Effects of myocardial contraction on ultrasonic backscatter before and after ischemia

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BARZILAI, BENICO, ERIC I. MADARAS, BURTON E. SOBEL, JAMES G. MILLER, AND JULIO E. PÉREZ. *Effects of myocardial contraction on ultrasonic backscatter before and after ischemia.* Am. J. Physiol. 247 (Heart Circ. Physiol. 16): H478–H483, 1984.—To determine whether contraction and relaxation influence quantitative myocardial ultrasonic backscatter we measured systolic and diastolic integrated backscatter separately in 10 pentobarbital-anesthetized dogs with defined, paced heart rates, before and after coronary occlusion. Data were acquired from intramural sites by coupling a broadband 5-MHz transducer to the left ventricular epicardium. Integrated backscatter was obtained from seven sequential ECG gated intervals throughout the cardiac cycle over the frequencies of 2.5–7.5 MHz and referenced to values obtained with a steel reflector. Before coronary occlusion myocardium in all dogs exhibited a decrease in integrated backscatter from end diastole to end systole ($P < 0.05$) in control zones and in zones destined to become ischemic ($P < 0.05$). Thirty minutes after occlusion integrated backscatter did not change in control zones but was elevated in ischemic zones with blunting of the diastolic-to-systolic variation. Thus myocardium undergoing contraction exhibits a decrease in integrated backscatter, and measurement of integrated backscatter at end systole differentiates ischemic from normal myocardium.

ultrasonic tissue characterization; experimental myocardial infarction in dogs; quantitative ultrasound

QUANTITATIVE TISSUE CHARACTERIZATION with ultrasound is a promising diagnostic application of ultrasonic technology (2, 6, 8, 9, 14). We have recently reported that coronary artery occlusion in dogs produces more than a 3-dB increase in frequency-averaged (integrated) backscatter in ischemic regions, representing more than a 100% elevation above preocclusion values (10). In these earlier studies, values of integrated backscatter were obtained by averaging data collected over several heart cycles. Thus detection of any effect of contraction or relaxation on parameters used for tissue characterization would not have been observed. However, to permit definitive characterization of myocardium, possible effects of conformational changes accompanying contractile protein interactions must be defined. Furthermore, delineation of such effects might ultimately permit assessment of contractile properties per se, independent of their effects on ventricular performance, which is modulated by loading conditions.

Since the present study was undertaken to delineate differences in backscatter in systole compared with diastole, a data acquisition system was developed to characterize integrated backscatter at selected intervals throughout the cardiac cycle in intact dogs. In addition, to determine the effects of cessation of normal contraction on this relationship, values of integrated backscatter were also measured during systole and diastole after coronary occlusion in ischemic and nonischemic zones.

METHODS

Experimental animal preparation. Ten adult dogs (15–25 kg) were anesthetized with pentobarbital sodium (25 mg/kg iv), ventilated with room air supplemented with O_2, and subjected to right thoracotomy. After the right side of the heart had been exposed, the sinus node was crushed, and electrodes were attached to the low right atrium to maintain heart rate constant with pacing. The right thoracotomy was closed, each animal was turned on its right side, and a left thoracotomy was performed to expose the anterior wall of the left ventricle. The left anterior descending coronary artery was dissected free, a snare was placed around the vessel beyond the origin of the first diagonal branch, and transient occlusions were initiated to identify regions of supply reflected by zones of induced cyanosis. Left ventricular pressure was measured with the use of catheters inserted via the left atrial appendage, and signals were amplified and differentiated with Honeywell Accudata 141 and 132 differentiation amplifiers. The peak negative first derivative of ventricular pressure was used to define the end of ventricular systole (1). The surface electrocardiogram was monitored continuously. All physiological parameters were recorded on a 1858 Honeywell Visicorder.

Ultrasonic data acquisition. To measure integrated backscatter in seven selected intervals (windows) evenly spaced throughout the cardiac cycle, a system shown

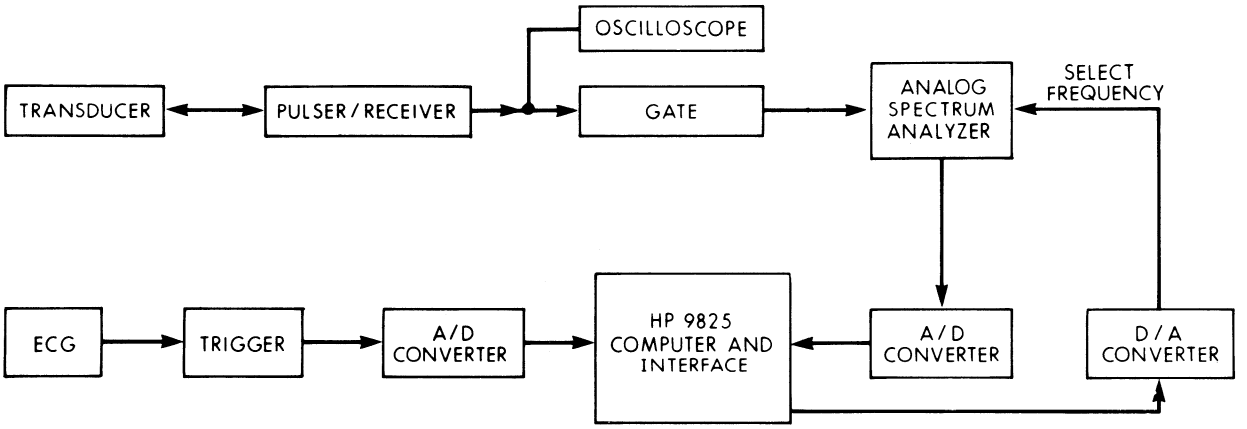

FIG. 1. Block diagram of data acquisition system.

diagrammatically in Fig. 1 was employed. Ultrasonic measurements were made with broadband pulses emanating from a 5-MHz focused transducer (Panametrics model V309) mounted in a water-filled Plexiglas tubular device with a latex rubber tip as previously described (10). The transducer was coupled directly to the epicardial surface of the heart in each dog, at 6–10 sites destined to become ischemic and 6–10 regions destined to remain well perfused. The pulser generated broadband signals at a rate of 1 kHz. A 4-μs portion of the backscattered rf signal corresponding to a 3-mm portion of the anterior myocardial wall was selected by an electronic gate. The gate location was set to ensure that the specular surface reflections were not in the region included by the gate. The gated signal was then processed with a Hewlett-Packard model 9825 desk-top computer that controlled an analog spectrum analyzer. The computer sensed the R wave of the surface electrocardiogram and determined the appropriate intervals for sampling signals from the spectrum analyzer. Figure 2 depicts the distribution of the sampling windows throughout the R-R interval in a dog with a heart rate of 150 beats/min. Due to inherent constraints of the interface of the analog and digital equipment, each window was selected to be 34 ms in duration, with a delay of 18 ms between windows. During the sampling interval, the computer sampled the spectrum analyzer that had been set to a frequency between 2.5 and 7.5 MHz in 500-kHz steps. The output of the spectrum analyzer characterized ultrasonic backscatter at that particular frequency which was referenced to the backscatter of a steel plate representing a nearly perfect reflector. Integrated backscatter for each sampling window was defined as the average of the backscatter transfer function (13) for all the frequencies (2.5–7.5 MHz) for the interval throughout 20 cardiac cycles. Thus, although the value of integrated backscatter for each sampling window was obtained for 20 cardiac cycles, it reflected information exclusively from a specific phase of the cardiac cycle. In a given dog the measured sites were then averaged in the logarithmic domain (dB scale) to produce a mean integrated backscatter for each window and zone (control, destined to become ischemic, or subsequently ischemic) for that dog. Averaging was carried out in the logarithmic domain because the ultrasonic data is measured in decibels, and in that domain the data approximate a normal distribution.

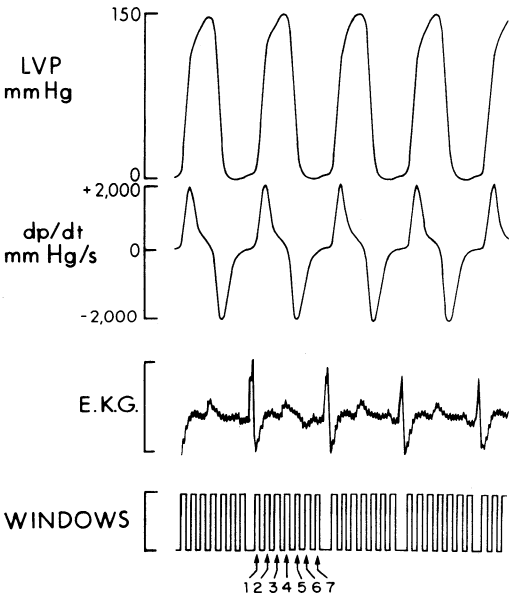

FIG. 2. Temporal distribution of windows for backscatter measurement throughout cardiac cycle. *Window 1* corresponds to end diastole and *windows 4* and *5* to end systole. LVP, left ventricular pressure; dP/dt, 1st time derivative of ventricular pressure; EKG, electrocardiogram.

Experimental protocol. After base-line backscatter data were acquired for all 12–20 myocardial regions in each dog, the coronary occlusive snare was tied and a series of measurements obtained from ischemic and nonischemic zones at intervals of 30 min and 1, 2, and 4 h after coronary occlusion. Each of the preocclusion and postocclusion sets of measurements was obtained while heart rate was maintained constant at 150 beats/min.

Statistical methods. Statistical analysis was carried out with a Bonferroni procedure applied to paired data (19). The data were analyzed in the following two fashions: *1*) comparison of the values for the first window (end diastole) to the fourth window (end systole) in control and ischemic zones, respectively, and *2*) comparison of the values for the control zones to the values in the ischemic zones for end-diastolic and end-systolic time intervals, respectively. Thus each animal served as its own control. A Fisher's F test was applied to the two sets of paired data representing backscatter values before coronary oc-

clusion (Table 1). Furthermore, a two-way analysis of variance was applied to data after induction of ischemia to compare *1*) differences between end systole and end diastole in the control and ischemic zones (*columns 1* and *2*, Table 1), *2*) end-systolic to end-diastolic differences at the various time intervals after occlusion (left-hand rows of data against each other within *columns 1* and *2*), and *3*) differences between control and ischemic zones and end diastole or end systole (*columns 3* and *4*).

RESULTS

Preocclusion values. Values of integrated backscatter obtained in systole and diastole conformed to a consistent pattern throughout the cardiac cycle characterized by elevated values at end diastole and significantly lower values near end systole. Mean values of integrated backscatter at end diastole and end systole for control zones (10 dogs) and for zones destined to become ischemic (10 dogs) are shown in Fig. 3*A* and Table 1. Results are similar for both zones, i.e., with maximal values of integrated backscatter occurring at end diastole in control zones and in zones destined to become ischemic and minimal values occurring at end systole in control zones and in zones destined to become ischemic. The differences between values at end diastole (*window 1*) and end systole (*window 4*) (2.7 and 3.8 dB, respectively) were statistically significant ($P < 0.05$ in both zones, Bonferroni procedure). In addition, there were no significant differences between backscatter values in control zones and zones destined to become ischemic at either end diastole or end systole (Fig. 3*B*, Table 1).

Before ischemia, differences in backscatter values from systole to diastole in control zones were not significantly different at the $P < 0.05$ level (Fisher's F test, Table 1) compared with differences in zones destined to become ischemic. Furthermore, the backscatter differences at end systole between control zones and zones destined to become ischemic were not significantly different at the $P < 0.05$ level (Fisher's F test) from the differences observed between the zones at end diastole.

Values after coronary occlusion. As early as 30 min after coronary occlusion, values of integrated backscatter were significantly elevated in ischemic compared with control zones when averaged over the seven sampling windows. Furthermore, myocardial ischemia induced a remarkable alteration in the previously observed variation of integrated backscatter values throughout the cardiac cycle in zones subserved by the occluded artery. The reduction of integrated backscatter during systole was maintained in control zones but was markedly attenuated in ischemic zones (Fig. 4*A*) with end-diastolic to end-systolic variation of only 0.6 dB (not significant) 30 min after coronary occlusion.

TABLE 1. *Time dependence of integrated backscatter after coronary occlusion*

Time of Measurement	n	Differences Between End Systole and End Diastole, dB		Differences Between Control and Ischemic Zones, dB	
		Control zones	Ischemic zones	End systole	End diastole
Preocclusion	10	−2.7 ± 1.0*	−3.8 ± 1.8*	0.7 ± 2.3	1.7 ± 2.5
Ischemia					
30 min	7	−2.7 ± 0.8*	−0.6 ± 0.9	5.9 ± 1.6*	3.7 ± 0.9*
1 h	9	−2.7 ± 1.3*	−1.2 ± 1.0	5.1 ± 1.4*	3.6 ± 1.9*
2 h	8	−2.5 ± 1.6*	−0.8 ± 1.0	5.4 ± 2.6*	3.6 ± 2.6
4 h	6	−2.9 ± 0.7*	−1.0 ± 1.1	4.4 ± 1.7*	2.6 ± 1.7

All values are means ± SD. *Significant at $P < 0.05$ (Bonferroni's procedure).

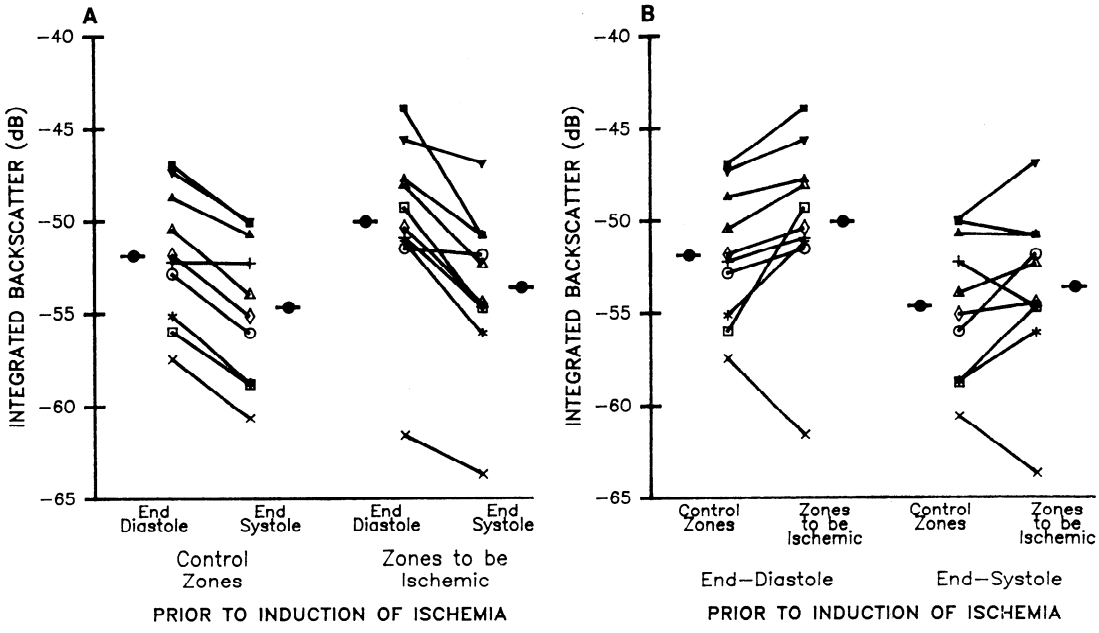

FIG. 3. Each symbol represents values for a given dog at each time interval in each myocardial zone. Mean values from end diastole to end systole are significanty different ($P < 0.05$); mean values from either zone are not significantly different from each other. *A*: diastolic-to-systolic variation in integrated backscatter values before coronary occlusion. *B*: differences in values of integrated backscatter between zones at each time interval before coronary occlusion were not significantly different.

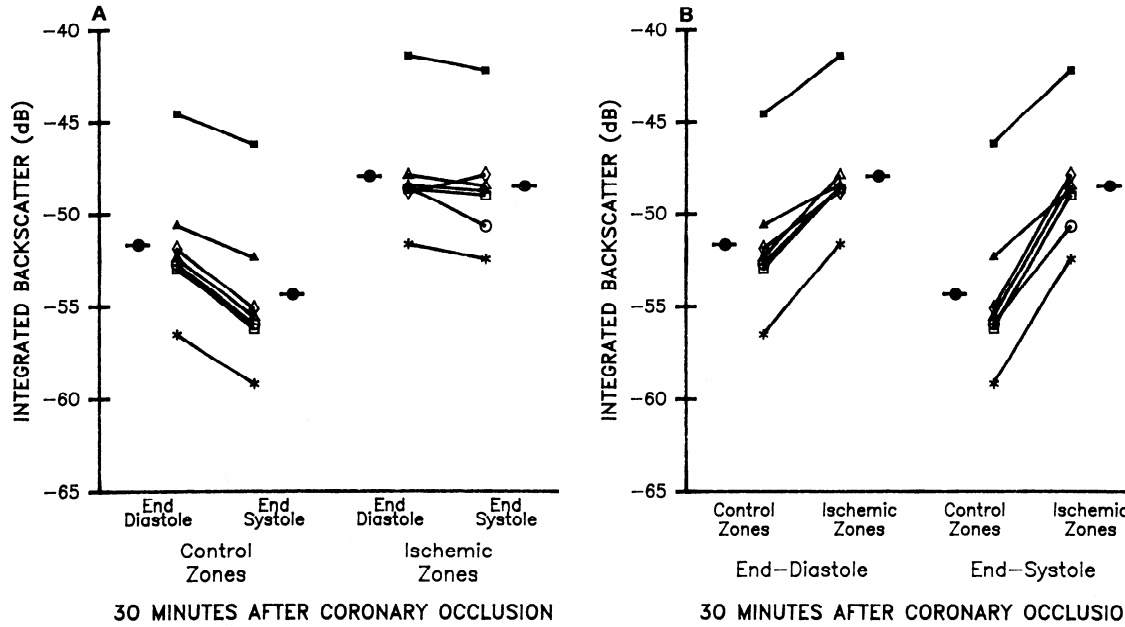

FIG. 4. Each symbol represents values for a given dog at each time interval in each myocardial zone. *A*: differences in values for individual dogs and mean values of integrated backscatter remain significantly different in control zones after occlusion (as in Fig. 3*A*, *left panel*). In contrast, ischemic zones exhibit mean values that are not only elevated but with a blunted diastolic-to-systolic variation (compared with Fig. 3*A*, *right panel*). These latter differences are not statistically significant. *B*: after ischemia mean values of integrated backscatter are significantly different among control and ischemic zones (compared with Fig. 3*B*), in particular at end systole.

Values of integrated backscatter in ischemic compared with nonischemic zones were elevated in each of the seven sampling windows. Differences were maximal in sampling *window 4* corresponding to near end systole ($P < 0.05$, Bonferroni procedure). Although variation from dog to dog was considerable for absolute values of integrated backscatter, end-systolic values (*window 4*) in nonischemic and ischemic zones were significantly different in each dog when compared as early as 30 min after coronary occlusion (Fig. 4*B*; Table 1). Thus effects of myocardial ischemia on integrated backscatter were reproducible despite biological variation from animal to animal.

To evaluate integrated backscatter throughout the cardiac cycle, results of measurements of integrated backscatter at preocclusion and 30 min, 1, 2, and 4 h are compared in Table 1. Analysis was conducted with a Bonferroni procedure applied to the paired data, and significance is reported at a level of $P < 0.05$. Differences between the end-systolic (*window 4*) and end-diastolic (*window 1*) values of integrated backscatter in each dog in the control zones are presented in *column 1*. The end-systolic values are significantly lower ($P < 0.05$) than the end-diastolic values at all time intervals. Differences between end-systolic and end-diastolic values of integrated backscatter in the ischemic zones are presented in *column 2*. In contrast to the results from control zones, no significant difference in backscatter is observed at any interval after coronary artery occlusion, indicating a marked blunting of the cyclic pattern. When ischemic and nonischemic zones are compared at end systole (*column 3*), a significant elevation in backscatter is observed at all intervals. In contrast, when ischemic and nonischemic zones are compared at end diastole (*column 4*), the differences are smaller. Thus the elevation in backscatter accompanying ischemia is most readily detected by comparisons at end systole.

Two-way analysis of variance (ANOVA) was utilized to compare the two sets of data following coronary occlusion. The first ANOVA compared control zones and ischemic zones. The differences in backscatter values between end systole and end diastole were not significantly different at any time interval during ischemia, (comparison between rows within *columns 1* and *2*, Table 1), but strong significance ($P < 0.005$) was evident between the differences in backscatter from end systole to end diastole among ischemic and control zones (comparison between *columns 1* and *2*). The second ANOVA was applied to the time intervals after ischemia (*columns 3* and *4*) comparing the backscatter differences between control and ischemic zones measured at end systole vs. end diastole. A strong significance ($P < 0.005$) was detected for the comparison of backscatter differences between control and ischemic zones measured at end systole vs. end diastole (comparison between *columns 3* and *4*).

DISCUSSION

Ultrasonic methods are utilized extensively for diagnosis of cardiac diseases in patients. Assessment of parameters with conventional techniques that measure myocardial geometry and dimensions such as segmental shortening (15, 17, 18), wall thickening (3–5), and ventricular diameter (7, 16) facilitates characterization of pathophysiology.

Ultrasonic tissue characterization is a qualitatively different approach designed to characterize the physical state of intramural cardiac tissue. We have previously shown that the slope of attenuation (12) and values of

integrated backscatter (11) differentiate normal myocardium from zones of infarction in vitro. Furthermore, characterization of integrated backscatter distinguishes ischemic from nonischemic myocardium in intact open-chest dogs as early as 1 h after coronary occlusion (10). Because the values of integrated backscatter were obtained by averaging data collected over several heart cycles, detection of any effect of contraction or relaxation would not have been observed. Thus we performed the present study to assess integrated backscatter throughout systole and diastole.

The results of the current study demonstrate the dependence of integrated backscatter on the cardiac cycle. Values of integrated backscatter are significantly reduced when myocardium is undergoing contraction compared with values typical of myocardium in relaxation. The pattern appears to depend on intact contractile function since ischemia promptly blunts differences.

Although direct coupling of the transducer to the epicardium of zones with different mechanical behavior might produce artifacts in the ultrasonic signal, the electronic gate was carefully selected to assure that the interrogated volume of tissue did not include any specular reflections from the endo- or epicardium. The rf signal was monitored throughout the experiment to assure maintenance of interrogation of midmyocardial samples. Furthermore, the same pattern was observed in additional experiments performed in closed-chest dogs with the transducer placed against the intact chest wall. Accordingly, the cycle-dependent pattern of integrated backscatter appears to reflect intrinsic properties of actively contracting myocardium.

The nature of the primary ultrasonic scatterers in the myocardium has not been elucidated definitively. Therefore, it is difficult to delineate the factors responsible for the variation observed. Several factors might be responsible, including *1*) alterations in the density of scatterers, *2*) changes in the size of the scatterers, *3*) modification in the effective density and compressibility of the scatterers resulting in variations of scattering efficiency, and *4*) anisotropy exhibited by the medium accompanying contractions and relaxation with resultant variation in the backscatter. It is also possible that the state of contraction may affect the attenuation, which could result in apparent variations in backscatter. The present experiments do not permit an assessment of the possible roles of these or other mechanisms. Elucidation of factors responsible will require studies with other types of experimental preparations in which coronary flow, contractility, heart rate, and intraventricular volume can be controlled independently.

Results of the present study confirm previous observations from our laboratory demonstrating that myocardial infarction in dogs is associated with elevations in integrated backscatter in involved regions. In the present study, with analysis of backscatter at selective intervals throughout the cardiac cycle, elevations were observed as early as 30 min after coronary artery ligation. The distinction between normal and ischemic areas was enhanced, since differences between backscatter values were greatest at end systole. Thus gated acquisition of data enhanced discrimination between ischemic and nonischemic myocadium.

Alterations of the cardiac cycle-dependent pattern of backscatter typical of normal tissue persist in ischemic myocardium for at least 4 h after coronary occlusion. Such regions were found to be grossly ischemic judging from inspection at the end of each experiment. Our earliest ultrasonic measurements were obtained 30 min after coronary occlusion to avoid artifacts due to the frequent ectopic beats encountered earlier. Hence, the blunted pattern of the integrated backscatter throughout the cardiac cycle may appear even earlier.

Results of this study indicate that quantitative ultrasonic backscatter conforms to a consistent pattern throughout the cardiac cycle, that variation from systole to diastole is blunted promptly in ischemic tissue, and that end-systolic measurements enhance differentiation of ischemic from normal myocardium. Assessment of integrated backscatter at selected intervals, gated to the cardiac cycle, should improve quantitative ultrasonic tissue characterization through intervening tissue such as the chest wall by reducing a previously unrecognized source of variation masked by averaging data throughout the cycle. Perhaps of most importance, the apparent dependence of regional integrated ultrasonic backscatter on local contraction of interrogated myocardium suggests that tissue characterization with ultrasound may ultimately provide a noninvasive means for evaluating the contraction of the heart.

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