diastole, because the aorta is compliant and therefore has a low pulse wave velocity (PWV). In the stiffer, old or hypertensive aorta (right-hand side) the PWV is higher, so the reflected wave returns soon enough to add to the heart-generated wave during systole, thus increasing systolic pressure and reducing diastolic pressure. The steeper pressure rise occurring in early systole is caused directly by the loss in the buffering ability of the proximal aorta, due to its increased stiffness.

As the conduit arteries age, changes in their composition and structure lead inexorably to an increase in the stiffness of their walls [12–15], resulting, as outlined above, in raised systolic and pulse pressure and greater mechanical load on the left ventricle, the systemic circulation as a whole and a consequent increase in the risk of stroke, myocardial infarction, renal failure and other sequelae of essential hypertension [16]. Indeed, the incidence of systolic hypertension in those aged 65 or more is close to 65% [17]. The fall in diastolic pressure seen in late middle age, which results in reduced coronary arterial perfusion (which occurs only during diastole), adds further to the demands on the left ventricle.

Unlike the localized remodelling and changes in arterial elasticity seen in atherosclerotic disease (essentially a disease of the arterial intima), this generalized age-related stiffening (arteriosclerosis), confined primarily to the media of the conduit arteries, appears to be inevitable, at least in the developed world [18–20], although its severity is linked to a number of well-known risk factors. The predictive and diagnostic power for cardiovascular morbidity and mortality of changes in arterial stiffness or its several surrogate measures (see below) is now widely recognized (see eg [16,21]) and this predictive ability, although effective in the young [22], itself increases with age [23,24].
Figure 1. The combination of the forward-going wave generated by the heart (thin grey line) and the sum of many reflected waves from various distal sites (dashed line) gives rise to the measured wave (thick black line). The shape of this wave (ie the pressure, and therefore mechanical force, ‘felt’ by the artery), depends on the timing and magnitude of the reflection and hence on the relative position of the heart and the reflection sites. It will therefore change progressively with position along the arterial tree. Late reflection in young subjects (left panel) augments diastolic pressure. In the stiffer, old or hypertensive aorta (right panel), the reflected wave returns during systole because the pulse wave velocity is higher in older, stiffer arteries, thus increasing systolic pressure and reducing diastolic pressure. The steeper pressure rise occurring in early systole is caused by the higher impedance of the proximal aorta, due to its increased stiffness in older or hypertensive subjects. The magnitude of the reflection seen in the older subject may be quantified by the augmentation index (AI), defined as $b/(a + b) \times 100\%$. The timing of the reflection which, as explained above, provides an estimate of the combined conduit artery PWV, is given by $\Delta t$. Both these parameters have been used as surrogates of conduit arterial stiffness [218], although their reliability when compared to PWV measurements has been questioned [219,220], as has their theoretical basis [4].

It is worth noting that peripheral vascular resistance is the main determinant and a better predictor of cardiovascular risk in subjects younger than 50, but that large artery stiffness is the more important in older subjects [23]. Arterial elasticity measurements have been used as part of a recently described formal risk assessment scheme [25], and a pharmacological approach to limiting or reversing increases in large artery stiffness now forms the basis of novel treatments for cardiovascular disease [26].

Although the association between increased arterial stiffness and cardiovascular disease is now well established, the underlying causes of arteriosclerosis remain the subject of debate. (For a detailed review, see [27]). The aim of this review is to describe several putative mechanisms for causes of age-related changes in arterial elasticity. Before doing so the structural basis of large arterial elasticity and is briefly outlined.

Structural basis of arterial elasticity

The healthy intima of the conduit arteries, consisting of the endothelium and a fine basement membrane composed predominantly of type IV collagen [28], contributes little structurally to their elastic properties. The media and, as is now becoming more widely recognized, the adventitia [29–34] are responsible for arterial stiffness and resilience (the ability to recover its original dimensions when the pressure returns to its original value).

The main structural components of the media are elastin, collagen, vascular smooth muscle cells (VSMCs) and ground substance in the form of a mucopolysaccharide gel. Elastin resembles rubber, at least in its ability to undergo large recoverable stretch, and confers resilience and extensibility on arteries as well as skin, lung and tendon [35,36]. Elastin comprises 90% of arterial elastic fibres, although at least 19 other proteins make up their microfibrillar and amorphous components [37]. The arrangement of the structural components in the vascular wall is complex and varies according to location within the arterial tree. The ratio of elastin to collagen falls with increasing distance from the heart, while the number of VSMCs per unit volume increases [38–40]. This reduction in elastic tissue and increase in muscularity continues via ‘transitional’ vessels [41], such as the common iliac, into the so-called muscular arteries and on into the arterioles.

In transverse histological sections of conduit arteries, a layered structure is evident consisting of what appear to be concentric rings of elastic tissue (lamellae), between which are found smooth muscle cells and surrounding which are collagen fibres, the whole being imbued with a matrix of ground substance (see Clarke and Glagov [42] for a lucid description). The three-dimensional microstructure of this arrangement and the nature of the connections between the components is not fully understood, although recent work suggests that the VSMCs are anchored to the surrounding extracellular proteins via extensions of the cytoskeleton which allow the contracting muscle to transmit tension to the vessel wall [43–45]. In resistance vessels, VSMC contraction allows large variation in the vessel lumen and is therefore able to
distribute flow to vascular beds according to metabolic demand. However, in conduit arteries, the limited amount of VSMCs relative to that of the other structural components and their disposition within the media allows only a modest reduction in lumen diameter, even when maximally contracted [46–49]. Rather, the effect of smooth muscle contraction is to redistribute tensile force between elastin and collagen and therefore to modulate stiffness in the short term [50,51].

Arteries are non-linearly elastic, becoming stiffer as they are distended, typically by a factor of 100 between mean pressures of 60 and 180 mmHg [51]. In 1957 Roach and Burton proposed a qualitative model of arterial elasticity in which it was supposed that at low pressures (and therefore low degrees of circumferential stretch) tension is born by elastin, while the much stiffer collagen fibres remain folded [52]. As pressure and stretch increase, the gradually unfolding collagen fibres take on an increasing fraction of the tension and the vessel becomes progressively stiffer, thus preventing over-distension at high pressures. Early attempts to quantify this process [38,53,54] have recently been superseded by comprehensive numerical models, based on the histological structure of the vessel wall, which account for the contribution of VSMCs, the viscoelastic properties of the matrix proteins, the presence of residual stresses due to growth and remodelling and, most importantly, the gradual engagement with increasing pressure of a population of collagen fibres of varying lengths and/orientations [55–58]. Currently there is a shortage of quantitative data concerning changes in collagen orientation with distension, although, as Figure 2 shows, novel microscopic techniques may help to overcome this. The aim of these studies was to explain changes in arterial elasticity associated with growth, ageing and pathological remodelling, allowing reliable prediction of aneurismal rupture and suggesting novel treatment for arterial disease based on

Figure 2. Inner adventitia of the rat carotid artery viewed by two-photon excited fluorescence and second harmonic generation microscopy. Both imaging modes are confocal and are combined to show collagen fibres in green and the underlying external elastic lamella in red. The collagen fibre bundles progressively unfold with increasing circumferential stretch (0%, 20%, 30% and 40% in panels A–D, respectively). With thanks to professors A Yeh and JE Moore Jr. of Texas A&M University for access to the equipment used to obtain these pictures.
modifying conduit artery structure and properties. The interrelationship between arterial structure, elasticity, wave reflections and pulse pressure is summarized qualitatively in Figure 3.

Age changes in conduit arterial morphology, composition and stiffness

During ageing and in the absence of observable vascular disease, the migration of medial VSMCs and proliferation causes the intima of the conduit arteries to thicken [59]. This is accompanied by a progressive increase in lumen diameter [60] and lengthening of loosely tethered vessels, such as the abdominal aorta, leading to tortuosity [61].

With a few exceptions (see eg [62]) it is generally agreed that in the adult aorta and pulmonary and carotid arteries, collagen content relative to the wet weight of the vessel increases with age, giving rise to a corresponding reduction in elastin content and the number of VSMCs [63–68]. Data for age changes of matrix protein in smaller conduit arteries are limited [69], although in more muscular vessels the content of elastin (relative to the dry weight of the vessel) appears to increase with age, due probably to a decrease in cellularity, as does that of collagen, although to a lesser extent [63,70] (for a detailed review, see eg [71]).

Post mortem and animal studies of arterial elasticity and its changes with age go back at least two centuries [72], although only in the last 30 years, since the refinement of ultrasonic techniques for the estimation of pulse wave velocity and/or vessel wall distension, has it been technically feasible to carry out large-scale non-invasive in vivo measurements in man. Early measurements of PWV have been reviewed by Haynes et al [73], more recent studies by Hickler [74] and in detail by Hayashi et al [71].

Almost all studies have shown that compliance in the aorta decreases steadily with age [14,18,75], a result that is consistent with an increase in the ratio of collagen to elastin and the increased stiffness of the scleroproteins themselves (see below). It should be emphasized that the absolute amounts of both matrix proteins fall with age, due to an increase in fat content and extracellular material such as calcium [76] (see below). Similarly, the carotid and pulmonary arteries stiffen with age [77–80]. However, some have reported an increase in aortic PWV in early childhood followed by a subsequent fall with age [75,81–83], although in measurements on 480 Chinese subjects aged 2–85 years, Avolio and his co-workers [14] did not observe this fall in PWV during childhood.

Figure 3. Interrelationships between vessel composition, structure, elasticity, geometry, elastic reservoir function and cardiac work. Functional stiffness, as measured non-invasively by PWV, for instance, depends on the combined effects of composition and geometry. Thus, a vessel with given material properties and a thick wall relative to its lumen will have a greater functional stiffness than a vessel of the same material but a thinner wall. Functional stiffness, coupled with the effects of reflection, determines the pulsatile components of pressure, flow and hence cardiac work. The dotted line completes a feedback loop indicating that changes in pulsatile pressure and flow can lead to remodelling, as explained in the text.
Results for the more peripheral conduit arteries are fewer and less straightforward. Several reports have shown that PWV in the leg (which is higher than aortic values) also increases with age, although more slowly than in the aorta [14,18,83,84], whereas others have found that the stiffness of the femoral arteries does not change significantly with age [85–87] and that the brachial and radial arteries may similarly retain their young adult values or, indeed, become more compliant [86–89]. Data for the iliac artery are sparse, although a single in vivo study suggests that it, too, does not become stiffer with age [75], a result confirmed by post mortem studies [82,90]. Bearing in mind the greater cellularity of more distal conduit arteries, it is possible that these contradictory results are due to short-term alterations in smooth muscle tone, causing greater changes in compliance within a given subject than are seen between subjects [87].

In summary, numerous studies have shown that, in adulthood at least, the large elastic arteries near the heart stiffen with age, although distal vessels which contain more VSMCs and less elastin may not be similarly affected. However, it should be emphasized that it is the large elastic arteries near the heart which make the dominant contribution to the elastic reservoir function of the arterial system, and therefore uncertainties about the effect of age on the stiffness of the more distal conduit arteries will have little impact on pulse pressure and cardiac preload.

Why do old or diseased conduit arteries become stiff?

Remodelling of arteries as they grow, age or become diseased leads to changes in their composition, their geometry and the manner in which mechanical forces are distributed within their walls. Figures 1 and 3 show how these changes can lead to altered haemodynamics and cardiac load. In general, it is found that an increase in mean and/or pulse pressure stretches the vessel wall, a change which is ‘sensed’ directly by the VSMC, leading to changes in its contractile state and/or its synthetic activity [91]. On the other hand, an increase or decrease in blood flow velocity affects the VSMC indirectly via the vascular endothelial cell (VEC), which senses the shear or frictional force between the blood and the vascular endothelium. This, in turn, releases vasoactive mediators and growth factors [92] which diffuse into the vessel and then interact with the VSMCs, causing, in the short term (minutes to hours), a change in their state of contraction, and in the longer term (hours to weeks), their synthetic, mitotic and migratory activity, resulting in both intimal and medial remodelling (see [93] for a review).

In addition to the age-related decrease in conduit artery elastin content, the structure of the elastic lamellae themselves changes with age, becoming sparser and showing clear signs of fragmentation and disorganization. At the same time the remaining elastin may become calcified, while the collagen molecules progressively acquire cross-links (see below). The remainder of this article reviews the contribution of these processes to the reduced compliance and resilience seen in aged conduit arteries.

Fatigue failure of elastin

In 1976 O’Rourke suggested that the age-related thinning and fragmentation of arterial elastin is due to fatigue failure [94]. Since the rate of elastin synthesis in adulthood is thought to be negligible, with a half-life in man of more than 40 years [95–97], and in the mouse, no detectable synthesis after the age of 3 weeks [98], there is little likelihood that the undamaged elastin will be replaced.

Although this attractive hypothesis is strongly supported by the histological and biochemical evidence, there is little direct evidence in the literature that arterial elastin does fracture when subjected to repeated cyclic loading. We have recently carried out measurements of elasticity on specimens of purified pig aorta following cyclic loading [99] and have found that the elastin undergoes a structural change while being cyclically stretched. Figure 4 shows that the specimens subjected to the greatest stretch fracture after a reduced number of cycles, a characteristic of materials fracturing as a result of fatigue and a pattern of behaviour similar to that seen in conventional elastomers [100].

Although the results of this study suggest that arterial elastin undergoes fatigue failure in vitro, they do not of course reveal anything about the way in which this process may be modified in a living artery. Nevertheless, they suggest that increased stretch, such as that due to raised blood pressure, coupled with the cumulative effect of cyclic stress, leads to accelerated fracture and ectasia, resulting in transfer of mechanical load to collagen and the consequent arterial stiffening seen in the aged, as well as the early
onset of this stiffening seen in essential hypertension. They also suggest a simple mechanism for the link between high heart rate and mortality from cardiovascular disease [101]. Biochemical and cellular mechanisms have been reviewed by Atkinson [102], who suggests that, acting in concert with the mechanical stress, disintegration may be mediated by integrins and metabolic factors such as oxidative stress [103,104].

Elastocalcinosis

In addition to the destruction due to repeated mechanical loading and oxidative stress, there is evidence that chemical degradation and calcification can cause the remaining elastic tissue to stiffen.

Apart from the calcification associated with atherosclerosis in the arterial intima (see [105] for a recent review), a more diffuse accretion of calcium salts, termed medial elastocalcinosis (MEC) [106], is seen in the aortic media, where it is associated with arteriosclerosis of the aged and of diabetics [104,107]. The mechanisms of mineralization have been reviewed recently by Dao [106] and Shao [104]. In the former review, Dao cites a report of medial calcification from a century ago [108] and makes the point that this type of degeneration can not therefore be ascribed to the lifestyle changes of the twentieth century. This view is supported by an extensive post mortem study in 1944 [109], which showed that the incidence of aortic calcification increases steadily with age, affecting only 4% in their third decade of life and almost all subjects by the age of 50. In approximately 30% of subjects over the age of 60 it is sufficiently severe to be detected by echocardiography [110].

There is strong circumstantial evidence that nonatherosclerotic arterial calcification is associated with elastic tissue, because large arteries tend to be more severely affected than smaller more muscular and collagennous vessels [102,109] and it is probable that the calcium salts bind specifically to elastin itself, rather than to the minor components of elastic tissue, such as fibrillin [111,112] and glycoproteins associated with microfibrils. At the cellular level the process resembles osteogenesis, with VSMCs expressing bone mineralization proteins BMP2 and 4 and, indeed, undergoing phenotypic changes into a mineralizing form [113] as well as losing their normal ability to inhibit calcification [114,115].

The evidence (confined for the moment to animal studies) that calcification is independent of elastic tissue degradation is conflicting. Bailey et al [112] suggest that calcification of elastin is associated with increased MMP-9 and MMP-2 mRNA expression (both enzymes having elastolytic properties), which implies that it is accompanied by remodelling. On the other hand, there is evidence that experimentally induced calcification of otherwise normal elastic lamellae does not lead to remodelling [116,117].

Whatever the precise biochemical mechanisms for the calcification of elastic tissue, there is little doubt that it is strongly correlated with increased arterial stiffness in two distinct rat models of calcium overload [116,117] as well as in patients with end-stage renal disease [113] and hypertension [118].

Given the parallels between mineralization of bone and MEC, investigations into the pharmacological control of MEC have been started as a possible new approach to the treatment of essential systolic hypertension. Dao et al treated warfarin/vitamin K1-induced calcification in the rat with darusentan, an inhibitor of endothelin, for 4 weeks and observed a reduction in aortic calcium content and a concomitant fall in pulse pressure and collagen : elastin ratio [119], whereas stopping the warfarin/vitamin K1 treatment did not itself lead to demineralization or a fall in blood pressure. They also report unpublished observations that suppression of endothelin by sinitrodil, a nitric oxide (NO) donor, induced mineral loss in the aorta. The mechanisms of mineral loss are currently under active investigation [106].

Matrix metallo-proteases (MMPs)

During growth and development, the balance between matrix protein synthesis and degradation is tightly controlled and proteases are constitutively synthesized only in very small amounts. As arteries age or undergo pathological changes, the balance between proteases and their inhibitors is lost and protease release increases by ‘the induction of MMP gene expression, the activation of zymogens or the secretion of enzymes by inflammatory cells’ [37].

Animal studies have shown that the gradual reduction in the relative elastin content of the ageing conduit arteries is associated with an increase in MMP-2 localized primarily in the thickened intima, perhaps due to an exaggerated VSMC response to cytokines [120] and MT1-MMP (a membrane-bound elastase), with no increase in the expression of their respective inhibitors [121]. Under pathological conditions, for instance, hypertension induced by chronic inhibition of NO production with L-NAME, MMP activity is inhibited by the combined effect of pro-inflammatory molecules, such as interleukin-6 and leukocyte stimulating factors, as well as tissue inhibitors of metalloproteases, allowing extracellular matrix production to increase and leading to medial and/or intimal hypertrophy [122]. On the other hand, in aneurysms, a condition characterized by medial degeneration, whose incidence is strongly correlated with age, the activity of neutrophil elastase and MMPs 2, 9 and 12 (the latter two produced by macrophages) is enhanced; whereas that of tissue inhibitors of metalloproteinase 1 and 2 is inhibited [123,124].

These and similar data show that the nature (and probably the rate) of age- and disease-related arterial remodelling is determined by specific proteolytic
enzymes and their inhibitors, and that the balance between protease production and inhibition is shifted in a way which is consistent with the proliferative or degenerative nature of the remodelling. In atherosclerosis, increased total serum elastase and MMP-9 levels [125] are associated with reduced aortic compliance (assessed by measuring by PWV) [126]. Interestingly, an association between MMP-9 and PWV is also seen in young normotensive subjects, which suggests that elastolysis may be an underlying cause of systolic hypertension before overt symptoms are detectable. The same group showed that those with the highest levels of serum elastase activity and aortic PWV were carriers of rare alleles for elastin gene polymorphisms, suggesting a genetic basis for systolic hypertension and stiff aortas [127]. The genetic aspects of arterial stiffness have been reviewed recently by Laurent et al [128].

**Advanced glycation end-products**

The products of glycation and oxidizing reactions between sugars and the amino groups in protein molecules undergo a slow and chemically irreversible rearrangement, the Maillard reaction, akin to caramelization in cooked food [129], to form so-called advanced glycation end-products or AGEs [130]. In arteries, the less appetizing accumulation of AGEs over time leads to cross-linking of collagen and consequent increases in its material stiffness [131,132]. In vitro studies suggest that elastin is similarly affected [133,134], although the literature yields no evidence of a link between AGEs and elastin cross-linking in vivo. AGE cross-link formation is enhanced under hyperglycaemic conditions and is therefore more severe in diabetics [135]. Dietary intake of AGEs in fatty [136] and browned foods [137] is associated with high serum AGE levels and increased protein cross-linking in diabetics [138].

In addition to the direct stiffening effect of collagen cross-linking, activation of receptors for AGEs (RAGEs) leads to the initiation of an inflammatory response via nuclear factor κB. Soluble AGEs activate monocytes, suppress macrophage migration through the basement membrane, increase endothelial permeability, inhibit NO activity and increase the expression of endothelin (for a detailed review of these processes, see [139]).

Suppression of AGE formation and inhibition or breakage of the resulting cross-links may form the basis of novel approaches to the treatment of atherosclerosis and isolated systolic hypertension. For instance, *alagebrium* (ALT-711), a thiazolium compound that breaks AGE-induced cross-links, has been found to reduce arterial stiffness in diabetic rats [140], old monkeys [141] and old spontaneously hypertensive rats [142] (for further details, see [143]). In aged patients with systolic hypertension, a high-dose regime (210 mg/day) of ALT-711 led to a reduction in pulse pressure 3 days after starting the treatment, a change that was maintained for at least 8 weeks [144], although after an initial fall there was no significant change in PWV during this time. However, patients with severe hypertension, including those who had not responded to more conventional treatments, responded to a low dose (35 mg/day), with a significant reduction in SBP after 6 months of treatment [145].

In addition to these cross-link breakers, compounds such as aminoguanidine and ACE inhibitors, which inhibit the formation of AGE-induced cross-links, also reduce the stiffness of large arteries in both diabetic [146,147] and hypertensive [148] rats. Their effectiveness in man remains to be established. Similarly, the possibility of modifying the cellular effects of AGEs, mentioned above, has not yet been thoroughly investigated.

**Homocysteine**

Serum total homocysteine (Hcy) increases with age, is higher in men than in women [149] and is an independent risk factor for myocardial infarction [150], stroke [151], atherosclerosis [152,153], carotid artery disease [154] and aneurysms [155,156] (for a thorough review, see [157]). Possible mechanisms for these associations include an enhanced tendency for thrombosis, due to impaired endothelial function [158], platelet activation, reduced cell expression of thrombomodulin, and inhibition of activated protein C [159]. There is also evidence from animal studies of a relationship between hyperhomocysteinaemia (Hhcy) and disturbances in arterial elastin metabolism and stiffness. For instance, the aorta of pigs fed an Hhcy-inducing diet contained less elastin than normal, had larger fenestrae in their elastic laminae and demonstrated increased activity of MMP [160]. Similar effects seen in the femoral and, more severely, in the coronary arteries were partially reversed following treatment with an ACE inhibitor (captopril), [161], suggesting a possible mechanism for the therapeutic effect of these agents in the treatment of coronary atherosclerosis. Severe disruption of elastic tissue, including separation of lamellae, VSMC proliferation and aneurysms, is also seen in young chicks fed diets rich in homocysteine and methionine. The damage is thought to be due to disordered microfibrils (specifically, loss of fibrillin-2 immunoreactivity), rather than elastin cross-linking *per se*, because no change in desmosine content or lysyl oxidase levels was observed [162]. In rats, increased carotid artery stiffness and reduced contractile ability in coronary arteries was associated with Hhcy (induced by reduced dietary folate) as well as increased glycoxidative stress, suggesting that this, too, is a possible mechanism for the link between Hhcy and increased vascular stiffness [163].

In man, Hhcy is associated with systolic hypertension (SBP ≥ 160 mmHg) [153], although in this study the ‘normotensive’ control group contained subjects
with SBP up to 159 mmHg. In patients with occlusive vascular disease, a strong independent correlation was found between plasma Hcy and aortic PWV after adjustment for age, BP and creatinine clearance rate [164]. A similar independent correlation, although only in the femoral artery, has been reported in patients with end-stage renal disease [165]. Even in healthy men, acute Hhcy evoked by oral methionine administration is associated with reduced femoral and brachial artery compliance [166], although this study was performed on only 12 patients.

Given the link between Hhcy and several age-related cardiovascular pathologies, the therapeutic potential of reducing Hhcy and its metabolites in the treatment of vascular disease is clear. Folic acid fortification of wheat products in the Framingham heart study population [167] reduced mean plasma Hcy levels by 7%. There is good evidence that dietary supplementation with folic acid and vitamin B12 improves endothelial function in homocysteinaemic adults [168] and in middle-aged men with CAD [169], as well as reducing the risk of stroke and CAD in subjects with no history of CV disease [170,171]. Folic acid treatment undoubtedly reduces blood pressure and arterial stiffness in hypertensive subjects, although the falls in these variables did not correlate with plasma levels of Hcy, or indeed with folic acid [172]. In contrast, folic acid in concert with pyroxidine treatment administered to the normotensive siblings of patients with premature atherothrombotic disease did reduce Hcy levels and blood pressure. However, no significant effect on carotid artery stiffness or flow-mediated vasodilatation in the brachial artery was found. Clearly, in this case the reduction of BP was not dependent on changes in arterial elasticity or contractility [173].

Taken together, most of these results show that Hhcy, whether it is induced experimentally or associated with vascular disease, is correlated with abnormal arterial metabolism and the familiar triad of disturbed elastic tissue structure, increased conduit artery stiffness and raised systolic blood pressure. Putative mechanisms of the link between them have been reviewed by Kuo et al [157] and include reduced production of endothelially derived NO, endothelial damage and LDL modification due to oxidation products of Hcy, and interference with collagen and/or elastin cross-linking. However, it remains to be convincingly shown that the therapeutic vascular effects of reducing plasma Hcy levels are dependent on changes in large artery elasticity and/or endothelial function, when considered independently of the link between age-related pathology and Hcy.

**The role of the endothelium**

There is good evidence that endothelial function, as assessed by the availability of NO, vascular production of \( \text{O}_2^- \) and vascular reactivity, falls with age [174] (see [175] for a recent review and a discussion of the interesting observations that endothelial cell proliferation due to injury or angiogenesis leads to inhibition of telomerase reverse transcriptase, consequent accelerated telomere shortening and endothelial cell senescence [176]). In resistance vessels, endothelium-dependent vasodilatation falls with age [174]. The consequent increase in resting VSMC tone due to reduced availability of NO leads to increased mean blood pressure and greater stretch of the conduit arteries. Thus, they become stiffer (elastic non-linearity) and pulse pressure is increased.

In the conduit arteries impaired endothelial function augments the development of atherosclerosis (for a review, see [177]). Although animal experiments suggest that increased VSMC tone in conduit arteries results in increased stiffness (see above), there is no evidence that large artery stiffness is changed by manipulation of NO synthesis in vivo [178]. However, in the longer term reduced NO availability may be associated with structural changes, due to its effect on the synthesis of matrix proteins [179,180]. Accurate assessment of endothelial function in vivo remains a technical challenge and further clarification of its role in age-related changes in large arteries must await improved methods for its measurement [181].

**Fetal programming**

It is clear that fatigue failure and the enzymatic remodelling of elastic tissue appear to be inexorable consequences of ageing and among the main causes of increasing pulse pressure with age. However, there is a wide variation in the steepness of this increase. Events in utero affecting the synthesis of elastin may provide a possible explanation of this variation, based on the concept of the fetal origins of metabolic disease (fetal programming) developed by David Barker and his colleagues. This notion has provided a link between the many reports of an association between various indices of impaired fetal growth, for example, low birth weight or a high ratio of head to abdominal circumference, and an increased incidence of coronary heart disease [182], stroke [183], hypertension [184], type 2 diabetes [185] and atherosclerosis [186].

Fetal programming may be described as a stimulus or insult at a critical period of early life, often when rates of growth are maximal, leading to irreversible changes in the structure and function of target organs [187,188]. Expressed briefly and applied to essential hypertension: the lower one’s birth weight, the more likely one is to have high blood pressure in middle age. Although the evidence for this association is now incontestable (see eg [189]), doubt remains about the underlying mechanism(s).

We have proposed a possible explanation for this association [190]. The idea is that, in fetuses whose growth is retarded, there is impairment in the synthesis of elastin during a critical period of blood vessel development. This impairment may be a consequence...
of haemodynamic changes in the fetal circulation that accompany intra-uterine growth retardation. As a result of the relative deficiency in elastin, the compliance of the aorta and large arteries is reduced. This in turn leads to higher pulse pressures, as explained above. Over time, elastin fragmentation due to fatigue failure and the transfer of mechanical load to collagen will tend to amplify the increase in blood pressure and may also independently predispose to left ventricular hypertrophy and impaired cardiac function. Up to now the evidence is sparse but it is accumulating. Earlier findings [191] have been confirmed that, after due allowance has been made for confounding factors, such as obesity, salt intake, heredity and alcohol consumption, higher than average blood pressure in middle age is inversely and strongly correlated with birth weight [192], and it has also been observed that conduit artery stiffness in middle age, as well as mean and pulse pressure, are higher in those with low birth weight or short stature. Others have found similar associations between impaired growth in early life and increased vascular stiffness [193], although some studies have found no evidence of this association [194]. A pilot study has recently been completed of arterial stiffness and blood pressure in a group of Zambian children aged 5–9 years and it was found that those with the lowest birth weight do indeed have higher systolic pressure and stiffer femoral arteries than those with the highest birth weights [195]. Furthermore, the mothers of the low birth weight children tended to be malnourished during pregnancy. The hypothesis is also being tested in an animal model of growth restriction. Measurements of aortic stiffness and protein composition in rats aged 4 weeks, whose mothers were fed a low-protein diet during pregnancy, clearly show that their aortas are stiffer and contain less elastin than controls born to mothers fed a normal diet [196]. Similar experiments on rats aged 6 weeks–1 year are in progress.

There is little direct evidence that elastin synthesis may be vulnerable to restriction before birth, although in a post mortem study of the human aorta Berry et al [197] have shown that early in prenatal development collagen is formed, whereas the elastin content remains at a lower level. During the perinatal period, the rate of elastin synthesis increases by a factor of 2 and aortic elastin content approaches postnatal values. Similar findings have been reported in the sheep [198]. The longevity of elastin and its lack of synthesis (at least in the healthy arterial system) in adulthood implies that reduced synthesis in early life may not be correctable during subsequent growth and development.

The idea that vessel development is modulated by changes in mechanical load, or that lasting perturbations in normal vessel growth may be caused by abnormal levels of this load due to disturbed pressure and/or flow, is supported by several strands of evidence. First, at birth, when pulmonary and systemic pressures are equal, the ratios of collagen to elastin in the rabbit aorta and the pulmonary artery are similar. By the age of 2 months, when systemic blood pressure has increased from 40 to 80 mmHg and pulmonary pressure has decreased to approximately 15 mmHg, the elastin:collagen ratio in the aorta is 1.75 times greater than that in the pulmonary artery [199]. Corresponding changes in stiffness have been observed in the pig pulmonary artery and aorta during the same period [200]. Second, many investigators have shown that infants with higher than average blood pressure tend to remain in a given percentile throughout childhood and adulthood [201–204] (blood pressure tracking). Third, in children born with a single umbilical artery (in whom the entire placental flow during fetal life passes through the common iliac artery on one side only, whilst that on the other side experiences almost no flow), it was observed that the stiffness of one common iliac artery was approximately 1.7 times greater than that of the other, although it was not known which side had been exposed to the full placental flow [205]. In a post mortem investigation on a similar group of children, it was found that the vessel that had been exposed to high flow had a normal lamellar structure rich in elastin; whereas the contralateral vessel was depleted in elastin and had a thin, predominantly muscular wall [206]. Finally, recent measurements of brachial artery pulse wave velocity in identical twins with twin-to-win transfusion syndrome (TTTS) have revealed intriguing differences between the two siblings. In this disorder, both twins suffer from abnormal haemodynamic loading in utero due to transfusion via placental anastomoses. The donor twin, which is characteristically small and hypovolaemic, typically has brachial artery PWV 25% less than that of its hypervolaemic sibling, whereas this disparity is not seen in non-TTTS twins. Furthermore, removal of the intertwin transfusion by laser treatment of the placental anastomoses alters (but does not remove) the disparity, resulting in PWV values similar to those of non-identical twins [207].

In summary, the perinatal changes in the pulmonary artery and aorta, the single umbilical artery and TTTS studies all suggest that arterial composition, morphology and elasticity may undergo lasting changes following a brief exposure to abnormal levels of pressure and flow in utero. The tracking data show that, once established in early life, relative blood pressure values are maintained into adulthood. All these results, together with the observations that low birth weight babies have stiffer conduit arteries and higher blood pressure in adulthood, support the notion that fetal programming of arterial structure by diminished elastin synthesis, and premature failure of elastin, may account in part for the association between fetal growth restriction and essential hypertension in adulthood.

Several alternative hypotheses have been proposed. For instance, because expression of the elastin gene is regulated by, among other things, insulin-like growth factor-1 and glucocorticoids, other non-mechanical pathways may be involved [208]. It is also likely that
impaired renal development in utero leads to disturbances in the renin–angiotensin system [209–212], as well as compromised endothelial function [213,214] (for reviews of mechanisms investigated in animal studies, see [215,216]). Whatever the underlying causes, the results of the epidemiological and animal studies mentioned above do support the hypothesis that the link between low birth weight and high blood pressure may be due to raised conduit artery stiffness resulting from impaired elastin production in early life.

Conclusion

It has now been established beyond reasonable doubt that there are strong independent associations between increased arterial stiffness or pulse pressure and increased morbidity, and mortality from cardiovascular disease. These correlations, together with recent technical innovations, account for the rapidly increasing number of studies involving direct (pulse wave velocity) and indirect (pulsewave analysis) measurements of arterial stiffness, both as a diagnostic tool and as a prognostic indicator, not only in patients with established cardiovascular disease but in healthy populations as well (see [217] for a critical review). Conduit arteries become stiffer with age because elastin becomes fragmented, degraded and replaced by much stiffer collagen. Furthermore, both proteins become stiffer as a result of cross-linking and calcification, changes which are accelerated by uraemia, hyperglycaemia and oxidative stress. Although there is much evidence for the link between arterial stiffening and the degradation and remodelling of collagen and elastin, much remains unknown about the detailed mechanisms. Elucidating these mechanisms will lead not only to novel and perhaps more effective treatments of vascular degenerative disease but also to a better general understanding of ageing in connective tissue.

References


Ageing of the conduit arteries


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