Angle dependence of ultrasonic backscatter in arterial tissues: a study in vitro

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ABSTRACT The object of this study was to obtain quantitative data on the angle dependence of reflected ultrasound signals in freshly excised normal human arterial walls and those with different degrees of atherosclerotic involvement (fatty, fibrofatty, fibrous, or calcified). Fifteen specimens were evaluated in each pathologic subset. The backscatter coefficient (BS, expressed as cm⁻¹ × steradians⁻¹), measured at the single frequency of 10 MHz, was evaluated at a normal angle of incidence of the interrogating beam to the tissue sample and over an angular span of 60 degrees (± 30 degrees around normal incidence, 2 degree steps). BS measured at normal incidence separated normal (10⁻² × 0.155 ± 0.018; mean ± SE) from fibrofatty (10⁻¹ × 0.0103 ± 0.008), fibrous (10⁻¹ × 0.182 ± 0.016), and calcified (0.202 ± 0.016) specimens; normal and fatty (10⁻³ × 0.759 ± 0.142) and fibrofatty and fibrous samples could not be distinguished from each other in a statistically significant way. Angular scattering measurements identified two patterns: (1) A "directive" pattern, characterized by a strongly angle-dependent BS that falls abruptly when the beam is moved slightly away from normal incidence. This pattern was typical of calcified, fibrous, and less markedly, fibrofatty and normal samples. (2) A "nondirective" pattern, characterized by a BS that is not significantly angle dependent and fluctuates throughout the entire angular range. This was typical of fatty samples.


ULTRASONIC tissue characterization has recently proved experimentally useful in different fields of cardiovascular pathology.¹⁻³ The usefulness of ultrasonic indexes — based either on backscatter⁴ or on attenuation⁵ — for characterization of atherosclerotic disease has also been demonstrated recently. In contrast to the echoes arising from the myocardium, which are relatively independent of the angle of incidence of the ultrasonic beam to the tissue, those arising from arterial walls are generally said to be of a "specular type."⁶ This is a major limitation to any application based on a quantitative diagnostic approach in vivo, since specular reflectors give rise to a signal the amplitude of which is highly dependent on the angle of incidence of the ultrasonic beam to the tissue target. The purpose of this study was to obtain quantitative data on the angle dependence of reflected signals from normal arterial walls and those with different degrees of atherosclerotic involvement.

Materials and methods

Experimental procedure. Fresh specimens of arterial wall were taken from human aortas at autopsy and those with one of four distinct kinds of lesions (fatty, fibrofatty, fibrotic, and calcified) and with regions, whenever possible, of relatively normal tissue were chosen for study. To deal with potential problems of methodology, such as variation in time after death at which the tissue was obtained and examined by ultrasound or variation in the temperature of the tissue during examination,⁷ samples were used only when the interval between death and excision ranged from 10 to 15 hr, and the ultrasonic examination was started immediately after excision and was performed while tissue was in a water bath at a constant temperature of 20°C.

Excised samples of aorta were cut down the anterior midline, opened flat, mounted on a sample holder (consisting of a rectangular metallic hollow rim with pins around its periphery), and placed on an angular scanning system in a distilled water bath at 20°C. This device enabled the aortic sample to be stabilized while maintaining it in the focus of the transducer, so avoiding interference with ultrasonic measurements. Once the ultrasonic studies were completed, the tissue was removed from the water bath and wall thickness was measured with a micrometer (at each site of ultrasonic study) before the tissue was prepared for histologic examination. Thickness values in each of the five groups studied were as follows: normal walls, 2.0 ± 0.9 mm (mean ± SD); fatty plaques, 2.6 ± 0.4 mm; fibrofatty plaques,
3.3 ± 0.3 mm; fibrotic plaques, 2.6 ± 0.4 mm; and calcific plaques, 3.8 ± 0.3 mm. For each pathologic subset, the first 15 consecutive values obtained were considered in the analysis.

**Ultrasonic technique.** Ultrasonic measurements were obtained with the system shown in figure 1. It comprises a single transducer (Aerotech, model gamma, 15 MHz nominal frequency, 0.5 cm diameter, 8.5 cm focal distance, 2 cm focal length) acting as both transmitter and receiver. The use of a small-diameter transducer reduces the phase cancellation artifacts caused by phase-sensitive transducers. The ultrasonic beam width (4 mm evaluated at −3 dB) was determined at 10 MHz (continuous-wave signal) by means of a metallic Rayleigh reflector. The transducer was excited with a broadband pulser/receiver (Panametric, model 5052 PR).

To evaluate the orientation dependence of tissue scattering, a single frequency from the incident beam was analyzed. Accordingly, the received signal was analyzed by fast-Fourier transform, in which frequency component of 10 MHz is derived by gating the broadband spectrum of the received signal in the frequency domain. The transducer was fixed on a half-crown scanning system; in this way the mechanical axis of the transducer was able to cross the geometric center of the crown, where the holder was placed, at all angles. For measurements of angular scattering, the transducer was rotated over an angle of ±30 degrees with respect to the perpendicular axis of the sample holder (0 degrees), moving in steps of 2 degrees (within a total range of 60 degrees). The received signal was captured with a transient recorder (Tektronik, model 7912 AD, conversion rate of 10 nsec, 9 bits of amplitude resolution).

**Ultrasonic data analysis.** The backscatter analysis involved a tissue volume approximately equal to the product of the beam area and the thickness of the specimen. During the acquisition, the gate length was fixed at 5 μsec, to allow digitization of the signal from the full thickness of the specimen. Before ultrasonic analysis, a masking operation was performed on each ultrasonic record under computer control so that echoes could be distinguished from water-tissue interfaces. The mask length was of 40 data points, corresponding to the time length of the transmitted pulse (about 400 nsec). Afterwards, a thickness normalization procedure was performed for each specimen. Thus, the measurement obtained was independent of biological variability in wall thickness. The backscatter coefficient (BS) was calculated in the frequency domain by squaring the amplitude of the spectral component at 10 MHz and by normalizing for the volume of insonated tissue; it was expressed as inverse centimeters times inverse steradians. A correction was performed with an almost perfect reflector (a stainless steel plate) placed in the focus of the transducer. A simple quantitative analysis of the angular distribution function was also performed. To express quantitatively the angular behavior of the integrated backscatter, a curve of BS as a function of the angle was derived: on the abscissa the range of values is 0 degrees (i.e., normal incidence) to 14 degrees; on the ordinate, BS values are displayed (see figure 3). The slope of the best-fitting straight line (S) was calculated from the data obtained from each specimen.

**Pathologic characterization.** After ultrasonic measurements each aortic region was studied histologically with Weigert-Van Gieson stain. As stated above, according to generally accepted criteria, five pathologic subsets were identified: normal aortic walls, fatty plaques (characterized by the accumulation of lipids in the intima), fibrofatty plaques (usually characterized by a fibrous cap and a lipid core), fibrous plaques (i.e., wall thickened by connective tissue), and calcified plaques (walls in which the atheromata were calcified).

**Statistical analysis.** For each index the mean and the SEM were measured. Differences were tested for significance by analysis of variance, with subgroup analysis by the Newman-Keuls test.

**Results**

The BS, measured at a normal angle of incidence, can differentiate normal specimens from fibrofatty, fibrous, and calcified specimens (table 1), although differences between normal and fatty and between fibrous and fibrofatty samples do not reach statistical significance. The BS value varies as a function of the angle differently in the various subsets. As shown in figure 2, the value falls steeply in normal, fibrofatty, fibrous, and calcified plaques when the beam departs from 0 degree (a behavior typical of the so-called directive or specular reflectors). Fatty plaques, however, have a peculiar "plateau-like" profile of angular scattering (typical of nonspecular or nondirective reflectors). When the data obtained for each subset at each angle of incidence are pooled (figure 3), it becomes apparent that a change of as little as a few degrees in the angle of incidence may completely blunt differences recorded at normal incidence. It is also apparent from figures 2 and 3 that the highest variation in the

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**TABLE 1**

<table>
<thead>
<tr>
<th>BS values recorded at a normal angle of incidence (mean ± SE)</th>
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<tr>
<td>BS (cm⁻¹ × sterad⁻¹)</td>
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<tr>
<td>NL (10⁻⁴) × 0.155 ± 0.018</td>
</tr>
<tr>
<td>FA (10⁻⁴) × 0.759 ± 0.142</td>
</tr>
<tr>
<td>FF (10⁻⁴) × 0.103 ± 0.008</td>
</tr>
<tr>
<td>FI (10⁻⁴) × 0.182 ± 0.016</td>
</tr>
<tr>
<td>CL 0.202 ± 0.016</td>
</tr>
</tbody>
</table>

NL = normal wall; FA = fatty plaque; FF = fibrofatty plaque; FI = fibrous plaque; CL = calcified plaque.

^p < .01.

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The angular scattering behavior in one specimen from each subset. In normal, fibrofatty, fibrous, and calcified specimens the BS value falls abruptly — with increasing steepness — when the beam is moved slightly away from the normal (θ = 0) angle of incidence (specular reflectors). The BS in the fatty plaque fluctuates throughout the entire angular range (non specular reflectors).

was more negative) in fibrous and calcified walls, it was intermediate in fibrofatty and normal samples, and it was almost flat in fatty plaques.

**Discussion**

The angle of incidence between the ultrasonic beam and the tissue target is a critical factor in the quantitative characterization of atherosclerosis based on reflected signals. A change of as little as a few degrees in the angle of incidence may blunt the difference (recorded at a normal incidence, table 1) between noncalcified plaques and normal fatty specimens. However, such angle dependence is not a complete drawback: fatty components of the atherosclerotic plaque show a peculiar pattern of angle dependence that may be considered their acoustical "fingerprint," a potentially useful feature.

The histogram of angular scattering in normal, fibrofatty, fibrous, and calcified walls is clearly peaked, i.e., it falls abruptly when the beam departs from a normal angle of incidence. It appears more plateau-like (less angle dependent) in fatty plaques. Simple statistical indexes (such as the slope of the scattering decay as a function of the angle of incidence) can quantitatively discriminate these different shapes. The histologic architecture and biochemical composition of the arterial wall might be a reasonable morphologic substrate for the recorded difference in angular scattering, which is determined by size and orientation of the scatterers relative to the ultrasonic beam (figure 4).

A directive angular response may be due to simple planar organization of the targets within the tissue. Scatterers in the normal wall might be physically identified in the thin elastic membranes present within the normal media layer and oriented perpendicularly to the beam axis. They give rise to directional scattering typical of that in structures in which large plane interfaces exist within the scattering volume. In fibrous and calcified specimens, the scatterers might be physically identified in thick collagen bundles and calcium lam-

**TABLE 2**

Slopes of angular backscatter decay

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SE</th>
<th>Comparisons</th>
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<tr>
<td></td>
<td></td>
<td>NL FA FF FI CL</td>
</tr>
<tr>
<td>NL</td>
<td>(10^{-5}) × 9.8 ± 0.6</td>
<td>^</td>
</tr>
<tr>
<td>FA</td>
<td>(10^{-5}) × 0.3 ± 1.0</td>
<td>^</td>
</tr>
<tr>
<td>FF</td>
<td>(10^{-5}) × 46.1 ± 5.8</td>
<td>^</td>
</tr>
<tr>
<td>FI</td>
<td>(10^{-5}) × 102.1 ± 22.9</td>
<td>^</td>
</tr>
<tr>
<td>CL</td>
<td>(10^{-5}) × 636.9 ± 46.9</td>
<td>^</td>
</tr>
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^p < .01.
fibrous inae, which are, like elastic membranes, oriented perpendicular to the beam (figure 4). This might explain the very high directivity of these plaques. In fatty plaques, lipids accumulate in the intima, mainly in the amorphous state but also as cholesterol crystals. Such crystals are comparable in size to the wavelength of the beam, and are spatially arranged in a random fashion (figure 4). The absence of a spatial orientation and the small size of the scatterers both contribute to the non-directive type of angular scattering in these plaques.

In the fibrofatty plaque the markedly directive response is probably due to the fibrous cap; however, the coexistence within the scattering volume of a non-directive structure (the fatty core, absent in the purely fibrous plaques) partially blunts the directivity of the angular response (which is substantially less than in the fibrous samples, as shown in figure 3 and table 2).

To evaluate the orientation dependence of tissue scattering, a single frequency was used for the incidental beam.12 Since the angular scattering behavior is a function of the wavelength of the incidental beam, it is important to stress that in the present study a single 10 MHz fixed frequency was used. The chosen frequency was the one that was potentially most useful with respect to clinical applications. In fact, “an ideal, hypothetical carotid scanning device would be a B mode imaging system, functioning at 8 to 10 MHz”.13 Furthermore, coronary intraoperative imaging systems also work with either 9 or 12 MHz central frequency.14 Since “any commercial device that is ultimately developed will probably have both conventional imaging and quantitative tissue characterization imaging built into the same basic hardware,”11 the frequency chosen for the present study was the one most compatible with conventional imaging needs.

In conclusion, the angular dependence of arterial backscatter — potentially a considerable limitation to quantitative ultrasonic diagnosis — may itself become a tool for atherosclerosis characterization. The BS at a normal angle of incidence cannot distinguish normal from fatty or fibrous from fibrofatty samples. Such a distinction can be accomplished, however, if simple statistical parameters describing angular scattering are used. Lipid tissue deposition, which occurs in the atherosclerotic plaque without being spatially organized in specific geometric patterns, introduces a non-specular component to the structure of the plaque, and gives a peculiar non-directive distribution to the angular scattering. On the basis of their angular responses, fatty plaques can be differentiated from normal walls.
and fibrofatty plaques can be distinguished from fibrous samples.

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References


FIGURE 4. Top, Typical histologic appearance of a specimen from each subset (Weigert-Van Gieson stain, original magnification × 32). Bottom, Schematic representation of a specimen from each pathologic subset. Directive scatterers are represented by lines that indicate the planar organization of the target within the scattering volume. The width of the line is proportional to the echo value at a normal angle of incidence. Nondirective scatterers are represented by circles. In normal, fibrous, and calcific samples the scatterers can be physically identified in thin elastic membranes, thick collagen bundles, and calcium laminae, respectively. In all these cases the scatterers represent plane interfaces within the tissue, which are relatively large if compared with the wavelength, and which give rise to directional scattering. In fatty plaques, scatterers can be physically identified in amorphous lipid tissue and cholesterol crystals, which are spatially oriented at random and of various sizes, but which in many cases are smaller than the wavelength of the incidental beam (nondirective reflectors). In the fibrofatty plaque, both a fibrous cap (directive) and a lipidic core (nondirective) are present. The end product is a directive response, but a less marked one than in the purely fibrous plaque.
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