

Review Article

Ageing of the conduit arteries

SE Greenwald*

Pathology Group, Institute of Cell and Molecular Science, Barts and the London School of Medicine and Dentistry, Queen Mary, University of London

*Correspondence to:

SE Greenwald, Pathology and
Pharmacy Building, Royal London
Hospital, 80 Newark Street,
London E1 2ES, UK.

E-mail:

s.e.greenwald@qmul.ac.uk

No conflicts of interest were
declared.

Abstract

Conduit arteries become stiffer with age due to alterations in their morphology and the composition of their 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 metallo proteinases (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.

Copyright © 2007 Pathological Society of Great Britain and Ireland. Published by John Wiley & Sons, Ltd.

Keywords: elastin; collagen; vascular endothelial cell; arterial compliance; elasticity; AGE; MMP; elastocalcinosis; homocysteine; fetal programming

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. ie 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].

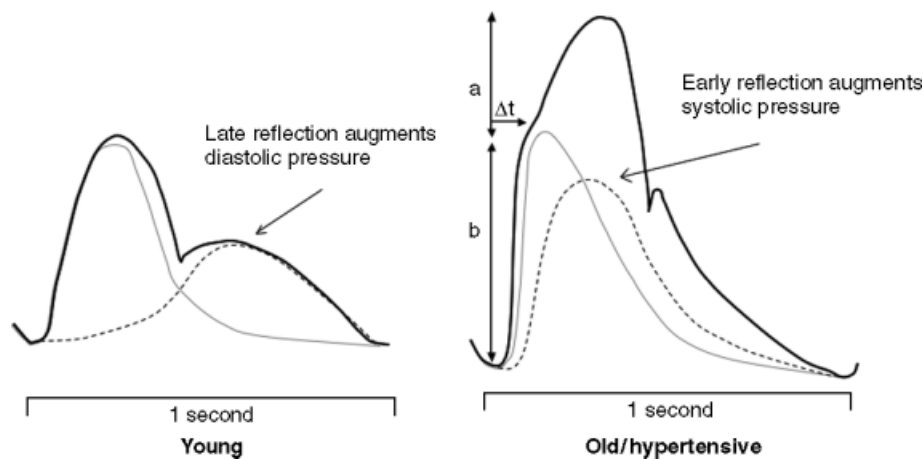


Figure 1. The combination of the forward-going wave generated by the heart (thin grey line) and the sum of many reflected waves from various distal sites (dashed line) gives rise to the measured wave (thick black line). The shape of this wave (ie the pressure, and therefore mechanical force, 'felt' by 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_x), 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 Δt . 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/or 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

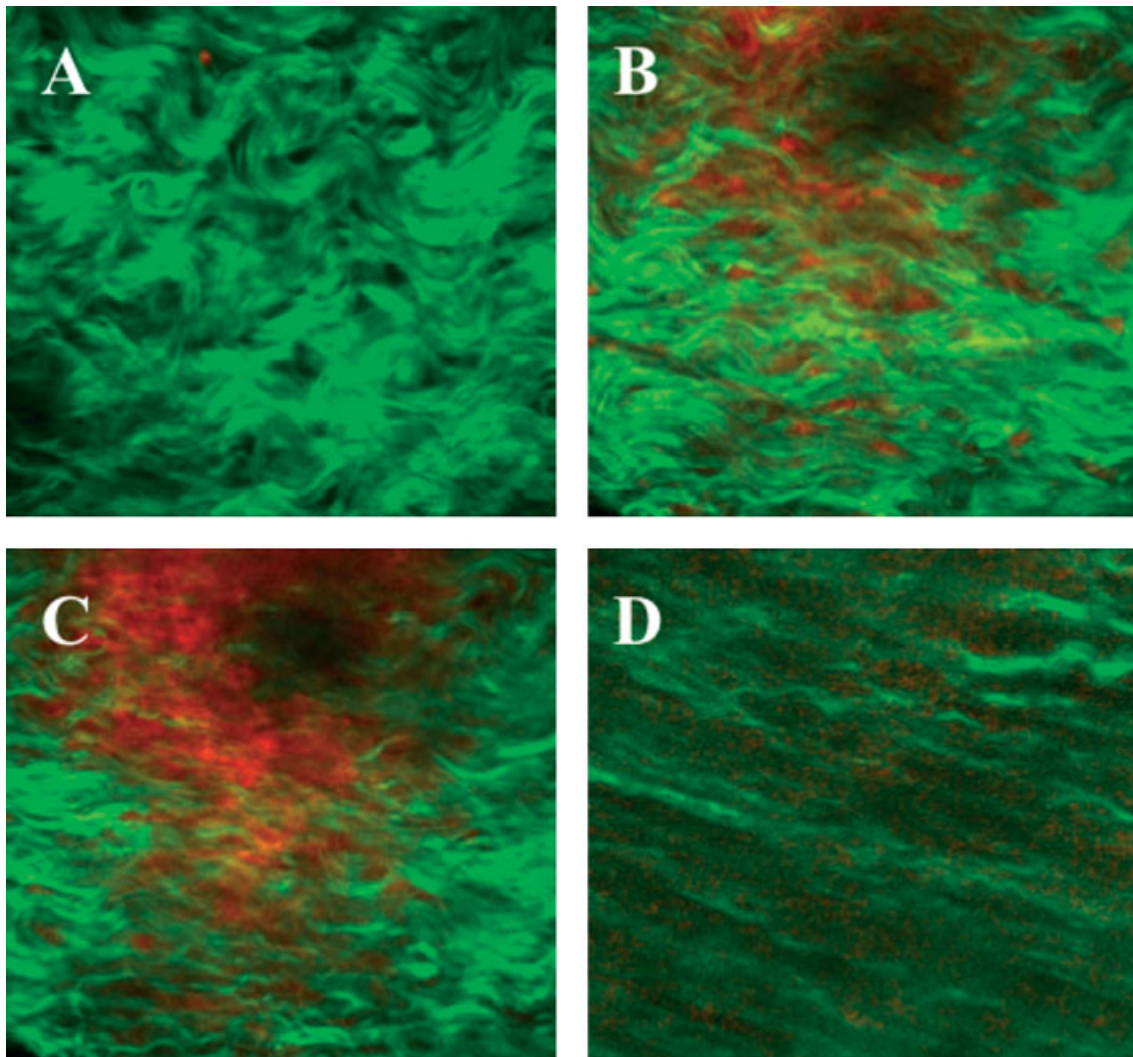


Figure 2. Inner adventitia of the rat carotid artery viewed by two-photon excited fluorescence and second harmonic generation microscopy. Both imaging modes are confocal and are combined to show collagen fibres in green and the underlying external elastic lamella in red. The collagen fibre bundles progressively 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

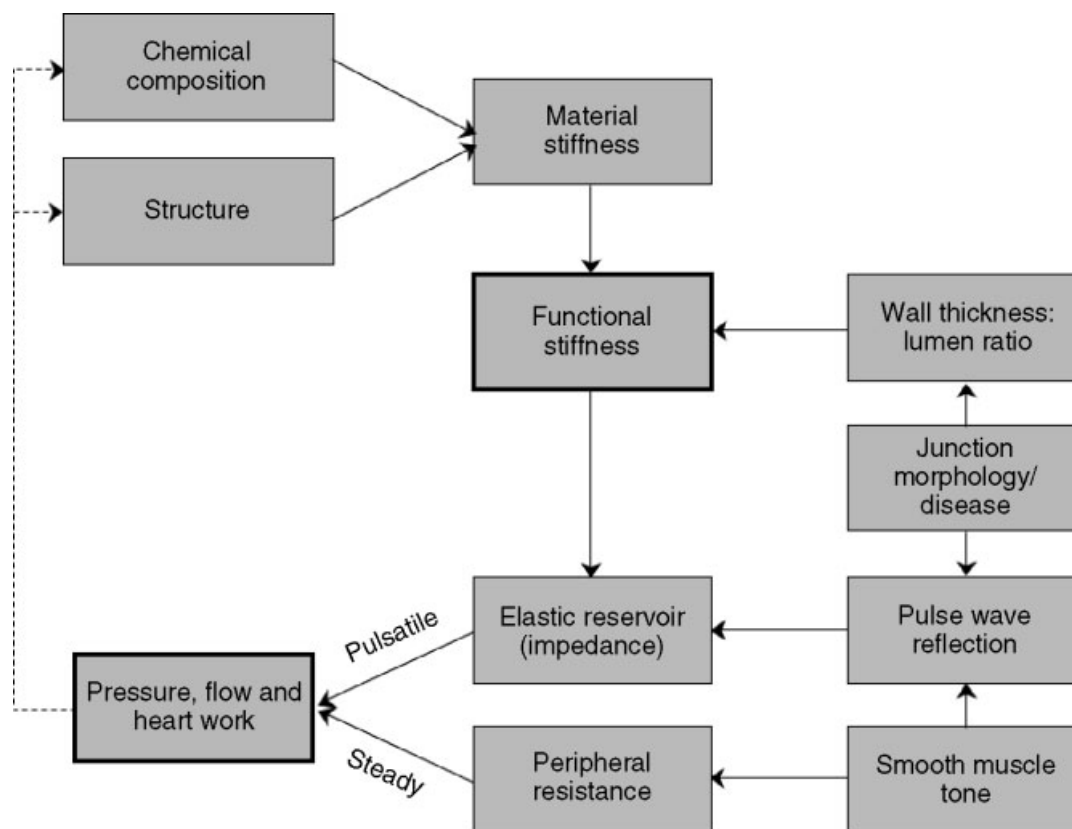


Figure 3. Interrelationships between vessel composition, structure, elasticity, geometry, elastic reservoir function and cardiac work. Functional stiffness, as measured non-invasively by PWV, for instance, depends on the combined effects of composition and geometry. Thus, a vessel with given material 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

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.

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 *in vivo*, 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

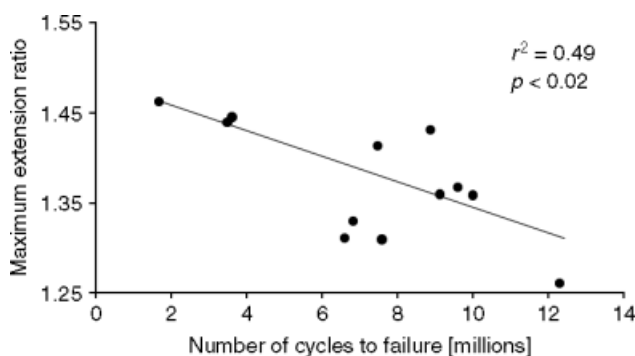


Figure 4. The number of cycles to failure of pig aortic elastin rings increases as the maximum extension of the ring during each stretch cycle is reduced. In other words, the greater the stretch, the sooner the failure. Such behaviour is characteristic of elastomeric fatigue fracture

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 non-atherosclerotic arterial calcification is associated with elastic tissue, because large arteries tend to be more severely affected than smaller more muscular and collagenous 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 sinitrodimil, 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 metalloproteinases, 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 κ B. 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 arteriosclerosis 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 \geq 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 Hcy 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 Hcy and several age-related cardiovascular pathologies, the therapeutic potential of reducing Hcy 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 Hcy, 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 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-twin 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 inter-twin 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

- Vogel S. *Vital Circuits. On Pumps, Pipes and the Workings of Circulatory Systems*. Oxford University Press: New York, Oxford, 1992.
- Lehmann ED. Noninvasive measurements of aortic stiffness: methodological considerations. *Pathol Biol (Paris)* 1999;**47**(7):716–730.
- Loukogeorgakis S, Dawson R, Phillips N, Martyn CN, Greenwald SE. Validation of a device to measure arterial pulse wave velocity by a photoplethysmographic method. *Physiol Measurment* 2002;**23**(3):581–596.
- Greenwald SE. Pulse pressure and arterial elasticity. *Qu J Med* 2002;**95**(2):107–112.
- Westerhof N, Stergiopoulos N, Noble MIM. *Snapshots of Haemodynamics. An Aid for Clinical Research and Graduate Education*. Kluwer Academic: Dordrecht, 2005.
- Pedley TJ. *The Fluid Mechanics of Large Blood Vessels*. Cambridge University Press: Cambridge, 1980; 106–108.
- Asakura T, Karino T. Flow patterns and spatial distribution of atherosclerotic lesions in human coronary arteries. *Circ Res* 1990;**66**(4):1045–1066.
- Cornhill JF, Herderick EE, Stary HC. Topography of human aortic sudanophilic lesions. *Monogr Atheroscler* 1990;**15**:13–19.
- Deng X, King MW, Guidoin R. Localization of atherosclerosis in arterial junctions. Concentration distribution of low density lipoproteins at the luminal surface in regions of disturbed flow. *ASAIO J* 1995;**41**(1):58–67.
- Nichols WW, O'Rourke MF. *McDonald's Blood Flow in Arteries* (5th edn). London: Hodder Arnold; 2005; p. 165–191.
- O'Rourke M. Arterial stiffness, systolic blood pressure, and logical treatment of arterial hypertension. *Hypertension* 1990;**15**(4):339–347.
- Roy CS. The elastic properties of the arterial wall. *Phil Trans R Soc B* 1880;**99**:1–31.
- Karnbaum S. Elastizität und Morphologie des Aortenwindkessels beim Blüthochdruck. *Archiv Kreislaufforsch* 1961;**34**:18–74.
- Avolio AP, Chen SG, Wang RP, Zhang CL, Li MF, O'Rourke MF. Effects of aging on changing arterial compliance and left ventricular load in a northern Chinese urban community. *Circulation* 1983;**68**(1):50–58.
- Asmar R, Benetos A, London G, Hugue C, Weiss Y, Topouchian J, *et al.* Aortic distensibility in normotensive, untreated and treated hypertensive patients. *Blood Press* 1995;**4**(1):48.
- Franklin SS. Blood pressure and cardiovascular disease: what remains to be achieved? *J Hypertens Suppl* 2001;**19**(3):S3–8.
- Hajjar I, Kotchen TA. Trends in prevalence, awareness, treatment, and control of hypertension in the United States, 1988–2000. *J Am Med Assoc* 2003;**290**(2):199–206.
- Avolio AP, Deng FQ, Li WQ, Luo YF, Huang ZD, Xing LF, *et al.* Effects of aging on arterial distensibility in populations with high and low prevalence of hypertension: comparison between urban and rural communities in China. *Circulation* 1985;**71**(2):202–210.
- Poulter NR, Khaw K, Hopwood BE, Mugambi M, Peart WS, Sever PS. Determinants of blood pressure changes due to urbanization: a longitudinal study. *J Hypertens Suppl* 1985;**3**(3):S375–377.
- Henry JP, Cassel JC. Psychosocial factors in essential hypertension. Recent epidemiologic and animal experimental evidence. *Am J Epidemiol* 1969;**90**(3):171–200.
- Domanski M, Norman J, Wolz M, Mitchell G, Pfeffer M. Cardiovascular risk assessment using pulse pressure in the first national health and nutrition examination survey (NHANES I). *Hypertension* 2001;**38**(4):793–797.
- Urbina EM, Srinivasan SR, Kieleyka RL, Tang R, Bond MG, Chen W, *et al.* Correlates of carotid artery stiffness in young adults: the Bogalusa Heart Study. *Atherosclerosis* 2004;**176**(1):157.
- Franklin SS, Larson MG, Khan SA, Wong ND, Leip EP, Kannel WB, *et al.* Does the relation of blood pressure to coronary heart disease risk change with aging? The Framingham Heart Study. *Circulation* 2001;**103**(9):1245–1249.
- Vaccarino V, Holford TR, Krumholz HM. Pulse pressure and risk for myocardial infarction and heart failure in the elderly. *J Am Coll Cardiol* 2000;**36**(1):130–138.
- Cohn JN, Duprez DA, Grandits GA. Arterial elasticity as part of a comprehensive assessment of cardiovascular risk and drug treatment. *Hypertension* 2005;**46**(1):217–220.
- Wilkinson IB, McEniery CM. Arterial Stiffness, Endothelial function and novel pharmacological approaches. *Clin Exp Pharmacol Physiol* 2004;**31**(11):795–799.
- Dart AM, Kingwell BA. Pulse pressure — a review of mechanisms and clinical relevance. *J Am Coll Cardiol* 2001;**37**(4):975–984.
- Jacob M. Extracellular matrix remodeling and matrix metalloproteinases in the vascular wall during aging and in pathological conditions. *Biomed Pharmacother* 2003;**57**(5–6):195.
- von-Maltzahn WW, Warriyar RG, Keitzer WF. Experimental measurements of elastic properties of media and adventitia of bovine carotid arteries. *J Biomech* 1984;**17**(11):839–847.
- Berry J, Moore JE Jr, Rachev A, Meister J-J. Analysis of the effects of a non-circular two layer stress-free state on arterial wall stresses. In IEEE-EMBS Conference, Paris; 1992; 65–66.

31. Xie J, Zhou J, Fung YC. Bending of blood vessel wall: stress-strain laws of the intima-media and adventitial layers. *J Biomech Eng* 1995;**117**(1):136–145.
32. Rachev A. Theoretical study of the effect of stress-dependent remodeling on arterial geometry under hypertensive conditions. *J Biomech* 1997;**30**(8):819–827.
33. Holzapfel G. Biomechanics of soft tissue. In *Handbook of Materials Behavior Models* (1st edn), Lemaitre J (ed.). Elsevier: Amsterdam, 2001.
34. Schulze-Bauer CA, Regitnig P, Holzapfel GA. Mechanics of the human femoral adventitia including the high-pressure response. *Am J Physiol Heart Circ Physiol* 2002;**282**(6):H2427–2440.
35. Dobrin PB. Mechanical properties of arteries. *Physiol Rev* 1978;**58**:397–446.
36. Gosline JM. The elastic properties of rubber-like proteins and highly extensible tissues. *Symp Soc Exp Biol* 1980;**34**:332–357.
37. Milewicz DM, Urban Z, Boyd C. Genetic disorders of the elastic fiber system. *Matrix Biol* 2000;**19**(6):471.
38. Apter JT, Rabinowitz M. Correlation of visco-elastic properties of large arteries with microscopic structure. (1) Methods used and their justification. (2) Elastin and muscle determined chemically. *Circ Res* 1966;**19**:104–121.
39. Harkness ML, Harkness RD, McDonald DA. The collagen and elastin content of the arterial wall in the dog. *Proc R Soc Lond B Biol Sci* 1957;**146**(925):541–551.
40. Looker T, Berry CL. The growth and development of the rat aorta. II. Changes in nucleic acid and scleroprotein content. *J Anat* 1972;**113**(1):17–34.
41. Janzen J. The microscopic transitional zone between elastic and muscular arteries. *Arch Mal Coeur Vaiss* 2004;**97**(9):909–914.
42. Clark JM, Glagov S. Transmural organization of the arterial media. The lamellar unit revisited. *Arteriosclerosis* 1985;**5**(1):19–34.
43. Nakamura T, Lozano PR, Ikeda Y, Iwanaga Y, Hinek A, Minamisawa S, *et al.* Fibulin-5/DANCE is essential for elastogenesis *in vivo*. *Nature* 2002;**415**(6868):171–175.
44. Chothia C, Jones EY. The molecular structure of cell adhesion molecules. *Annu Rev Biochem* 1997;**66**:823–862.
45. Labat-Robert J. Cell-matrix interactions, alterations with aging, involvement in angiogenesis. *Pathol Biol (Paris)* 1998;**46**(7):527–533.
46. Dobrin PB. Mechanical behavior of vascular smooth muscle in cylindrical segments of arteries *in vitro*. *Ann Biomed Eng* 1984;**12**(5):497–510.
47. Greenwald SE, Newman DL, Denyer HT. The effect of smooth muscle activity on the static and dynamic elastic properties of the rabbit carotid artery. *Cardiovasc Res* 1982;**16**:86–94.
48. Dobrin PB. Influence of initial length on length-tension relationship of vascular smooth muscle. *Am J Physiol* 1973;**225**(3):664–670.
49. Gow BS. The influence of vascular smooth muscle on the viscoelastic properties of blood vessels. In *Cardiovascular Fluid Dynamics*, Bergel DH (ed.). Academic Press: New York, 1972; 66–110.
50. Rachev A, Hayashi K. Theoretical study of the effects of vascular smooth muscle contraction on strain and stress distributions in arteries. *Ann Biomed Eng* 1999;**27**:459–468.
51. Nichols WW, O'Rourke MF. *McDonald's Blood Flow in Arteries* (5th edn). Hodder Arnold: London, 2005; 70–93.
52. Roach MR, Burton AC. The reason for the shape of the distensibility curves of arteries. *Can J Biochem Physiol* 1957;**35**:681–690.
53. Cox RH. Passive mechanics and connective tissue composition of canine arteries. *Am J Physiol* 1978;**234**:H533–541.
54. Greenwald SE, Berry CL. The effect of alterations of scleroprotein content on the static mechanical properties of the arterial wall. *Adv Physiol Sci* 1980;**8**:203–212.
55. Holzapfel GA, Gasser TC. A new constitutive framework for arterial wall mechanics and a comparative study of material models. *J Elasticity* 2000;**61**:1–48.
56. Gasser TC, Ogden RW, Holzapfel GA. Hyperelastic modelling of arterial layers with distributed collagen fibre orientations. *J R Soc Interface* 2006;**3**(6):15–35.
57. Zulliger MA, Fridez P, Hayashi K, Stergiopoulos N. A strain energy function for arteries accounting for wall composition and structure. *J Biomech* 2004;**37**(7):989–1000.
58. Driessen NJ, Wilson W, Bouten CV, Baaijens FP. A computational model for collagen fibre remodelling in the arterial wall. *J Theor Biol* 2004;**226**(1):53–64.
59. Glagov S, Zarins CK, Masawa N, Xu CP, Bassiouny H, Giddens DP. Mechanical functional role of non-atherosclerotic intimal thickening (review). *Frontiers Med Biol Eng* 1993;**5**(1):37–43.
60. Virmani R, Avolio AP, Mergner WJ, Robinowitz M, Herderick EE, Cornhill JF, *et al.* Effect of aging on aortic morphology in populations with high and low prevalence of hypertension and atherosclerosis. Comparison between occidental and Chinese communities. *Am J Pathol* 1991;**139**(5):1119–1129.
61. Wenn CM, Newman DL. Arterial tortuosity. *Australas Phys Eng Sci Med* 1990;**13**(2):67–70.
62. Hosoda Y, Kawano K, Yamasawa F, Ishii T, Shibata T, Inayama S. Age-dependent changes of collagen and elastin content in human aorta and pulmonary artery. *Angiology* 1984;**35**(10):615–621.
63. Hayashi K, Nagasawa S, Naruo Y, Okumura A, Moritake K, Handa H. Mechanical properties of human cerebral arteries. *Biorheology* 1980;**17**(3):211–218.
64. Kao KYT, McGavack TH. Connective tissue I. Age and sex influence on protein composition of rat tissues. *Proc Soc Exp Biol Med* 1959;**101**:153–157.
65. Sans M, Moragas A. Mathematical morphologic analysis of the aortic medial structure. Biomechanical implications. *Anal Quant Cytol Histol* 1993;**15**(2):93–100.
66. Clausen B. Influence of age on connective tissue. Hexosamine and hydroxyproline in human aorta, myocardium and skin. *Lab Invest* 1962;**11**:229–234.
67. Cleary EG. A correlative and comparative study of the non-uniform arterial wall. PhD Thesis, University of Sydney, 1963.
68. Farrar JF, Blomfield J, Reye RDK. The structure and composition of the maturing pulmonary circulation. *J Pathol Bacteriol* 1965;**90**:83–96.
69. Toda T, Tsuda N, Nishimori I, Leszczynski DE, Kummerow FA. Morphometrical analysis of the aging process in human arteries and aorta. *Acta Anat (Basel)* 1980;**106**(1):35–44.
70. Hayashi K, Handa H, Nagasawa S, Okumura A, Moritake K. Stiffness and elastic behavior of human intracranial and extracranial arteries. *J Biomech* 1980;**13**(2):175–184.
71. Hayashi K, Stergiopoulos N, Meister J-J, Greenwald SE, Rachev A. Techniques in the determination of the mechanical properties and constitutive laws of arterial walls. In *Cardiovascular Techniques (Biomechanical Systems: Techniques and Applications)*, Leondes CT (ed.). CRC Press: Boca Raton, FL, 2001; 6:1–6:61.
72. Young T. On the functions of the heart and arteries. *Phil Trans* 1809;**99**:1–31.
73. Haynes FW, Ellis LB, Weiss E. Pulsewave velocity and arterial hypertension, arteriosclerosis and related conditions. *Am Heart J* 1936;**11**:385–401.
74. Hickler R. Aortic and large artery stiffness: current methodology and clinical correlations. *Clin Cardiol* 1990;**13**:317–322.
75. Laogun AA, Gosling RG. *In vivo* arterial compliance in man. *Clin Phys Physiol Measur* 1982;**3**(3):201–212.
76. Cattell M, Anderson J, Hasleton P. Age-related changes in amounts and concentrations of collagen and elastin in normotensive human thoracic aorta. *Clin Chim Acta* 1996;**245**(1):73.
77. Gozna ER, Marble AE, Shaw A, Holland JG. Age related changes in the mechanics of the aorta and pulmonary artery of man. *J Appl Physiol* 1974;**36**(4):407–411.
78. Kelly R, Hayward C, Avolio A, O'Rourke MF. Noninvasive determination of age-related changes in the human arterial pulse. *Circulation* 1989;**80**:1652–1659.
79. Asmar R, Benetos A, London G, Hugue C, Weiss Y, Topouchian J, *et al.* Aortic distensibility in normotensive, untreated and treated hypertensive patients. *Blood Press* 1995;**4**(1):48–54.

80. Van Merode T, Hick PJ, Hoeks AP, Rahn KH, Reneman RS. Carotid artery wall properties in normotensive and borderline hypertensive subjects of various ages. *Ultrasound Med Biol* 1988;**14**(7):563–569.
81. Hass G. Relations between structure of the ageing aorta and the properties of isolated aortic elastic tissue. *Arch Pathol* 1943;**35**:29–54.
82. Butcher HR, Newton WT. The influence of age, arteriosclerosis and homotransplantation upon the elastic properties of major human arteries. *Ann Surg* 1958;**148**:1–20.
83. Gosling RG, King DH. Ultrasonic angiology. In *Arteries and Veins*, Hargis A, Adamson L (eds). Churchill Livingstone: Edinburgh, 1974; 61–98.
84. Learoyd BM, Taylor MG. Alterations with age in the viscoelastic properties of human arterial walls. *Circ Res* 1966;**18**:278–292.
85. Benetos A, Laurent S, Hoeks AP, Boutouyrie PH, Safar ME. Arterial alterations with aging and high blood pressure. A noninvasive study of carotid and femoral arteries. *Arterioscler Thromb* 1993;**13**(1):90–97.
86. Boutouyrie P, Laurent S, Benetos A, Girerd XJ, Hoeks AP, Safar ME. Opposing effects of ageing on distal and proximal large arteries in hypertensives. *J Hypertens Suppl* 1992;**10**(6).
87. Kawasaki T, Sasayama S, Yagi S, Asakawa T, Hirai T. Non-invasive assessment of the age related changes in stiffness of major branches of the human arteries. *Cardiovasc Res* 1987;**21**(9):678–687.
88. Smulyan H, Csermely TJ, Mookherjee S, Warner RA. Effect of age on arterial distensibility in asymptomatic humans. *Arteriosclerosis* 1983;**3**:199–205.
89. Hallock P. Arterial elasticity in man in relation to age as evaluated by the pulse wave velocity method. *Arch Intern Med* 1934;**54**:770–798.
90. Greenwald SE, Carter AC, Berry CL. The effect of age on the reflection coefficient of the aorto-iliac junction in man. *Circulation* 1990;**82**:114–123.
91. Kamiya A, Togawa T. Adaptive regulation of wall shear stress to flow change in the canine carotid artery. *Am J Physiol* 1980;**239**(1):H14–21.
92. Tronc F, Wassef M, Esposito B, Henrion D, Glagov S, Tedgui A. Role of NO in flow-induced remodeling of the rabbit common carotid artery. *Arterioscler Thromb Vasc Biol* 1996;**16**(10):1256–1262.
93. Langille BL. Arterial remodeling: relation to hemodynamics. *Can J Physiol Pharmacol* 1996;**74**(7):834–841.
94. O'Rourke MF. Pulsatile arterial haemodynamics in hypertension. *Austral NZ J Med* 1976;**6**(suppl 2):40–48.
95. Lefevre M, Rucker RB. Aorta elastin turnover in normal and hypercholesterolemic Japanese quail. *Biochim Biophys Acta* 1980;**630**:519–529.
96. Schapiro SD, Endicott SK, Province MA, Pierce JA, Campbell EJ. Marked longevity of human lung parenchymal elastic fibres deduced from prevalence of D-aspartate and nuclear weapons-related radiocarbon. *J Clin Invest* 1991;**87**:1828–1834.
97. Powell JT, Vine N, Crossman M. On the accumulation of D-aspartate in elastin and other proteins of the ageing aorta. *Atherosclerosis* 1992;**97**:201–208.
98. Davis EC. Elastic lamina growth in the developing mouse aorta. *J Histochem Cytochem* 1995;**43**(11):1115–1123.
99. Hillery CB. Mechanical and morphological characteristics of elastin from conduit arteries. PhD Thesis, Queen Mary College, University of London, 2005.
100. Nichols WW, O'Rourke MF. Structural changes in arteries with age. In *McDonald's Blood Flow in Arteries* (4th edn). Arnold: London, 1998; 356–376.
101. Sa Cunha R, Pannier B, Benetos A, Siche JP, London GM, Mallion JM, et al. Association between high heart rate and high arterial rigidity in normotensive and hypertensive subjects. *J Hypertension* 1997;**15**(12, pt 1):1423–1430.
102. Atkinson J. Aging of arterial extracellular matrix elastin: etiology and consequences. *Pathol Biol (Paris)* 1998;**46**(7):555–559.
103. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature* 2000;**408**(6809):239–247.
104. Shao JS, Cai J, Towler DA. Molecular mechanisms of vascular calcification: lessons learned from the aorta. *Arterioscler Thromb Vasc Biol* 2006;**26**(7):1423–1430.
105. Abedin M, Tintut Y, Demer LL. Vascular calcification: mechanisms and clinical ramifications. *Arterioscler Thromb Vasc Biol* 2004;**24**(7):1161–1170.
106. Dao HH, Essalihi R, Bouvet C, Moreau P. Evolution and modulation of age-related medial elastocalcinosis: impact on large artery stiffness and isolated systolic hypertension. *Cardiovasc Res* 2005;**66**(2):307–317.
107. Proudfoot D, Shanahan CM. Biology of calcification in vascular cells: intima versus media. *Herz* 2001;**26**(4):245–251.
108. Klotz O. Studies upon calcareous degeneration: I. The process of pathological calcification. *J Exp Med* 1905;**7**:633–675.
109. Blumenthal H, Lansing A, Wheeler P. Calcification of the media of the human aorta and its relation to intimal atherosclerosis, ageing and disease. *Am J Pathol* 1944;**20**:665–679.
110. Otto CM, Lind BK, Kitzman DW, Gersh BJ, Siscovick DS. Association of aortic-valve sclerosis with cardiovascular mortality and morbidity in the elderly. *N Engl J Med* 1999;**341**(3):142–147.
111. Rucker RB. Calcium binding to elastin. *Adv Exp Med Biol* 1974;**48**(0):185–209.
112. Bailey M, Pillarisetti S, Jones P, Xiao H, Simionescu D, Vyavahare N. Involvement of matrix metalloproteinases and tenascin-C in elastin calcification. *Cardiovasc Pathol* 2004;**13**(3):146.
113. Guerin AP, London GM, Marchais SJ, Metivier F. Arterial stiffening and vascular calcifications in end-stage renal disease. *Nephrol Dialysis Transpl* 2000;**15**(7):1014–1021.
114. Schurgers LJ, Teunissen KJ, Knapen MH, Kwaijtaal M, van Diest R, Appels A, et al. Novel conformation-specific antibodies against matrix gamma-carboxyglutamic acid (Gla) protein: undercarboxylated matrix Gla protein as marker for vascular calcification. *Arterioscler Thromb Vasc Biol* 2005;**25**(8):1629–1633.
115. Price PA, Faus SA, Williamson MK. Warfarin causes rapid calcification of the elastic lamellae in rat arteries and heart valves. *Arterioscler Thromb Vasc Biol* 1998;**18**(9):1400–1407.
116. Essalihi R, Dao HH, Yamaguchi N, Moreau P. A new model of isolated systolic hypertension induced by chronic warfarin and vitamin K1 treatment. *Am J Hypertens* 2003;**16**(2):103–110.
117. Niederhoffer N, Lartaud-Idjouadiene I, Giummelly P, Duvivier C, Peslin R, Atkinson J. Calcification of medial elastic fibers and aortic elasticity. *Hypertension* 1997;**29**(4):999–1006.
118. Bielak LF, Turner ST, Franklin SS, Sheedy PF II, Peyser PA. Age-dependent associations between blood pressure and coronary artery calcification in asymptomatic adults. *J Hypertens* 2004;**22**(4):719–725.
119. Dao HH, Essalihi R, Graillon JF, Lariviere R, De Champlain J, Moreau P. Pharmacological prevention and regression of arterial remodeling in a rat model of isolated systolic hypertension. *J Hypertens* 2002;**20**(8):1597–1606.
120. Li Z, Froehlich J, Galis ZS, Lakatta EG. Increased expression of matrix metalloproteinase-2 in the thickened intima of aged rats. *Hypertension* 1999;**33**(1):116–123.
121. Wang M, Lakatta EG. Altered regulation of matrix metalloproteinase-2 in aortic remodeling during aging. *Hypertension* 2002;**39**(4):865–873.
122. Gonzalez W, Fontaine V, Pueyo ME, Laquay N, Messika-Zeitoun D, Philippe M, et al. Molecular plasticity of vascular wall during N(G)-nitro-L-arginine methyl ester-induced hypertension: modulation of proinflammatory signals. *Hypertension* 2000;**36**(1):103–109.
123. Curci JA, Liao S, Huffman MD, Shapiro SD, Thompson RW. Expression and localization of macrophage elastase (matrix metalloproteinase-12) in abdominal aortic aneurysms. *J Clin Invest* 1998;**102**(11):1900–1910.
124. Tamarina NA, McMillan WD, Shively VP, Pearce WH. Expression of matrix metalloproteinases and their inhibitors in aneurysms and normal aorta. *Surgery* 1997;**122**(2):264–271; discussion 271–272.
125. Blankenberg S, Rupprecht HJ, Poirier O, Bickel C, Smieja M, Hafner G, et al. Plasma concentrations and genetic variation

- of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. *Circulation* 2003;**107**(12):1579–1585.
126. Yasmin, McEniery CM, Wallace S, Dakham Z, Pulsalkar P, Maki-Petaja K, *et al.* Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. *Arterioscler Thromb Vasc Biol* 2005;**25**(2):372.
 127. Yasmin, McEniery CM, O'Shaughnessy KM, Harnett P, Arshad A, Wallace S, *et al.* Variation in the human matrix metalloproteinase-9 gene is associated with arterial stiffness in healthy individuals. *Arterioscler Thromb Vasc Biol* 2006;**26**(8):1799–1805.
 128. Laurent S, Boutouyrie P, Lacolley P. Structural and genetic bases of arterial stiffness. *Hypertension* 2005;**45**(6):1050–1055.
 129. Kaanane A, Labuza TP. The Maillard reaction in foods. *Progr Clin Biol Res* 1989;**304**:301–327.
 130. Monnier VM, Sell DR. Prevention and repair of protein damage by the Maillard reaction *in vivo*. *Rejuv Res* 2006;**9**(2):264–273.
 131. Wollfenbittel BH, Boulanger CM, Crijns FR, Huijberts MS, Poitevin P, Swennen GN, *et al.* Breakers of advanced glycation end-products restore large artery properties in experimental diabetes. *Proc Natl Acad Sci USA* 1998;**95**(8):4630–4634.
 132. Aronson D. Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes. *J Hypertens* 2003;**21**(1):3–12.
 133. Konova E, Baydanoff S, Atanasova M, Velkova A. Age-related changes in the glycation of human aortic elastin. *Exp Gerontol* 2004;**39**(2):249–254.
 134. Winlove CP, Parker KH, Avery NC, Bailey AJ. Interactions of elastin and aorta with sugars *in vitro* and their effects on biochemical and physical properties. *Diabetologia* 1996;**39**(10):1131–1139.
 135. Vlassara H, Bucala R, Striker L. Pathogenic effects of advanced glycosylation: biochemical, biologic, and clinical implications for diabetes and aging. *Lab Invest* 1994;**70**(2):138–151.
 136. Vlassara H, Cai W, Crandall J, Goldberg T, Oberstein R, Dardaine V, *et al.* Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. *Proc Natl Acad Sci USA* 2002;**99**(24):15596–15601 [erratum appears in *Proc Natl Acad Sci USA* 2003 Jan 21; **100**(2): 763].
 137. Goldberg T, Cai W, Peppas M, Dardaine V, Baliga BS, Uribarri J, *et al.* Advanced glycoxidation end-products in commonly consumed foods. *J Am Diet Assoc* 2004;**104**(8):1287–1291 [erratum appears in *J Am Diet Assoc* 2005 Apr; **105**(4): 647].
 138. Koschinsky T, He CJ, Mitsuhashi T, Bucala R, Liu C, Buenting C, *et al.* Orally absorbed reactive glycation products (glycotoxins): an environmental risk factor in diabetic nephropathy. *Proc Natl Acad Sci USA* 1997;**94**(12):6474–6479.
 139. Goldin A, Beckman JA, Schmidt AM, Creager MA. Advanced glycation end-products: sparking the development of diabetic vascular injury. *Circulation* 2006;**114**(6):597–605.
 140. Wollfenbittel BHR, Boulanger CM, Crijns FRL, Huijberts MSP, Poitevin P, Swennen GNM, *et al.* Breakers of advanced glycation end-products restore large artery properties in experimental diabetes. *Proc Natl Acad Sci USA* 1998;**95**(8):4630–4634.
 141. Vaitkevicius PV, Lane M, Spurgeon H, Ingram DK, Roth GS, Egan JJ, *et al.* A cross-link breaker has sustained effects on arterial and ventricular properties in older rhesus monkeys. *Proc Natl Acad Sci USA* 2001;**98**(3):1171–1175.
 142. Susic D, Varagic J, Ahn J, Frohlich ED. Cardiovascular and renal effects of a collagen cross-link breaker (ALT 711) in adult and aged spontaneously hypertensive rats. *Am J Hypertens* 2004;**17**(4):328–333.
 143. Vasan S, Foiles P, Founds H. Therapeutic potential of breakers of advanced glycation end-product–protein crosslinks. *Arch Biochem Biophys* 2003;**419**(1):89–96.
 144. Kass DA, Shapiro EP, Kawaguchi M, Capriotti AR, Scuteri A, deGroof RC, *et al.* Improved arterial compliance by a novel advanced glycation end-product crosslink breaker. *Circulation* 2001;**104**(13):1464–1470.
 145. Bakris GL, Bank AJ, Kass DA, Neutel JM, Preston RA, Oparil S. Advanced glycation end-product cross-link breakers: a novel approach to cardiovascular pathologies related to the aging process. *Am J Hypertens* 2004;**17**(12, suppl 1):S23.
 146. Corman B, Duriez M, Poitevin P, Heudes D, Bruneval P, Tedgui A, *et al.* Aminoguanidine prevents age-related arterial stiffening and cardiac hypertrophy. *Proc Natl Acad Sci USA* 1998;**95**(3):1301–1306.
 147. Forbes JM, Cooper ME, Thallas V, Burns WC, Thomas MC, Brammar GC, *et al.* Reduction of the accumulation of advanced glycation end-products by ACE inhibition in experimental diabetic nephropathy. *Diabetes* 2002;**51**(11):3274–3282.
 148. Chang KC, Hsu KL, Peng YI, Lee FC, Tseng YZ. Aminoguanidine prevents age-related aortic stiffening in Fisher 344 rats: aortic impedance analysis. *Br J Pharmacol* 2003;**140**(1):107–114.
 149. Nygard O, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE, *et al.* Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. *J Am Med Assoc* 1995;**274**(19):1526–1533.
 150. Stampfer MJ, Malinow MR, Willett WC, Newcomer LM, Uppson B, Ullmann D, *et al.* A prospective study of plasma homocysteine and risk of myocardial infarction in US physicians. *J Am Med Assoc* 1992;**268**(7):877–881.
 151. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *J Am Med Assoc* 1995;**274**(13):1049–1057.
 152. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. *N Engl J Med* 1998;**338**(15):1042–1050.
 153. Sutton-Tyrrell K, Bostom A, Selhub J, Zeigler-Johnson C. High homocysteine levels are independently related to isolated systolic hypertension in older adults. *Circulation* 1997;**96**(6):1745–1749.
 154. Aronow WS, Ahn C, Schoenfeld MR. Association between plasma homocysteine and extracranial carotid arterial disease in older persons. *Am J Cardiol* 1997;**79**(10):1432–1433.
 155. Mohan IV, Adam DJ, Kurian KM, Ruckley CV. Isolated external iliac aneurysm associated with hyperhomocysteinemia. *Eur J Vasc Endovasc Surg* 1997;**14**(6):506–508.
 156. Brunelli T, Prisco D, Fedi S, Rogolino A, Farsi A, Marcucci R, *et al.* High prevalence of mild hyperhomocysteinemia in patients with abdominal aortic aneurysm. *J Vasc Surg* 2000;**32**(3):531–536.
 157. Kuo HK, Sorond FA, Chen JH, Hashmi A, Milberg WP, Lipsitz LA. The role of homocysteine in multisystem age-related problems: a systematic review. *J Gerontol A Biol Sci Med Sci* 2005;**60**(9):1190–1201.
 158. Hassan A, Hunt BJ, O'Sullivan M, Bell R, D'Souza R, Jeffery S, *et al.* Homocysteine is a risk factor for cerebral small vessel disease, acting via endothelial dysfunction. *Brain* 2004;**127**(1):212–219.
 159. Rodgers GM, Conn MT. Homocysteine, an atherogenic stimulus, reduces protein C activation by arterial and venous endothelial cells. *Blood* 1990;**75**(4):895–901.
 160. Rolland PH, Friggi A, Barlatier A, Piquet P, Latrille V, Faye MM, *et al.* Hyperhomocysteinemia-induced vascular damage in the minipig. Captopril-hydrochlorothiazide combination prevents elastic alterations. *Circulation* 1995;**91**(4):1161–1174.
 161. Charpiot P, Bescond A, Augier T, Chareyre C, Fraternali M, Rolland PH, *et al.* Hyperhomocysteinemia induces elastolysis in minipig arteries: structural consequences, arterial site specificity and effect of captopril-hydrochlorothiazide. *Matrix Biol* 1998;**17**(8–9):559–574.
 162. Hill CH, Mecham R, Starcher B. Fibrillin-2 defects impair elastic fiber assembly in a homocysteinemic chick model. *J Nutr* 2002;**132**(8):2143–2150 [erratum appears in *J Nutr* 2002 Nov; **132**(11): 3431].
 163. Symons JD, Mullen AE, Ensuna JL, Ma AA, Rutledge JC. Hyperhomocysteinemia evoked by folate depletion: effects on coronary and carotid arterial function. *Arterioscler Thromb Vasc Biol* 2002;**22**(5):772–780.
 164. Bortolotto LA, Safar ME, Billaud E, Lacroix C, Asmar R, London GM, *et al.* Plasma homocysteine, aortic stiffness, and renal function in hypertensive patients. *Hypertension* 1999;**34**(4, pt 2):837–842.

165. Blacher J, Demuth K, Guerin AP, Safar ME, Moatti N, London GM. Influence of biochemical alterations on arterial stiffness in patients with end-stage renal disease. *Arterioscler Thromb Vasc Biol* 1998;**18**(4):535–541.
166. Arcaro G, Fava C, Dagradi R, Faccini G, Gaino S, Degan M, et al. Acute hyperhomocysteinemia induces a reduction in arterial distensibility and compliance. *J Hypertens* 2004;**22**(4):775–781.
167. Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *N Engl J Med* 1999;**340**(19):1449–1454.
168. Woo KS, Chook P, Lolin YI, Sanderson JE, Metreweli C, Celermajer DS. Folic acid improves arterial endothelial function in adults with hyperhomocystinemia. *J Am Coll Cardiol* 1999;**34**(7):2002–2006.
169. Chambers JC, Ueland PM, Obeid OA, Wrigley J, Refsum H, Kooner JS. Improved vascular endothelial function after oral B vitamins: an effect mediated through reduced concentrations of free plasma homocysteine. *Circulation* 2000;**102**(20):2479–2483.
170. Rimm EB, Willett WC, Hu FB, Sampson L, Colditz GA, Manson JE, et al. Folate and vitamin B6 from diet and supplements in relation to risk of coronary heart disease among women. *J Am Med Assoc* 1998;**279**(5):359–364.
171. Bazzano LA, He J, Ogden LG, Loria C, Vupputuri S, Myers L, et al. Dietary intake of folate and risk of stroke in US men and women: NHANES I Epidemiologic Follow-up Study. National Health and Nutrition Examination Survey. *Stroke* 2002;**33**(5):1183–1188.
172. Williams C, Kingwell BA, Burke K, McPherson J, Dart AM. Folic acid supplementation for 3 wk reduces pulse pressure and large artery stiffness independent of MTHFR genotype. *Am J Clin Nutr* 2005;**82**(1):26–31.
173. van Dijk RA, Rauwerda JA, Steyn M, Twisk JW, Stehouwer CD. Long-term homocysteine-lowering treatment with folic acid plus pyridoxine is associated with decreased blood pressure but not with improved brachial artery endothelium-dependent vasodilation or carotid artery stiffness: a 2-year, randomized, placebo-controlled trial. *Arterioscler Thromb Vasc Biol* 2001;**21**(12):2072–2079.
174. Gerhard M, Roddy MA, Creager SJ, Creager MA. Aging progressively impairs endothelium-dependent vasodilation in forearm resistance vessels of humans. *Hypertension* 1996;**27**(4):849–853.
175. Brandes RP, Fleming I, Busse R. Endothelial aging. *Cardiovasc Res* 2005;**66**(2):286–294.
176. Minamino T, Miyauchi H, Yoshida T, Ishida Y, Yoshida H, Komuro I. Endothelial cell senescence in human atherosclerosis: role of telomere in endothelial dysfunction. *Circulation* 2002;**105**(13):1541–1544.
177. Brunner H, Cockcroft JR, Deanfield J, Donald A, Ferrannini E, Halcox J, et al. Endothelial function and dysfunction. Part II: Association with cardiovascular risk factors and diseases. A statement by the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. *J Hypertens* 2005;**23**(2):233–246.
178. Stewart AD, Millasseau SC, Kearney MT, Ritter JM, Chowienczyk PJ. Effects of inhibition of basal nitric oxide synthesis on carotid–femoral pulse wave velocity and augmentation index in humans. *Hypertension* 2003;**42**(5):915–918.
179. Friedman H. Infection of endothelial cells by common human viruses. *Infect Dis* 1989;**11**(suppl 4):S700–704.
180. Henrion D, Kubis N, Levy BI. Physiological and pathophysiological functions of the AT(2) subtype receptor of angiotensin II: from large arteries to the microcirculation. *Hypertension* 2001;**8**(5):1150–1157.
181. Wilkinson IB, Franklin SS, Cockcroft JR. Nitric oxide and the regulation of large artery stiffness: from physiology to pharmacology. *Hypertension* 2004;**44**(2):112–116.
182. Barker DJP, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *Lancet* 1993;**341**:938–941.
183. Barker DJ. Intrauterine programming of coronary heart disease and stroke. *Acta Paediatr Suppl* 1997;**423**:178–182.
184. Barker DJ, Martyn CN. The fetal origins of hypertension. *Adv Nephrol Necker Hosp* 1997;**26**:65–72.
185. Phipps K, Barker DJ, Hales CN, Fall CH, Osmond C, Clark PM. Fetal growth and impaired glucose tolerance in men and women. *Diabetologia* 1993;**36**(3):225–228.
186. Martyn CN, Gale CR, Jespersen S, Sherriff SB. Impaired fetal growth and atherosclerosis of carotid and peripheral arteries. *Lancet* 1998;**352**(9123):173–178.
187. Barker DJ. Intra-uterine programming of the adult cardiovascular system. *Curr Opin Nephrol Hypertens* 1997;**6**(1):106–110.
188. Barker DJ, Clark PM. Fetal undernutrition and disease in later life. *Rev Reprod* 1997;**2**(2):105–112.
189. Law CM, Shiell AW. Is blood pressure inversely related to birth weight? The strength of evidence from a systematic review of the literature. *J Hypertens* 1996;**14**(8):935–941.
190. Martyn CN, Greenwald SE. Impaired synthesis of elastin in the walls of the aorta and large conduit arteries during early development may be an initiating event in the pathogenesis of systemic hypertension. *Lancet* 1997;**350**:953–955.
191. Barker D, Bull A, Osmond C, Simmonds S. Fetal and placental size and risk of hypertension in adult life. *Br Med J* 1990;**301**:259–262.
192. Martyn CN, Barker DJP, Jespersen S, Greenwald SE, Osmond C, Berry CL. Growth *in utero*, adult blood pressure and arterial compliance. *Br Heart J* 1995;**73**:116–121.
193. te Velde SJ, Ferreira I, Twisk JW, Stehouwer CD, van Mechelen W, Kemper HC. Birthweight and arterial stiffness and blood pressure in adulthood — results from the Amsterdam Growth and Health Longitudinal Study. *Int J Epidemiol* 2004;**33**(1):154–161.
194. Montgomery AA, Ben-Shlomo Y, McCarthy A, Davies D, Elwood P, Smith GD. Birth size and arterial compliance in young adults [comment]. *Lancet* 2000;**355**(9221):2136–2137.
195. Mutiti A, Kelly MP, Greenwald SE. Nutritional status and arterial stiffness in Zambian children. 4th International Congress of the African Association of Physiological Science, Tetouan, Morocco; 2004.
196. Greenwald SE, Martyn CN. Impaired synthesis of elastin during fetal and infant life as a cause of raised adult blood pressure. *Am J Hypertens* 1999;**12**(4):169A.
197. Berry CL, Looker T, Germaine J. Nucleic acid and sclero-protein content of the developing human aorta. *J Pathol* 1972;**108**:265–274.
198. Bendeck MP, Langille BL. Rapid accumulation of elastin and collagen in the aortas of sheep in the immediate perinatal period. *Circ Res* 1991;**69**(4):1165–1169.
199. Leung DYM, Glagov S, Mathews MB. A new *in vitro* system for studying cell response to mechanical stimulation. Different effects of cyclic stretching and agitation on smooth muscle cell biosynthesis. *Exp Cell Res* 1977;**109**:285–298.
200. Greenwald SE, Berry CL, Howarth SG. Changes in the distensibility of the intrapulmonary arteries in the normal newborn and growing pig. *Cardiovasc Res* 1982;**16**:716–726.
201. Raitakari OT, Porkka KV, Rasanen L, Ronnemaa T, Viikari JS. Clustering and six year cluster-tracking of serum total cholesterol, HDL-cholesterol and diastolic blood pressure in children and young adults. The Cardiovascular Risk in Young Finns Study. *J Clin Epidemiol* 1994;**47**(10):1085–1093.
202. Wilsgaard T, Jacobsen BK, Schirmer H, Thune I, Lochen ML, Njolstad I, et al. Tracking of cardiovascular risk factors: the Tromso study, 1979–1995. *Am J Epidemiol* 2001;**154**(5):418–426.
203. Voors AW, Webber LS, Berenson GS. Time course studies of blood pressure in children — the Bogalusa Heart Study. *Am J Epidemiol* 1979;**109**(3):320–334.
204. Clarke WR, Schrott HG, Leaverton PE, Connor WE, Lauer RM. Tracking of blood lipids and blood pressures in school age children: the Muscatine study. *Circulation* 1978;**58**(4):626–634.

205. Berry CL, Gosling RG, Laogun A, Bryant E. Anomalous iliac compliance in children with a single umbilical artery. *Br Heart J* 1976;**38**:310–315.
206. Meyer WW, Lind J. Iliac arteries in children with a single umbilical artery. Structure, calcifications, and early atherosclerotic lesions. *Arch Dis Child* 1974;**49**(9):671–679.
207. Gardiner HM, Taylor MJ, Karatza A, Vanderheyden T, Huber A, Greenwald SE, *et al.* Twin–twin transfusion syndrome: the influence of intrauterine laser photocoagulation on arterial distensibility in childhood. *Circulation* 2003;**107**(14):1906–1911.
208. Bendeck MP, Keeley FW, Langille BL. Perinatal accumulation of arterial wall constituents: relation to hemodynamic changes at birth. *Am J Physiol* 1994;**267**(6, pt 2):H2268–2279.
209. McMullen S, Gardner DS, Langley-Evans SC. Prenatal programming of angiotensin II type 2 receptor expression in the rat. *Br J Nutr* 2004;**91**(1):133–140.
210. Woods LL. Fetal origins of adult hypertension: a renal mechanism? *Curr Opin Nephrol Hypertens* 2000;**9**(4):419–425.
211. Ashton N. Perinatal development and adult blood pressure. *Braz J Med Biol Res* 2000;**33**(7):731–740.
212. Wintour EM, Johnson K, Koukoulas I, Moritz K, Tersteeg M, Dodic M. Programming the cardiovascular system, kidney and the brain — a review. *Placenta* 2003;**24**(suppl A):S65–71.
213. Goodfellow J, Bellamy MF, Gorman ST, Brownlee M, Ramsey MW, Lewis MJ, *et al.* Endothelial function is impaired in fit young adults of low birth weight. *Cardiovasc Res* 1998;**40**(3):600–606.
214. Leeson CP, Kattenhorn M, Morley R, Lucas A, Deanfield JE. Impact of low birth weight and cardiovascular risk factors on endothelial function in early adult life. *Circulation* 2001;**103**(9):1264–1268.
215. McArdle HJ, Andersen HS, Jones H, Gambling L. Fetal programming: causes and consequences as revealed by studies of dietary manipulation in rats — a review. *Placenta* 2006;**27**(suppl 1):S6.
216. Alexander BT. Fetal programming of hypertension. *Am J Physiol Regul Integr Comp Physiol* 2006;**290**(1):R1–10.
217. Izzo JL Jr. Arterial stiffness and the systolic hypertension syndrome. *Curr Opin Cardiol* 2004;**19**(4):341–352.
218. Wilkinson IB, Cockcroft JR, Webb DJ. Pulse wave analysis and arterial stiffness. *J Cardiovasc Pharmacol* 1998;**32**(suppl 3):S33–37.
219. Kelly RP, Millasseau SC, Ritter JM, Chowienczyk PJ. Vasoactive drugs influence aortic augmentation index independently of pulse-wave velocity in healthy men. *Hypertension* 2001;**37**(6):1429–1433.
220. Davies JI, Struthers AD. Pulse wave analysis and pulse wave velocity: a critical review of their strengths and weaknesses. *J Hypertens* 2003;**21**(3):463–472.