Carotid Wall Viscosity Increase Is Related to Intima-Media Thickening in Hypertensive Patients

Ricardo L. Armentano, Sebastián Graf, Juan G Barra, Gerardo Velkovsky, Hugo Baglivo, Ramiro Sánchez, Alain Simon, Ricardo H Pichel, Jaime Levenson

Abstract—Increases in arterial wall viscosity and intima-media thickness (IMT) were found in hypertensive patients. Because smooth muscle cells are responsible for the viscous behavior of the arterial wall and they are involved in the process of thickening of the intima-media complex, this study evaluates the relationship between carotid thickness and wall viscosity. The simultaneous and noninvasive assessment of the intima-media complex and arterial diameter was performed using high-resolution ultrasonography. This technique was contrasted against sonomicrometry in sheep, showing that the waveforms obtained by both methods were similar. The common carotid arteries of 11 normotensive subjects (NTA) and 11 patients with mild to moderate essential hypertension (HTA) were measured noninvasively by using tonometry and an automatic densitometric analysis of B-mode images to obtain IMT and instantaneous pressure and diameter loops. A viscoelastic model was used to derive the wall viscosity index (η) using the hysteresis loop elimination criteria. In NTA, η was 2.73 ± 1.66 (mm Hg · s/mm) and IMT was 0.58 ± 0.08 (mm), whereas in HTA, η was 5.91 ± 2.34 (P < 0.025) and IMT was 0.70 ± 0.12 (P < 0.025), respectively. When all data of η versus IMT of NTA and HTA were pooled in a linear regression analysis, a correlation coefficient of r = 0.71 (P < 0.05) was obtained. Partial correlation between η and IMT holding constant pressure was r = 0.59 (P < 0.05). In conclusion, wall viscosity increase was associated with a higher IMT even maintaining blood pressure fixed, suggesting that the intima-media thickening might be related to smooth muscle alterations manifested as an increase in viscous behavior (Hypertension. 1998;31[part 2]:534-539.)

Key Words: wall thickness ■ arterial wall viscosity ■ hypertension ■ tonometry

Complete characterization of geometric, elastic, and viscous properties of arteries has been proposed on the basis of invasive recordings of pressure and diameter pulses, allowing the determination of the pressure-diameter hysteresis loop in conscious chronically instrumented dogs. Moreover, another study provided a complete description of geometric, elastic, and viscous properties of carotid and femoral arteries in human hypertension in which recent noninvasive vascular techniques, sonomicrometry, and Doppler postprocessing M-mode images were applied for measuring pressure and diameter pulses. Wall viscosity was estimated as the area of the diameter-pressure hysteresis loop during the procedure of loop elimination. Compared to normotensive subjects, hypertensive patients had an increased wall viscosity index in the carotid artery. This increased wall viscosity might be the consequence of hypertensive vascular hypertrophy and particularly of the participation of the smooth muscle in that hypertrophy. Moreover, the smooth muscle component among the arterial wall constituents has been demonstrated to be responsible for the viscous behavior of the diameter-pressure relationship. An increased wall thickness in carotid arteries of asymptomatic hypertensive patients has been recently documented. The intima-media thickness (IMT) increases in cross-sections of the arteries provided evidence of vascular growth in human hypertension, at least in conduit arteries.

The aim of this study was to assess the carotid wall viscosity and its relationship to arterial wall thickening even when pressure is statistically held constant.

Methods

Study Subjects

Eleven normotensive subjects (NTA) and 11 patients with mild-to-moderate essential hypertension (HTA, hypertension defined as a supine diastolic pressure, Korotkov Phase V, from 95 to 114 mm Hg on an average of three outpatient visits) entered into the study. No patient had received treatment for at least 1 month before the study and essential hypertension had been documented for all patients by classical laboratory tests. All patients had uncomplicated hypertension and none presented with cardiac, neurological, or renal affection or peripheral vascular disease. The subjects were examined in a quiet room at a controlled temperature of 20 ± 1°C resting in the recumbent position. After 10 minutes of rest, brachial artery blood pressure was measured by sphygmomanometry as the average of three consecutive measurements. One physician specifically trained in vascular investigations made all echographic and tonometric measurements throughout the study. All subjects gave written consent to participate in this study. Clinical parameters of the normotensive and hypertensive groups are summarized in Table 1.
Arterial Diameter Waveform and IMT

Echographic studies were performed with a real-time B-mode ultrasound imager (Hewlett-Packard Sono 1500). The right common carotid artery, 3 cm proximal to the bifurcation of the vessel, was examined with a 7.5-MHz probe. Scanning of the carotid artery was performed in the anteroposterior projection with the patient lying on his back with the head in axis. Subjects with the presence of atherosclerotic lesions, such as an echogenic plaque encroaching into the vessel lumen at the sites of arterial investigation, were excluded from the study. During the scanning, the sound beam was adjusted perpendicular to the arterial surface of the far wall of the vessel to obtain two parallel echogenic lines corresponding to the lumen-intima and media-adventitia interfaces. When the two parallel echogenic lines of the far wall were clearly visible on the monitor, along at least 1 cm of the segment of measure presenting a net echogenic structure on the arterial adventitia, two kinds of image acquisition were performed. These were 1) a fixed image (end-diastolic ECG triggering) to assess IMT; and 2) a succession of images (sequence) to determine the instantaneous waveform of arterial diameter. The images were transferred to a computer (Power Macintosh 7100), digitized into 640x580 pixels with 256 gray levels, stored in a mass memory system, and analyzed off-line with appropriate software (Iotec System).

This software was based on the analysis of gray-level density and on specific tissular recognition algorithms. Briefly, the observer drew a rectangular measurement field around the area of interest using a mouse and an automatic four-step detection process was then used. For the first step, the computer analyzed globally the statistical distribution of the pixel gray densities and identified automatically the locations of the blood-intima and media-adventitia interfaces by computing the rate of intensity change in gray level (Fig 1). For the second step, the computer validated the points consistent with the expected behavior at the two interface levels and substituted the points of discontinuity or the aberrant points for the use of appropriate algorithms. For the third step, the vertical and horizontal coherence of each pixel labeled as an interface was performed. For the fourth step, the whole set of interface points was traced on the monitor of the computer. Two continuous lines were visualized and the computer automatically determined the intima-media thickness as the distance between the two lines calculated by their average difference over 100 successive points.

To obtain the diameter waveform, the sequence of images was analyzed automatically frame by frame. Using a procedure similar to that described above, the anterior and posterior walls were detected. After analyzing the overall sequence, the software output the internal diameter waveform, calculated as the difference between the far and near wall movement (Fig 2).

### Arterial Pressure Waveform

The arterial pressure wave was recorded at the same site as the diameter wave but after the echographic recording. To measure arterial pressure waveform, we used a probe that incorporates a Millar micromanometer in its tip and has the same high-frequency response as the conventional Millar catheter. It has been stated that when the curved surface of the artery is flattened (or applanated) by the probe, the circumferential stress in the wall of the vessel was balanced and the pressure registered by the sensor is identical to the intra-arterial pressure. In practice, we considered that the applanation of the vessel was achieved when a reproducible pulsatile pressure with a large pulse pressure amplitude could be obtained. Indeed, when the hold-down force needed to achieve applanation was excessive, the pressure wave showed a gradual increase in late diastole, with minimal change in the systolic pressure; this phenomenon tended to reduce the pulse pressure amplitude. Subjects who exhibited a distorted signal, principally caused by large amounts of overlying tissue, were excluded from the study. We eliminated obese subjects with a body mass index greater than 29 kg/m². At the carotid site, distortion of tonometric pressure was found in two normal subjects and one hypertensive patient. In these cases, the pressure–diameter relationships became an 8-shaped loop without any mechanical sense. The instantaneous pressure waveforms of four cardiac cycle were digitized every 1 ms and the signal average was calculated. The pressure signal was calibrated by assigning the diastolic pressure measured by brachial sphygmomanometry to the minimum value, and the mean pressure was calculated as one-third of pulse pressure plus diastolic pressure assessed by brachial sphygmomanometry to the average. We assume that mean pressure did not change in large conduit arteries and that the diastolic pressure

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**TABLE 1. Clinical Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensive Subjects (n=11)</th>
<th>Hypertensive Patients (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>49±14</td>
<td>51±7</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25±1</td>
<td>27±2</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>118±12</td>
<td>146±9†</td>
</tr>
<tr>
<td>Diastolic</td>
<td>80±9</td>
<td>102±67</td>
</tr>
<tr>
<td>Mean</td>
<td>93±9</td>
<td>115±67</td>
</tr>
<tr>
<td>Diameter (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>0.615±0.074</td>
<td>0.688±0.072*</td>
</tr>
<tr>
<td>Diastolic</td>
<td>0.576±0.077</td>
<td>0.654±0.073*</td>
</tr>
<tr>
<td>Mean</td>
<td>0.597±0.075</td>
<td>0.671±0.072*</td>
</tr>
</tbody>
</table>

*Values are mean±SD.

*P<.05; †P<.01.
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Figure 3. Plot of pressure-diameter loop in the carotid artery in one patient shows the measured pressure-diameter viscoelastic loop and the purely elastic relationship calculated after elimination of the viscous components.

(as opposed to the systolic pressure) did not substantially differ between brachial and carotid arteries.

During both arterial diameter and pressure measurements, the spikes corresponding to the QRS complex of the ECG were acquired and stored along with the diameter and pressure signals.

Arterial Wall Viscosity Index
A computerized procedure was used to determine the pressure-diameter hysteresis loop and calculate the purely elastic pressure-diameter relationship using an original system developed in our laboratory (Software Borland C++). The pressure and diameter waveforms were identified according to the QRS complex of the ECG. Each cardiac cycle, both for pressure and diameter, was interpolated in time, to obtain the same number of data points, allowing calculation of the averaged cardiac cycle corresponding to the pressure signal and the averaged cardiac cycle corresponding to the diameter signal. This operation allowed the construction of the pressure-diameter hysteresis loop, involving elastic and viscous components in its area (Fig 3). To obtain the purely elastic pressure-diameter relationship, we transformed real pressure (P) into elastic pressure (Pelastic) by using a first-order differential equation, which characterizes the viscoelastic behavior of the arterial wall.

\[
\text{P}_{\text{elastic}} = P - \eta \cdot \frac{dP}{dt}
\]

where \(\eta\) is the viscosity index and \(\frac{dP}{dt}\) is the first derivative of the diameter with respect to time.

By definition, the purely elastic pressure-diameter relationship runs along the same curve both for increasing and decreasing diameters; therefore, in this diagram, no hysteresis loop appears (Fig 3) and the optimal value of \(\eta\) must be assessed by the criterion of disappearance of the hysteresis loop. The value of \(\eta\) was increased by iteration to obtain reduction of the hysteresis loop area until it reached the minimum value, which preserved the clockwise rotation of the loop equation.1,2

Experimental Validation of Arterial Diameter Waveform
Three adult Corriedale sheep (2 to 3 years of age, 40±5 kg) were prepared for this study. Diet restriction was carried out at least 48 hours before surgery. Anesthesia was induced with intravenous thiopental sodium (20 mg/kg) and phentanyl (4 μg/kg) and, after intubation, was maintained with 3% enflurane carried in pure oxygen (4 L/min) through a Bain tube connected to a Bird Mark VIII respirator. With the animal in the right lateral decubitus position, a polyvinyl chloride catheter was inserted into the left jugular vein and a 5% dextrose drip (0.25 mL/min) was started. Minimal incisions were made to expose the left carotid artery, the right femoral artery, and the abdominal aorta. Each artery was carefully and minimally dissected and a pair of ultrasonic crystals (5 MHz, 2-mm-diameter) was sutured on the adventitia with 7-O silk to measure external arterial diameter. Before repairing skin incisions, all cables were tunneled subcutaneously to emerge beyond the measurement areas. The transit time of the ultrasonic signal (1580 m/s) was converted into distance using a sonomicrometer (Trion Technology Inc., 160-Hz frequency response) and optimal signal quality was confirmed on the screen of an oscilloscope (Tektronix 463B).

Noninvasive measurements were carried out using a Hewlett-Packard Sonos 1500 echograph. The left carotid and the right femoral arteries were examined with a 7.5-MHz probe and the abdominal aorta was examined with a 5-MHz probe. Studies were performed with the animal in the right lateral decubitus position. The probe was positioned adjacent to the ultrasonic crystals, which presented a clear echogenic structure on the arterial adventitia. The animal was disconnected from the respirator for 15 s to perform the data acquisition. Images were treated as described above, and simultaneously, the arterial external diameter signal provided by sonomicrometry was acquired at a sample rate of 330 Hz in a PC-486 computer equipped with an analog-to-digital converter (Metrabyte DAS 16).

The 15-s series obtained from sonomicrometry was analyzed beat-to-beat to calculate the average external diameter. Both internal (noninvasive) and external (invasive) average diameter waveforms of abdominal aorta, carotid arteries, and femoral arteries were resampled to 256 samples by interpolation. Having obtained the invasive and noninvasive arterial diameters, both signals were compared in terms of temporal domain (correlation coefficient and residual analysis).

Reproducibility of Measurements
One of the major problems related to the technique used to measure IMT and arterial diameter is the variability in the specific positioning of the ultrasound probe by the technician. To optimize the reproducibility of measurements of successive examination, we used an original procedure based on the automatic computerized generation of the anatomic profile of the arterial examination. During the first examination, an anatomic mask of the artery under study was formed and its surrounding structure was automatically generated by the computer software and stored in the memory mass system. During subsequent examination, the mask allowed the sonographic physician to adjust the probe in the same position as at the first.

We repeated the scanning of the artery in 14 subjects (6 from NTA group and 8 from HTA group). The IMT and the systolic, mean, and diastolic values of the arterial diameter waveforms were averaged, and the variation coefficient was calculated for each pair of measurements. The results of this procedure is summarized in Table 2.

Statistical Analysis
Group data were expressed as mean±SD. The unpaired t test was used to compare parameters between normotensive and hypertensive groups as well as between invasive and noninvasive techniques.

| TABLE 2. Reproducibility of Measurements of Carotid Diameter and Intima-Media Thickness (Expressed as Variation Coefficient, VC) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | Systolic Diameter |     Mean Diameter |     Diastolic Diameter |     Intima-Media |
|                  | VC (%)           |         VC (%)   |          VC (%)   |       Thickness VC (%) |
| All patients (n=14) | 3.92±3.17       |       3.96±3.11   |          4.08±3.26   |       4.46±3.37   |
| NTA group (n=6)    | 4.45±3.68       |       4.66±3.70   |          5.10±3.94   |       3.96±3.55   |
| HTA group (n=8)    | 3.51±2.91       |       3.44±2.73   |          3.31±2.66   |       4.05±3.54   |
Differences were considered significant at a value of $P < 0.05$. Correlation was performed by the least-squares method.

To evaluate the nature of the error when contrasting noninvasive against invasive measurements, a residual analysis was performed.

To evaluate the relationship between IMT and wall viscosity index at fixed level of arterial pressure, i.e., when pressure is statistically held constant, we used the partial correlation coefficient.

**Results**

The normalized diameter waveforms for the carotid, femoral, and abdominal aorta in the three sheep investigated are shown in Figure 4. Because invasive (sonomicrometry) and noninvasive techniques measure external and internal diameter, respectively, data were normalized to a range between 0 and 1. The regression coefficients were $0.95 \pm 0.04$ (range 0.88 to 0.98) for abdominal aorta, $0.88 \pm 0.08$ (range 0.81 to 0.98) for carotid artery, and $0.91 \pm 0.10$ (range 0.75 to 0.99) for femoral artery. In all cases, the residual analysis showed a gaussian behavior.

In hypertensive patients, values of diameter were higher than those observed in the NTA group (Table 1), and values for IMT (0.70 ± 0.12 mm) were higher than those observed in the normotensive subjects (0.58 ± 0.08, $P < 0.025$). In agreement with this finding, the wall viscosity index presented in the hypertensive patients was also higher (5.91 ± 2.34 mm Hg·s/mm) than that observed in the NTA group (2.73 ± 1.66 mm Hg·s/mm, $P < 0.025$).

When all data of wall viscosity index versus IMT from NTA and HTA groups were pooled in a linear regression analysis (Fig 5), a significant correlation coefficient was obtained ($r = 0.71, P < 0.05$). The partial correlation between wall viscosity index and IMT holding mean blood pressure constant yielded a correlation coefficient of 0.59 ($P < 0.05$).

**Discussion**

The objective of the present study was to determine the carotid wall viscosity and its relationship to arterial IMT in hypertensive and normotensive subjects. The arterial wall viscosity and IMT of the carotid artery was assessed noninvasively using high-resolution ultrasonography and tonometric technique.

To measure internal diameter waveform, we used an automatic computerized device. This technique was contrasted against sonomicrometry, an accurate and reproducible technique used in animal experimentation. The time domain analysis showed that the arterial diameter waveforms obtained by both methods were similar. The regression analysis of both times series presented high correlation coefficients and normal residual distribution, suggesting that the differences between the methods are random rather than the result of systematic errors.

The applanation tonometry procedure has showed high accuracy and reproducibility for recording pressure waveform in peripheral vessels, given the condition that the arteries dilate symmetrically and that the elastic properties of surrounding tissues are high compared with those of the artery under study. The accuracy of the probe has been previously validated in animals and in human subjects. Comparisons both in the time
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domain and by spectral analysis show excellent correspondence between tonometric and intra-arterial pressure measurements. The tonometer provides a high-fidelity recording of arterial pressure wave contour under a wide variety of clinical conditions and pulse pressures.

We used the far wall to measure IMT because it is more constantly visualized and repeatable than the near wall. This is due to the different order in which the interfaces of the intima-lumen and media-adventitia are exposed to the incoming ultrasound beam, generating different B-mode images of the near and far walls. The values observed in our population ranged from 0.53 to 0.98 mm of the IMT, and the resolution of the 7.5-MHz probes used in this study ranged from 0.2 to 0.4 mm. This resolution is useless for estimating the smallest changes in the IMT. For this reason, we used the subpixel interpolation incorporated in the software, thereby increasing 5 to 10 times the resolution of the changes of the IMT.

An original procedure of hysteresis elimination, based on the characteristic of the viscoelastic behavior of the arterial wall, was used to determine the wall viscosity. It must be considered an index of arterial wall viscosity because the calculation of the viscous modulus requires the use of the stress-strain relationship, which is not so easy to obtain, because an unstressed value of arterial diameter must be used as a unique reference for both groups under study. Nevertheless, to accomplish the aim of the present work, we can deduce whether increased arterial wall viscosity is related to the IMT, we consider that the index of arterial wall viscosity represents an adequate estimation.

One of the important limitations of the IMT evaluation is the impossibility of differentiating the specific intima from the media layer. In hypertensive patients, an increase in the IMT complex might be caused by an increase in intima thickness, which implies atheromatous, as well as by media augmentation, which indicates hypertrophy phenomenon.

The concomitant augmentation of arterial wall viscosity and IMT in hypertension suggests that vascular growth observed in the carotid arteries is mainly caused by smooth muscle cell hypertrophy that alters the media more than the intima layer. Indeed, the smooth muscle component among the arterial wall constituents has been demonstrated to be responsible for the viscous behavior of the diameter-pressure relationship. However, smooth muscle tone, which is a major determinant of arterial wall viscosity, should be addressed. It has been demonstrated by our group that vascular smooth muscle activation by phenylephrine or the renin angiotensin system in renovascular hypertension in conscious dogs provokes an increase in arterial wall viscosity. These findings suggest that functional mechanisms might mediate wall viscosity augmentation. In addition, no changes were found in wall viscosity after mechanical increase in blood pressure (aortic cuff occlusion) in normotensive dogs. This suggests that in the normotensive state, pressure augmentation did not modify the smooth muscle behavior. Further studies performing this analysis after abolition of most of the vascular tone in both groups of patients by sublingual nitroglycerin are needed.

The blood pressure level may be implicated in viscosity and IMT increases. However, correlation analysis holding pressure level statistically constant showed a slight fall in the correlation coefficient, confirming the pressure independence of the relationship. This indicates that mechanisms other than blood pressure itself participate in the vascular growth of the carotid arteries.

Arterial wall viscosity alterations induced by renovascular hypertension in conscious dogs have been assessed invasively by using the solid-state pressure transducer and sonomicroscope technique, with a high degree of frequency response. Viscosity index of the thoracic aorta was increased by 30% in hypertensive dogs compared to normotensive dogs. It is conceivable that the use of tonometry to measure carotid pressure could provoke a blunting of the systolic peak alone or of the systolic rise time, which, in turn, would result in a reduced value of calculated wall viscosity, mainly in hypertensive patients, who show greater systolic peak as well as lower rise time. However, in our case, the viscous behavior of the carotid wall of the hypertensive patient was augmented by 115%, suggesting that blunting phenomena were not present.

Further studies are needed to analyze more precisely the physiopathological mechanisms of the increase in wall viscosity related to the vascular growth. Also, it is necessary to examine whether the simultaneous assessment of the viscosity index and IMT may be useful to monitor the reverting of the arterial wall alterations during antihypertensive treatment, as well as to ascertain whether such reversibility may have beneficial effects on the progression of cardiovascular disease.

In conclusion, the present study demonstrates that increased wall viscosity is associated with a higher IMT, even when pressure is statistically held constant, suggesting that in human hypertension, the intima-media thickening might be related to smooth muscle alterations manifested by the enlargement of viscous behavior.

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