Quantification of Alterations in Structure and Function of Elastin in the Arterial Media

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Abstract—The structure of medial elastin determines arterial function and affects wall mechanical properties. The aim of this study was to (1) characterize the structure of elastin in terms of textural features, (2) relate structural parameters to total number of cardiac cycles (TC), and (3) determine the contribution of medial elastin to lumen mechanical stress. Images of pressure-fixed aortic sections stained for elastin were obtained from specimens collected postmortem from 35 animals of different species with a wide range of age, heart rate, and TC and divided into 2 groups: $T_{C_{low}} = 3.69 \pm 0.38 \times 10^6 \ (n=17)$ and $T_{C_{high}} = 15.8 \pm 2.38 \times 10^6 \ (n=18) \ (P<0.001)$. A directional fractal curve was generated for each image, and image texture was characterized by directional fractal curve parameters. Elastin volume fraction and interlamellar distance were obtained by image analysis. Wall stress distribution was determined from a finite element model of the arterial wall with multiple layers simulating elastin lamellae. DFC amplitude was related to elastin volume fraction. Increased TC ($T_{C_{low}}$ versus $T_{C_{high}}$) was associated with lower directional fractal curve amplitude ($0.23 \pm 0.02$ versus $0.14 \pm 0.02; \ P<0.001$), reduced elastin volume fraction (36.5±2.6% versus 25.7±2.1%; $P<0.01$), and increased interlamellar distance (8.5±0.5 versus 11.5±1.0 μm; $P<0.05$). Loss of medial elastic function increased pressure-dependent maximal circumferential stress. Structural alterations of medial elastin, quantified by fractal parameters, are associated with cumulative effects of repeated pulsations due to the combined contribution of age and heart rate. Loss of medial functional elasticity increases luminal wall stress, increasing the possibility of endothelial damage and predisposition to atherosclerosis. (Hypertension. 1998;32:170-175.)

Key Words: fractals ■ stress, mechanical ■ fatigue ■ aging

The phylogenetic distribution of the presence of the elastin protein suggests that arterial elastin evolved as an adaptive response to mechanical stresses imposed on arteries by the high-pressure circulatory system achieved early in vertebrate evolution. Because of the inherent stability of the elastin protein, the unceasing application of these same pulsatile mechanical stresses due to oscillatory arterial pressure throughout the animal’s lifetime makes arterial elastin susceptible to the degenerative effects of mechanical fatigue. Medial elastin is a major determinant of arterial distensibility and capacitive effects of large arteries. Alteration of arterial elasticity due to structural modifications of the elastin matrix results in functional changes of arterial properties, affecting arterial pressure through altered vessel compliance, wave transmission properties, and secondary effects of wave reflection. Arterial elasticity also affects the mechanical load-bearing function of the arterial wall, and the structural orientation of the elastin fibers affects the stress distribution throughout the wall. In addition to and separate from the relative content of elastin, alteration in structure therefore is an important factor determining the functional properties of arteries.

Arterial elastin is usually characterized by concentric lamellar structures, and the lamellar unit, consisting of an elastin lamella associated with the two adjacent interlamellar zones, is considered a functional and structural unit of the arterial wall. Although lamellar elastin is what is essentially seen with conventional elastic stains using light microscopy, contrast stains and electron microscopy reveal that a substantial area of the interlamellar zones is occupied by elastin fibers. The interlamellar connections to the main lamellae could also determine functional properties, as has been shown in recent studies implicating changes in interlamellar elastin in the pathogenesis of aortic dissecting aneurysms.

The elastin structure can be quantified in terms of simple geometric lamellar parameters (e.g., lamella thickness, interlamellar distance) or texture-based parameters (e.g., fractal dimensions). The self-similarity property, which is the basis for fractal analysis, has been shown to be present from the low-level supramolecular structure to the macro-level structure of the lamellar and interlamellar fibers obtained from scanning electron microscope images. This investigation applies similar fractal analysis techniques to light microscopy images of aortic sections stained for elastin. In this study, structural parameters were obtained from images of the aortic wall acquired from a range of animal species with a wide range of heart rate and lifespans to provide quantitative comparisons of the effect of accumulated total number of cardiac cycles on the changes in elastin structure.

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The effects of altered medial elastic structure on arterial stiffness were modeled by finite element techniques to obtain the contribution of medial elasticity on wall stress distribution. This technique enabled calculation of stress contours in the presence of an intraluminal pressure, with different values of medial stiffness modeled by altered elasticity of concentric elastin layers and nonelastin layers. Change in luminal stress concentration at the intimal surface due to material property of the media indicates a possible causative effect of arterial stiffness and atherogenesis, independent of other intimal processes such as material transport.

Methods

Arterial Specimens

Aortic specimens were collected from a range of animal species from the Sydney Taronga Park Zoo within 24 hours after autopsy and fixed under pressure (100 mm Hg) in a buffered formalin solution. A fixation pressure of 100 mm Hg was chosen because this is the approximate average in vivo pressure for all animals in this study, and it has been previously shown\(^7\) that at this pressure, elastic lamellae are sufficiently distended and that higher pressures cause minimal distension to alter the lamellar pattern.

Histology

Histology sections for analysis were taken from the upper descending thoracic aorta. Blocks were processed overnight on an automatic tissue processor (Tissue-Tek VIP) and embedded in paraffin wax. Sections were cut at 5 µm on a rotary microtome and stained with Verhoeff’s iron hematoxylin for elastic fibers.

Light Microscopy and Image Acquisition

Aortic sections were examined by light microscopy (Olympus BX-50), and digital images were obtained by a commercial imaging system (PulnicX TM-6CN miniature high-resolution monochrome CCD camera; Data Translation DT3155 PCI monochrome frame grabber board; Data Translation DT3155 WiT hardware server [WiT-H-DT3155]; Logical Vision WiT (version 5.01) image analysis software) at ×200 magnification.

Statistics

Mean and standard errors were calculated for each parameter, and 2-tailed Student’s t tests were performed for comparisons between the groups. The level of significance was taken as \(P=0.05\). Analysis was performed on 2 groups of approximately equal numbers (\(n_1=17\), \(n_2=18\)) of high and low cardiac cycles, high and low heart rate, and old and young age. Groups were determined by ranking in terms of cardiac cycles, heart rate, and age.

Elastin Content

EVF (percentage) and interlamellar distance ILD (micrometers) were obtained by image analysis. EVF was determined using WiT image analysis software (Logical Vision). A threshold operation was performed on each image to select the elastic tissue and produce a binary image. Pixels corresponding to elastic tissue were counted and expressed as a percentage of the total image area. ILD was determined by geometric procedures as previously described.\(^1\)

Fractal Analysis

Light microscope images were analyzed using custom-written software.\(^5,11\) The fractal dimensions were determined from the Fourier power spectrum of the image.

A power-law relation is defined in terms of the rate at which the Fourier power spectrum of an image falls off with increasing spatial frequency\(^3\):

\[
A \approx f^{-(2H+1)}
\]

therefore

\[
\log A \approx -(2H+1) \log f
\]

where \(A\) is the amplitude of the Fourier spectrum, \(f\) is spatial frequency, and \(H\) is defined as the Hurst coefficient. The fractal dimension \(F\) is a function of \(H\):

\[
F = 3 - H
\]

The fractal dimension of the image can be obtained from the slope of the log-log plot of the amplitude \(A\) as a function of spatial frequency, \(f\).

Directional Fractal Dimensions

A 128×128-pixel mask was placed randomly on the 512×512 pixel image to select 10 locations for fast-Fourier transformation. The corresponding Fourier spectrum of each mask was obtained by Equation 4 below:

\[
A = R(f, \alpha) + iI(f, \alpha)
\]

where \(R(f, \alpha)\) and \(I(f, \alpha)\) are the real and imaginary components of \(F(f, \alpha)\), respectively, and \(\alpha\) is the angle defining the direction on the plane of the image where the Fourier spectrum was calculated.

For real natural surfaces, a single fractal dimension cannot be applied at all possible scales but rather only over a range of scales.\(^14\) That is, there is a particular range of \(f\) where the linear log-log relation of Equation 2 is satisfied (Figure 1). For the images obtained at the particular magnification, the range of the spatial frequency was

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**Selected Abbreviations and Acronyms**

- DFC = directional fractal curve
- EVF = elastin volume fraction
- ILD = interlamellar distance
- LEF = loss of elastic function
- Q = relative stiffness parameter defined as ratio of elastic modulus (\(E_1\)) of elastic (lamellar) component of the wall to the modulus (\(E_0\)) of the nonelastic (interlamellar) component (\(Q=E_1/E_0\))
- TC = total cardiac cycles ([heart rate]×[age])

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\(n\) the number of experiments.

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**Figure 1.** Logarithmic relation between amplitude of Fourier spectrum (\(\|\mathbf{A}\|\)) and spatial frequency (\(f\)) (see text for description). The fractal dimension is determined from the slope of the linear regression line in the section between the vertical lines [log(\(f\)) range, 2.54 to 4.1]. Regression equation: \(\log(\|\mathbf{A}\|) = -2.4\log(f) + 11.96\); \(r=0.79\); \(P<0.01\).
found to be between 12 and 60 bins (corresponding to 0.252 and 1.262 cycles/μm, respectively). A single regression line was fitted to the spectrum data at a specific angle \( \alpha \), and the fractal dimension was derived from the slope of the line (using Equations 2 and 3) and denoted as \( F_i(\alpha) \), with \( i \) indicating the sequence of the masks and equal to 1, 2, …, 10. Because of the symmetry of the power spectrum, \( F_i(\alpha) \) needs to be calculated only in the range 0° to 178°. Calculation was done at intervals of 2°. The mean value of 10 \( F_i(\alpha) \), denoted as \( F(\alpha) \), was calculated to generate the DFC for each image.

**Directional Fractal Curve**

The DFC (\( F \)) composed of 90 \( F(\alpha) \) was then fitted with a 2-term sinusoid of the form:

\[
F = A_1 \sin (B_1 \alpha + C_1) + A_2 \sin (B_2 \alpha + C_2) + D,
\]

where \( A_i \) is amplitude, \( B_i \) is period, \( C_i \) is phase (\( i = 1, 2 \)), \( D \) is offset, and \( \alpha \) is angle.

The best-fit sinusoidal wave was determined by Levenberg-Marguardt algorithm,15 with which the curve parameters were sought by an iterative process to minimize the sum of the squared difference between the values of the observed and predicted values of \( F(\alpha) \). Based on this method, a good fit was obtained by a 2-term sinusoid. Figure 2 shows an example of the histological image and associated curve for 2 animals of similar age but different heart rate (hence markedly different TC).

**Finite Element Modeling**

Effects of change in luminal pressure and elastic function of wall components were studied by means of a finite element stress analysis using MSC-Nastran software. Calculations were performed for an arterial geometry of 10 mm inner radius and 1 mm thickness. Medial structure was modeled by creation of lamellar and interlamellar layers. Interlamellar layers were assumed to be a network of collagen and elastin fibers with a nonlinear stress-strain relationship. The stress-strain curve for these layers was based on experimental data for the human aorta.16

Elastin lamellae were assumed to be uniform circumferential structures with a single Young’s modulus of elasticity. Functional change of arterial wall results in change of elastic moduli of layers. For example, functional loss of elastin may result in stiffening of the nonlamellar layers, which are composed of collagen and elastin. This may result in an increase in difference between elastic moduli of lamellar (\( E_1 \)) and nonlamellar (\( E_2 \)) layers. A nondimensional parameter (\( Q \)) was defined as the ratio \( E_1/E_2 \), so a change in \( Q \) simulates change of elastic function of arterial wall. Because interlamellar layers have a nonlinear stress-strain relationship with incremental Young’s modulus of elasticity, the slope of the initial part of the stress-strain curve was considered in the ratio. In addition to variation in \( Q \), stepwise increases in luminal pressure (\( P \)) were applied. Resultant stress values were obtained for different \( Q \) values (changing in the range of 1/10 to 1/100) and luminal pressures [0.005 MPa (37.5 mm Hg), 0.01 MPa (75 mm Hg), 0.0133 MPa (100 mm Hg), 0.016 MPa (120 mm Hg), and 0.02 MPa (150 mm Hg)]. Calculations were performed for a model consisting of 31 layers using a mesh of 1500 elements.

**Results**

**Fractal Parameters**

The Table shows results for 2 separate groups for TC, heart rate, and age. The significant parameters were DFC amplitude of the first sinusoid component, EVF, and ILD. Other
DFC parameters were not significant. The most significant difference was found for TC. Heart rate also showed a significant but smaller difference with a reduced P value. Age did not produce any significant difference for the 3 parameters. The 2 groups had a 4-fold difference in mean TC (TClow = 3.69 ± 0.37 × 10^8 [n = 17], TC_high = 15.85 ± 2.38 × 10^8 [n = 18]; P < 0.0001). Increased number of cardiac cycles was associated with a 40.4% decrease in the amplitude (A) of the first sinusoid component of the DFC (A_low = 0.23 ± 0.02; A_high = 0.14 ± 0.016; P < 0.0001), a 29.6% decrease in lamellar EVF (EVF_low = 36.5 ± 2.55%; EVF_high = 25.7 ± 2.14%; P < 0.0001), and a 34.6% increase in interlamellar distance (ILD_low = 8.75 ± 0.46 µm; ILD_high = 11.54 ± 1.0 µm; P < 0.002).

Figure 3 shows scatterplots for DFC amplitude as the dependent variable as a function of TC, EVF, and ILD. There is a trend for DFC amplitude to decrease with cardiac cycles, indicating an association between elastin structure and accumulated pulsations. This becomes significant when data are divided into 2 groups (Table). There is also a trend for DFC amplitude to decrease with ILD and increase significantly (r = 0.4, P < 0.05) with EVF, suggesting a structural association with elastin content in the arterial wall.

Finite Element Model
Figure 4 shows maximum circumferential stresses on the luminal surface for different Q and luminal pressures. Loss of functional elastin causes higher relative stiffness in nonlamellar layers and an increase in difference between elastic moduli of layers (ie, an increase in Q), and this elevates maximum circumferential stress. An increase in luminal pressure results in an increase in maximum circumferential stress on the luminal surface. Occurrence of both effects, ie, an increase in luminal pressure and functional loss of elastin, accelerates increase of maximum circumferential stress.

Discussion
Structural Quantification
Texture analysis of images of elastin allows quantification of structure of the elastin network by means of fractal parameters that facilitate comparison of arterial structure and function. Previous studies have shown that like other physiological textural images such as lung scans or mammographic parenchymal patterns, micrographs of arterial elastin exhibit properties of self-similarity and thus can be characterized in terms of fractal dimensions. It was also shown that...
because of the directional preference of elastin fibers, a single global fractal dimension is not sufficient to describe the image, but rather a DFC was developed to account for the anisotropic features. The components of the DFC were also shown to be associated with degree of “disorganization” of the image, due to disorientation and fragmentation of elastin fibers as seen with age or disease.

In this study, results in the 2 groups of animals show that increased number of TC are associated with decreased amplitude of DFC, an index of structural organization of elastin. This was obtained from the aorta of a whole range of animal species with a 5-fold range of heart rate (40 to 203 bpm) and a 15-fold range of age (3 to 45 years), resulting in a 28-fold range of TC (1.38\times10^3 to 38.08\times10^9). In relating the structural modifications to the fatiguing effects of the accumulated pulsations, the inherent assumption is that the elastin in all the animals studied undergoes similar fatiguing effects and, by implication, that the elastin is similar in all species. Any species difference that may exist regarding elastin isoforms has not been evaluated in this study. Although it has been shown that species differences in amino acid composition do exist, amino acid content also seems to be subject to effects of age, similar to the macrostructure of elastic fibers. Furthermore, since all arterial sections from all animals took up similar elastic stain, it is reasonable to assume that essentially similar elastic structures have been analyzed.

**Functional Quantification of Wall Stress**

Finite element modeling of the aortic wall has been used to calculate change in stress distribution due to change in functional elasticity of the wall material. This was done by modeling concentric layers composed of pure elastin with elastic modulus $E_1$ and interlamellar zones composed of a stiffer material with elastic modulus $E_2$ ($E_2 > E_1$). The change in functional elasticity was simulated by altering the ratio $E_1/E_2$. The caveat is that $E_1$ is essentially linear and that $E_2$ is nonlinear, hence the stress dependency due to increase in luminal pressure is not similar. This was resolved by obtaining values for the linear part of the stress-strain curve and interpolating values for each iteration of the model. The results indicate that structural changes in wall elastic components affect maximal stress at the lumen for a given blood pressure. That is, change in medial elastic properties can influence intimal stress concentration without change in luminal pressure.

Calculations from the finite element model do not include residual stress. It is known that residual circumferential stress does exist in arteries, with a suggestion that it contributes to vascular remodeling. It exhibits compression at the lumen and tension at the adventitial side and to some extent seems to be species dependent (comparison between pig and rat). Residual stress cannot be readily determined and is estimated indirectly by measurement of residual strain using the opening angle of cut arterial rings. Using nominal values for arterial Young’s modulus in porcine and bovine aortas, Vaishnav and Vassoughi estimated residual compressive stress at the lumen of the order of 14% of the mean circumferential stress. Thus, luminal stress calculated from the finite element model would be reduced by this amount. However, since increased medial stiffness or loss of elastic function would have the effect of decreasing the compressive residual stress, the calculated circumferential stress would be reduced by a smaller amount. In essence, this would increase the slopes of the curves in Figure 4.

**Effects of Elastin Structure on Wall Function**

From the fractal analysis, a conceptual framework could be developed where the effects of mechanical fatigue could be related to loss of elastic function. Because the amplitude of the DFC was shown to be related to structural disorganization associated with loss of elasticity with age, a relationship could be estimated between LEF and TC by assuming that DFC amplitude is inversely proportional to LEF. From the Table, a 327% increase in TC is related to a 64% increase in LEF (calculated from the reciprocal of DFC amplitude), that is, a doubling (100% increase) of TC is related to a 20% loss of elastic function. This assumption is supported by the fact that a 40.4% reduction in DFC amplitude is associated with a drop of 29.6% in EVF.

The LEF can also be related to the parameter Q describing the relative stiffness of elastic (lamellar) ($E_1$) and stiffer nonelastic (interlamellar) ($E_2$) components in the finite element model; ie, $LEF \propto 1/Q$ or $LEF \propto E_2/E_1$. That is, elastic function is decreased (increase in LEF) either by mechanisms that increase the relative stiffness of the nonlamellar components, as can happen when interlamellar connections are...
broken so that the load is taken up by the stiffer collagenous components. From finite element model results and these associations between LEF, total cardiac pulsations and relative stiffness (1/Q), the combined effect of blood pressure and total cardiac pulsations could be estimated. From Figure 2, the effect of luminal pressure on maximum luminal stress is therefore a function of LEF. For a 20% change in LEF (corresponding to the estimated effect of doubling TC and calculated from the values for 1/Q = 40 and 1/Q = 50), maximum luminal stress changes at a rate of 0.023%/mm Hg. For a 43% change in LEF (corresponding to approximately 200% increase in TC and calculated from the values of 1/Q = 40 and 1/Q = 70), the maximum luminal stress increases at a rate of 0.062%/mm Hg. That is, for a doubling of TC, the effect of a similar rise in blood pressure is 2.7 times greater. In fact, this would be an underestimation if residual stresses were taken into account. In other words, if blood pressure rises over a given period, the deleterious effects on intimal mechanical stress can be compensated for by a reduction of the total number of pulsations, i.e., by reduction of heart rate.

These results reinforce the association between wall medial properties and intimal stress. If intimal stress is a factor for atherogenesis, it links the structural elements of the load-bearing properties of the media with intimal processes such as atherosclerosis. Thus, in addition to high blood pressure being a risk factor for atherosclerosis through direct effect on intimal wall stress, arterial stiffness per se can also be considered a possible risk factor through its structural effects on maximum luminal stress. If the modification of wall elasticity is then related to the accumulated effects of cyclic fatigue, the deleterious effects of these processes could be minimized by reduction of heart rate. Because of the current possibilities of modification of heart rate through continued pharmacotherapy or physical exercise over long periods of time, this topic is receiving considerable interest with respect to heart rate and life expectancy, as is manifested by a recent editorial.  

References
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