Mechanics and Composition of Human Subcutaneous Resistance Arteries in Essential Hypertension

Hope D. Intengan, Li Y. Deng, Jin S. Li, Ernesto L. Schiffrin

Abstract—Mechanical properties of arteries are altered in some rat models of hypertension, and this may influence peripheral resistance and blood pressure as well as some of the complications of hypertension. It has usually been assumed that arterial wall stiffness is increased in hypertension, although recent studies suggest that this may not necessarily be the case in large arteries. We determined whether the mechanics of human resistance arteries are altered in hypertension. Subcutaneous resistance arteries (lumen diameter < 300 μm) were isolated from hypertensive and normotensive subjects of similar ages (46 ± 3 and 43 ± 4 years, respectively). Vessels were mounted in a pressurized myograph, deactivated, and exposed to intraluminal pressures ranging from 3 to 140 mm Hg. At each pressure, lumen and media dimensions were measured. Media-to-lumen ratio and media width were greater in hypertensive vessels, reducing wall stress (P < 0.01), whereas media cross section was similar in vessels from both groups. Isobaric elastic modulus (which is influenced by vessel geometry and by wall component stiffness) was lower in hypertensive vessels (P < 0.01). Stiffness of wall components (slope of incremental elastic modulus versus stress, which is geometry-independent) was significantly lower in hypertensive vessels (8.2 ± 0.7) versus normotensive vessels (11.0 ± 1.0, P < 0.05), whereas distensibility was unchanged. Electron microscopic analysis of the media of the small arteries showed a greater collagen to elastin ratio (P < 0.05) in the media of vessels from hypertensive patients. In conclusion, the stiffness of wall components (slope of elastic modulus versus stress) is not increased but is in fact decreased in subcutaneous resistance arteries from patients with mild essential hypertension. Reduced stiffness of resistance arteries from hypertensive patients does not appear to relate to changes in volume density of extracellular matrix components but may be the result of changes in extracellular matrix architecture or cell-matrix attachment, which remains to be established. (Hypertension. 1999;33[part II]:569-574.)

Key Words: arteries, small ■ remodeling ■ elasticity ■ elastic modulus ■ collagen

The structure1,2 and mechanical properties3–5 of arteries are altered in hypertension. In large arteries it has usually been assumed that structural changes result in increased stiffness of vessels. It has been shown, however, that this is not necessarily the case. Indeed, several recent studies demonstrate that compliance of large arteries is not uniformly reduced in hypertension and may even be increased.6,7 In experimental hypertensive models in the rat, the structure of small resistance size arteries is altered,1,2 and some studies have shown that in some vascular beds, this is associated with reduced rather than increased stiffness of the vessel wall.8,9 The structural alterations of small arteries may comprise a combination of growth (characterized by increased media thickness and cross-sectional area) and eutrophic remodeling (characterized by decreased lumen and external diameter, with unchanged media cross-sectional area).10 The changes in composition of small arteries may influence how the structural alterations result in changes in wall mechanics. In small arterioles of the cerebral circulation, genetic hypertension is associated with increased elastin and distensibility,11 whereas in the mesenteric circulation, there is increased collagen deposition, without a change in volume density.12 Alterations in small artery mechanics may exacerbate vascular damage resulting from hemodynamic changes (for example, pulse pressure, wave reflection, and shear stress). An understanding of the former provides important mechanistic information for choosing therapeutic targets in hypertension. Mechanical properties may also provide a parameter that may eventually be useful for the noninvasive evaluation of cardiovascular risk. Some studies of human resistance arteries in which their structure has been elucidated in hypertensive patients are available,13,14 but little is known of the mechanical changes that follow the alterations in structure that occur in hypertension. Changes in wall mechanics (stiffness) of resistance arteries may influence pressure-diameter relationships of blood vessels. We hypothesized that as in rat models of hypertension, mechanical properties of human resistance arteries from patients with mild hypertension would not present abnormal stiffness despite altered structure. There is only one previous study in which mechan-
ical properties of resistance arteries from patients with mild hypertension have been studied.\textsuperscript{15} It showed similar stiffness of wall components in resistance arteries from normotensive subjects and hypertensive patients, but that study was performed with the older technique of the wire myograph. There have been suggestions that the wire myograph may not accurately reflect properties of small arteries,\textsuperscript{16} although most of the data obtained with the wire myograph in studies of human small arteries have been reproduced in studies using more modern and physiological pressurized arteriographs.\textsuperscript{17} Therefore, to assess the mechanical properties of pressurized small arteries, we isolated small vessels (lumen diameter <300 μm) from gluteal subcutaneous biopsies performed on normotensive and hypertensive subjects and investigated their mechanics on a pressurized arteriograph. To understand mechanistically changes found in mechanical properties, we investigated the composition of the media of these resistance vessels by electron microscopy.

**Methods**

**Subjects**

The protocol was approved by the Ethics Committee of the Clinical Research Institute of Montreal. Written informed consent to participate in the study was obtained from each subject. Subjects aged 25 to 65 years were recruited from the Hypertension Clinic of the Clinical Research Institute of Montreal if they had essential hypertension and had never been treated with antihypertensive drugs. The hypertensive subjects had a history of recumbent systolic blood pressures of >140 mm Hg or diastolic blood pressure >95 mm Hg on more than two occasions. The diagnosis of essential hypertension was established by the absence of clinical evidence of secondary hypertension; normal serum electrolytes, creatinine, and urinalysis; normal abdominal ultrasound examination; and where indicated, normal renal scintiscan, renal arteriogram, and computed abdominal tomography. Individuals were excluded if they smoked more than 5 cigarettes per day, their fasting blood glucose level was abnormal, their serum creatinine concentration was >150 μmol/L, or they had any other systemic disease. Control subjects were recruited simultaneously with the hypertensive subjects and were matched for age. They had systolic blood pressure <140 mm Hg and diastolic blood pressure <85 mm Hg, smoked fewer than 5 cigarettes per day, and had no evidence of disease on physical or routine laboratory investigation. Subjects arrived between 7:30 and 9 AM, having fasted since the previous evening. Blood pressure was measured with a standard mercury sphygmomanometer after subjects had rested 15 minutes in the sitting position. Diastolic blood pressure was read as phase V of the Korotkoff sounds.

**Preparation and Study of Pressurized Small Arteries**

Biopsies of gluteal subcutaneous fat were obtained from all subjects by the same physician, with subjects under local anesthesia. Superficial subcutaneous tissue (1.0×0.5×0.5 cm) was obtained through a horizontal 1-cm-long incision of the skin in the upper external gluteal quadrant. Arteries were dissected from the sample of subcutaneous tissue under a dissecting microscope immediately after the biopsy was obtained, and 1 to 3 small arteries were chosen at random and isolated. Arteries were mounted in a servo-controlled pressurized myograph chamber, allowing a stable preset pressure as previously described.\textsuperscript{9} The vessels were slipped onto two glass microcannulas and secured with nylon ties. The axial length of the vessel was adjusted by moving one cannula until the vessel walls were parallel without stretch. Vessels were equilibrated under a constant intraluminal pressure of 60 mm Hg for 1 hour with physiological salt solution (PSS) (37°C) that was continuously bubbled with 95% air and 5% CO₂ to achieve a pH of 7.40 to 7.45. PSS contained (mmol/L) NaCl 120, NaHCO₃ 25, glucose 5.5, KCl 4.7, CaCl₂ 2.5, KHPO₄ 1.2, MgSO₄ 1.2, and EDTA 0.026. Vessels were deactivated of myogenic tone by perfusion with Ca²⁺-free PSS containing 10 mmol/L EGTA for 30 minutes. Lumen and media dimensions (for morphology) were measured while the intraluminal pressure was 60 mm Hg. Intraluminal pressure was then raised to 140 mm Hg three times, and the cannula was adjusted until the artery walls were straight and parallel to each other. Adjustments in length for the maintenance of parallel walls were minimal.

For calculation of vessel mechanics, intraluminal pressure was increased at several intervals up to 140 mm Hg, as previously described,\textsuperscript{2} and media thickness and lumen diameter were measured at each pressure level at 5 points along the vessel. The precision of the system was 0.7 μm. The initial diameter was measured at 3 mm Hg unless the vessel collapsed. In these cases, lumen diameter was estimated by fitting the intraluminal pressure-lumen diameter data (measured between 10 and 140 mm Hg) to a third-order polynomial equation.

**Determination of the Composition of Small Artery Walls**

Composition of the media of small artery walls was studied as we have previously described.\textsuperscript{12} Briefly, after morphometric determinations, pressurized arteries from 5 of the normotensive and 9 of the hypertensive subjects were perfused for 30 minutes with calcium-free PSS at an intravascular pressure of 60 mm Hg, fixed with 1.5% glutaraldehyde solution at room temperature for 60 minutes, and removed from the cannula. After three washes with calcium-free PSS, vessels were stored in 70% ethanol at 4°C. Arteries were then fixed with 1% osmium tetroxide for 30 minutes at room temperature, dehydrated in graded ethanol, and embedded in araldite CY212 epoxy resin (Ladd Research Industries Inc). Excess resin was trimmed so that the block face contained only the specimen. Sections were cut on a microtome (Reichert Ultratritm) at a thickness ranging from 70 to 90 nm and stained with 0.25% phosphotungstic acid for 15 minutes, 4% uranyl acetate for 10 minutes, and lead citrate for 3 minutes. The sections were examined with a JEM-1200EX electron microscope (JEOL Ltd). Electron micrographs were taken at an original magnification of ×4000 and enlarged by a factor of 3 for a final magnification ×12 000. Vessels were divided into four quadrants, and three electron micrographs were taken randomly for a total of 12 to 15 electron micrographs per vessel. Negatives of micrographs were scanned (Scan Jet 4C/T, Hewlett-Packard). The areas occupied by smooth muscle, collagen fibers, and elastin were measured by repeated tracing with a light pen and then use of an imaging program (Adobe Photoshop 3.0). Means of repeated tracings recorded in pixels were averaged and used to calculate the cross-sectional area occupied by each component.

**Data Analysis**

Calculation of arterial morphology and mechanics was done as previously described.\textsuperscript{9} Results are represented as mean±SEM. Statistical comparisons were performed by two-tailed Student’s \textit{t} test. Statistical evaluation of relationships between mechanical parameters of vessels from normotensive and hypertensive subjects was performed by repeated-measures analysis of variance. Where statistical significance was detected, interaction means were compared using a two-tailed Student’s \textit{t} test. Differences were considered statistically significant at a value of \textit{P}<0.05.

**Results**

Demographics of the study subjects are shown in Table 1. Seven normotensive and 14 hypertensive individuals were investigated. The normotensive group had proportionately more women; otherwise, there were no significant differences between the groups in age, body mass index, serum creatinine levels, and serum lipid profile. Blood pressure was signifi-
TABLE 1. Subject Demographics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensive Subjects</th>
<th>Hypertensive Subjects</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>3/4</td>
<td>11/3</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>42.7±3.6</td>
<td>46.0±2.6</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.3±1.5</td>
<td>26.0±1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>112.7±4.6</td>
<td>143.0±3.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>75.0±2.9</td>
<td>98.2±1.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Serum creatinine, μmol/L</td>
<td>92.1±4.0</td>
<td>97.5±2.4</td>
<td>NS</td>
</tr>
<tr>
<td>Total serum cholesterol, mmol/L</td>
<td>5.5±0.5</td>
<td>5.4±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Serum triglycerides, mmol/L</td>
<td>1.49±0.34</td>
<td>1.35±0.16</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SEM. BP indicates blood pressure.

The structure of subcutaneous small arteries of the subjects studied is depicted in Table 2. Media width and media-to-lumen ratio of the vessels of hypertensive subjects were increased (P<0.01), whereas media cross-sectional area and lumen diameter did not exhibit significant differences between the groups. The growth index, that is, the percent difference in cross-sectional area of the media between vessels from hypertensive and normotensive subjects, was 32.8%.

Vascular mechanics were studied by progressively increasing intraluminal pressure. The left panel of Figure 1 shows that wall distensibility plotted against intraluminal pressure was similar in arteries from both groups. The right panel of Figure 1 depicts the media stress-strain relationship, which was significantly shallower in the vessels from hypertensive subjects, indicating that isometric stress was lower in hypertensive vessels. From the stress-strain relationship, incremental elastic modulus was calculated and plotted against intraluminal pressure and circumferential stress (Figure 2). The left panel of Figure 2 shows that incremental elastic modulus plotted against intraluminal pressure was lower in vessels from hypertensive subjects. The right panel demonstrates that when plotted against circumferential stress, the slope of the incremental elastic modulus (β), which demonstrates the stiffness of wall components, was significantly lower in vessels from hypertensive subjects than in those from normotensive subjects. Since in the present study there was a marked imbalance in the number of male and female hypertensive subjects, the mechanics of vessels from male and female normotensive and hypertensive subjects were evaluated separately. Figure 3 shows that vessel mechanics in male and female hypertensive subjects were identical. No significant differences were found between vessels from male and female normotensive subjects. Differences between mechanics of all normotensive and hypertensive subjects were thus maintained within men and women.

Electron microscopic evaluation of the structure and composition of the media of pressurized small arteries from 5 of the 7 normotensive subjects and 9 of the 14 hypertensive subjects showed a trend toward a greater number of smooth muscle layers (P=0.07) and higher collagen volume density (P=0.08) in vessels from hypertensive subjects and a significant increase in the collagen-elastin ratio (P<0.05) (Table 2).

**Discussion**

This study evaluates the vascular mechanics of subcutaneous small resistance size arteries from hypertensive subjects for the first time using a pressurized system, which may represent a more physiological approach than other methods previously used for examining vascular structure and mechanics. The composition of the media of vessels was also studied to determine whether changes in the volume density of collagen (less distensible) and elastin or smooth muscle (more distensible) could explain the differences in mechanical properties. We show that subcutaneous small arteries of the hypertensive subjects studied present an altered structure, as previously reported by others and in our own studies. In this particular group of subjects, media-to-lumen ratio of small arteries was increased, as was media width, whereas media cross-sectional area was not. However, although differences between the media cross section of small arteries did not achieve statistical significance, the growth index was 32.8%, which suggested a trend toward what has been designated as hypertrophic remodeling. This finding is in contrast to previous studies from our own group and others in which the predominant finding was that of eutrophic remodeling, but is in agreement with the first results obtained from the study of human subcutaneous small arteries. The heterogeneity of individuals with essential hypertension suggests the need for caution in making our conclusions in view of the range of results that may be encountered.

The lumen diameter of a vessel will depend on opposing forces of transmural pressure and compliance of the vessel wall. Compliance, that is, the ability of a vessel to buffer changes in pressure, depends on the geometry and stiffness of the wall components of the vessel. Decreases in lumen size (resulting mainly from remodeling in hypertension) may reduce compliance, which is geometry-dependent. However, vascular compliance may be normalized by decreases in elastic modulus. Incremental elastic modulus is geometry-
independent and depends on the stiffness of the vessel wall components. Elastic modulus is determined by the combined elastic modulus of the structural components of the vascular wall: connective tissue, elastin and collagen fibers, smooth muscle cells, and endothelial cells. Previous studies of the mechanics of large arteries have demonstrated that vascular wall compliance is not necessarily reduced in hypertension both in animal models and in human essential hypertension. The mechanism for the absence of reduced vascular compliance and distensibility despite increased media thickness and collagen deposition has been obscure. In very small arteries from the brain, the pioneering studies of Baumbach et al showed for the first time that wall mechanics in hypertension might not be altered as expected. These investigators first reported that compliance and distensibility could actually be increased and that the slope of the incremental elastic modulus in relation to circumferential stress, that is, the stiffness of wall components, could be reduced in pial arterioles of approximately 80 mm in lumen diameter from stroke-prone spontaneously hypertensive rats (SHRSP). They found similar results in small arteries (<150 μm in lumen diameter) but not larger small arteries (150 to 300 μm in lumen diameter) in the brain. Their studies demonstrated that the composition of vessels with reduced stiffness was altered, with increased elastin content, which could explain the direction of the alteration in mechanics. We have found in some groups of SHR, and in Dahl-salt sensitive rats and deoxycorticosterone acetate (DOCA)–salt hypertensive rats, similar or lower stiffness of wall components of mesenteric small arteries than in equivalent vessels from normotensive rats. Together with previous findings in large arteries from animal models and humans, this may suggest that a reduced stiffness of wall components is indeed a common characteristic in hypertension. The mechanism may not be the same for all vascular beds, however, because in contrast to pial arterioles, in which elastin is increased, in large arteries in SHR most studies have shown an increase in collagen deposition (although the volume density of collagen was not increased). In mesenteric small arteries of SHR we have recently demonstrated increases in collagen as well. The present study we found that even though the stiffness of wall components is decreased in vessels from hypertensive subjects, the volume density of collagen in the media tends to be increased, although not achieving statistical significance, and the collagen-elastin ratio is significantly enhanced. This agrees with our previous findings in mesenteric arteries of similar dimension in SHR. It would be expected that an increased collagen-elastin ratio might increase stiffness. Thus it is likely that in these vessels other factors are involved, and it is possible that changes in extracellular matrix–smooth muscle cell attachments may play a role in the change in mechanical properties found in both human arteries and those of experimental models of hypertension. Recent studies have demonstrated that in aorta of SHR, fibronectin and α5-integrin are increased. It has been speculated that increases in cell–extracellular matrix attachment sites may contribute to the maintenance of equivalent mechanical properties even though SHR vessels, or human vessels in the present study, are subjected in vivo to higher pressures. The mechanisms involved in the reduction of the elastic modulus in relation to wall stress and pressure in resistance arteries from hyperten-

Figure 1. Left, Incremental distensibility plotted against intraluminal pressure in relaxed subcutaneous small arteries from normotensive and hypertensive subjects. The curves did not differ significantly. Right, Media stress plotted against strain at progressively higher intraluminal pressures. Curves were significantly different (*P<0.01). Error bars indicate SEM.

Figure 2. Left, Incremental elastic modulus plotted against intraluminal pressure in relaxed subcutaneous small arteries from normotensive and hypertensive subjects. **P<0.01. Right, Incremental elastic modulus plotted against media stress at progressively higher intraluminal pressures. The slope of both curves (β) was significantly different (P<0.05). Error bars indicate SEM.
inserted in the lumen. We have previously demonstrated that pressure rather than by stretching the vessels with two wires and distending them progressively by increasing intraluminal approach of examining small arteries in a pressurized system comparable results with the newer and more physiological present results extend the previous observations, showing modulus was not increased in hypertensive vessels. The wire myograph technique and showed that elastic modulus in vessels from male and female hypertensive subjects plots against media stress at progressively higher intraluminal pressures. The slopes of both curves ($\beta$) were similar. Error bars indicate SEM.

Elastic properties of subcutaneous small arteries from hypertensive and normotensive individuals have been the subject of one previous study. That study was carried out with the wire myograph technique and showed that elastic modulus was not increased in hypertensive vessels. The present results extend the previous observations, showing comparable results with the newer and more physiological approach of examining small arteries in a pressurized system and distending them progressively by increasing intraluminal pressure rather than by stretching the vessels with two wires inserted in the lumen. We have previously demonstrated that structural data obtained with the pressurized system are also comparable to data from observations using wire myography. With respect to the study of mechanical properties, both techniques may have limitations. Stretching a vessel with two wires may result in vessel shortening, which may affect the transversally measured elastic properties of the shortened vessel; and pressurization of vessels may result in elongation, which necessitates adjustment of the microcannulas to maintain parallel walls. This could also affect the mechanical characteristics measured. In the present study, however, very little or no adjustment was necessary in the distance between the microcannulas. Thus, it is unlikely that this played a role as a confounder in the results obtained. The loss of tethering by surrounding tissues results in different conditions when the vessels are studied in vitro relative to the in vivo situation. However, the present study does not pretend to examine the in vivo behavior of these vessels, but rather the in vitro characteristics of the vessel wall under similar conditions in arteries from normotensive and hypertensive subjects to help understand the changes that have occurred in the vessel wall. Furthermore, because the original length in vivo of the vessels studied cannot be known, axial stiffness cannot be evaluated whereas circumferential stiffness may be quantified. The arterial wall is anisotropic, that is, direction-dependent. Axial and circumferential compliance have been evaluated in carotid arteries in situ in SHR and shown to be similar. However, these studies may be performed only when the in vivo length of vessels can be evaluated, such as with the use of ultrasound techniques, which are not yet applicable to the study of human subcutaneous small arteries. Because mechanical properties may be altered biaxially, uniaxial stiffness as investigated in the current study has to be interpreted cautiously.

A limitation of this study is that the proportion of men and women differed in the normotensive and hypertensive groups. This resulted from the difficulties inherent in the recruitment of subjects for an invasive study such as this one, in which a biopsy of subcutaneous tissue was performed. This could potentially have contributed to the suggestion of hypertrophic growth in resistance arteries from this group of hypertensive subjects, who were predominantly male. However, the stiffness of small arteries from normotensive men and women was not significantly different, and the same was found for hypertensive men and women (Figure 3). Also, within men, the stiffness of vessels was greater in normotensive than in hypertensive individuals,
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and in women, the direction of the findings was similar, although not achieving statistical significance in the latter because of the small numbers. Thus, although this imbalance in the number of subjects from each sex is a recognized limitation, it may not have influenced the results.

The clinical relevance of these findings may result from the fact that altered mechanics of the vascular wall may affect the velocity of the pulse wave, wave reflection, pulsatility of vessels, blood flow velocity, and accordingly shear stress. Vascular wall damage, and with it cardiovascular risk, may be modified by changes in wall mechanics. Decreases in the stiffness of wall components may protect the vessel wall. The extent to which these changes in mechanics are protective and whether their absence may be an indicator of increased vascular risk remain to be determined.

In conclusion, the components of the wall of subcutaneous small arteries of hypertensive subjects are less stiff than in comparable vessels from normotensive subjects. The mechanism for the increased compliance of small arteries of hypertensive individuals remains unclear. The changes found in the composition of the media of the wall of small arteries, an increased collagen-elastin ratio, cannot explain the differences in mechanical properties between vessels from normotensive and hypertensive subjects. It is possible that changes in extracellular matrix architecture or in cell–extracellular matrix attachments may play a role, but this remains to be established. Whether similar changes occur in other vascular beds at the same level of the vasculature requires further study.

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References

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