Variation of left ventricular myocardial gray level on two-dimensional echocardiograms as a result of cardiac contraction

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ABSTRACT Integrated ultrasonic backscatter from normal myocardium has been shown to vary with the phase of cardiac contraction (decreasing from end-diastole to end-systole) in previous studies of open-chest dogs. If confirmed, this finding would have important implications for clinical application of ultrasonic tissue characterization. Our hypothesis was that a cardiac cycle–dependent variation in regional average gray level would be detected on analysis of digitized two-dimensional echocardiograms. We analyzed echocardiographic images from 16 subjects in whom normal, technically good studies were obtained with a commercial phased-array scanner and a 2.25 MHz transducer. Images from six subjects were digitized from stop-frame photographs and those from 10 subjects were obtained directly in digital format from the scanner. Average gray level was measured in a portion of the left ventricular posterior wall in parasternal long-axis images obtained at end-diastole and end-systole by both photographic and digital-image acquisition. In seven of the subjects from whom digital images were acquired, left ventricular posterior wall gray level and ventricular septal gray level were also evaluated on parasternal short-axis images. In images digitized by the photographic technique, mean posterior wall gray level decreased significantly from end-diastole (175 ± 5.8 SEM) to end-systole (167 ± 5.1, \( p < .05 \)). Similarly, in images digitized directly, mean posterior wall gray level in the long-axis view decreased from end-diastole (71 ± 3.4) to end-systole (59 ± 2.5, \( p < .005 \)). In directly digitized short-axis views, mean posterior wall gray level also decreased from end-diastole (70 ± 5.2) to end-systole (59 ± 4.0, \( p < .05 \)). Results in the ventricular septum were less consistent. We conclude that average gray level in the left ventricular myocardium on two-dimensional echocardiographic images decreases from end-diastole to end-systole in normal subjects.

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CLINICAL ECHOCARDIOGRAPHY, as it is presently employed, consists of evaluation of M mode patterns and two-dimensional images of the smooth, large interfaces of the heart, such as the endocardial and epicardial surfaces. Quantitative evaluation of echoes of lower amplitude, such as those from the myocardium, has not yet been widely used clinically. Preliminary evidence, however, suggests that such data may be useful in the diagnosis of abnormalities of myocardial structure and perfusion.\(^1\)-\(^3\) This type of evaluation, called "ultrasonic tissue characterization,"\(^4\) may be accomplished by analysis of radio frequency data to determine integrated ultrasonic backscatter\(^4\) or by analysis of echo amplitude (video) data from standard echocardiographic images.\(^5\)

Previous studies have shown that abnormalities in integrated ultrasonic backscatter occur in areas of ischemia and infarction.\(^6\)-\(^9\) Observed alterations may be due to changes in tissue water content or in myocardial blood flow early, or to increased deposition of collagen late after infarction. The exact factors causing these acoustic alterations are presently not completely understood. Changes in integrated ultrasonic backscatter from abnormal myocardium may reflect actual changes in the physical structure or perfusion of the myocardium. However, Madaras et al.\(^10\) have recently reported that integrated ultrasonic backscatter measured in open-chest dogs also varies with the phase of
cardiac contraction in normal myocardium. These investigators found that backscatter decreased by more than 3 dB from end-diastole to end-systole. Previous studies of myocardial structure utilizing integrated ultrasonic backscatter have averaged backscatter values throughout the cardiac cycle. We have measured regional echo amplitude (gray level) at a selected point in the cardiac cycle to avoid any potential change resulting only from cardiac contraction. The video data that we analyze (e.g., image gray level) corresponds to radio frequency data that have undergone several processing steps in the clinical echocardiographic instrument. These steps could either exaggerate or blunt the observed cardiac cycle-dependent changes in ultrasonic backscatter. The effect of cardiac contraction on echo image data would be important to determine since apparent ultrasonic tissue characteristics might change with the cardiac cycle independent of any pathologic abnormalities in myocardial structure.

In the present study we assumed a direct relationship between integrated ultrasonic backscatter (radio frequency data) and average gray level (video data) as measured from a standard two-dimensional echocardiogram. We therefore hypothesized that average ultrasonic backscatter amplitude (gray level) would decrease from end-diastole to end-systole in standard two-dimensional echocardiograms of normal men, in a direction similar to the previously noted changes in integrated ultrasonic backscatter.

Methods

Subjects. Sixteen young, normal, healthy men participated in this experiment. No subject had a history of cardiac disease or hypertension, and the standard echocardiogram of each subject was normal.

Echocardiographic studies. We obtained two-dimensional echocardiograms with a standard phased-array scanner operating at 2.25 MHz (Diasonics V3400R). The echocardiograms were performed in the left lateral decubitus position. Gain settings and gain compensation profiles were adjusted to obtain apparently uniform myocardial brightness throughout the echocardiogram in each subject. The gray scale transfer function was adjusted to be linear for the entire video signal range, and no reject was used. Parasternal long-axis (figure 1) and short-axis views of the heart were obtained at end-diastole and end-systole. End-diastole was defined as the point in the cardiac cycle at the onset of the electrocardiographic Q wave. End-systole was defined as the time of apparent minimal left ventricular chamber size and occurred near the peak of the T wave.

Image digitization. The echocardiographic images were digitized in two ways. In the first six subjects echocardiograms were recorded on 1/2 inch videotape. Photographs were obtained from stop-frame videotape data at end-diastole and end-systole during the same cardiac cycle. The images were photographed with Polaroid 665 positive-negative film with an f-stop of 2.9 and an exposure duration of 5 sec. Photographic negatives were cleared with a sodium sulfite solution. The photographic negatives were placed on a light table and digitized with use of a video camera (Hamamatsu C-1000) interfaced with a PDP 11/34 computer and to a Ramtek image display system. All photographs were digitized on the same day with constant camera settings and identical light box and ambient illumination. The data were digitized into a 256 x 256 pixel matrix with 8-bit gray level quantization. Theoretically this permits the echocardiographic intensity to be quantized to 256 gray levels. However, the effective number of gray levels that can be distinguished is less and depends on the dynamic range of the primary display monitor, film type, method of film processing, and video camera signal-to-noise ratio.

In the other 10 subjects, data were acquired directly from the freeze-frame memory of the digital scan converter of the echocardiograph. These data were digitized at 470 sampling points along each of 128 scan lines in a pseudopolar coordinate format. These digitized data are not affected by the gray scale transfer controls of the scanner, which only affect image display. The diastolic and systolic images were acquired from the same cardiac cycle. Data were directly transferred to our computer system and then reconstructed into a rectangular format by a geometric transformation technique that we developed. This
resulted in a 512 \times 512 pixel image with an 8-bit or 256 gray level quantization. As in the case of photographic digitization, these 256 levels represent a subset of the very wide amplitude dynamic range of the echo signal. The advantage of the latter digitization technique was that it avoided possible degradation in the quality of image data that might result from photographing videotape data displayed on a video screen and subsequent video camera digitization.

For both photographic and directly acquired digital data, the specific gray level values reported here were dependent on our particular experimental system and thus should be interpreted as arbitrary, not absolute, values.

**Image analysis.** The image data were analyzed to determine the echo amplitude (gray level) in the posterior left ventricular wall (figure 2) and in the ventricular septum. A region of interest was interactively positioned in the posterobasal left ventricular wall just inferior to the atrioventricular groove in both long- and short-axis images. In short-axis images, a region of interest was placed in the ventricular septum. Regions of interest were individually selected in diastole and systole such that the region enclosed as much of the transmural myocardium as possible, excluding the endocardial and epicardial specular reflections and avoiding obvious side lobe artifacts or areas of echo dropout. Thus, regions of interest were typically slightly less than 1 cm in transmural dimension at end-diastole. Regions of interest were identified at similar locations at the two points in the cardiac cycle by use of anatomic landmarks in the images. Positioning of regions of interest in long-axis images was accomplished by consensus of three observers who were not blinded to the hypothesis of the study. Positioning of region of interest in short-axis images was done independently by two observers. Gray level was averaged within each region of interest so that the average gray level was measured at both end-diastole and end-systole.

Interobserver variability was evaluated by two independent observers who chose regions of interest in the short-axis images of the posterior wall at end-diastole and end-systole. Intraobserver variability was evaluated by one observer who chose regions of interest in the short-axis images at end-diastole and end-systole on 2 different days. Since our digital data acquisition system allowed the storage of only two frames, reproducibility studies were done by choosing different regions of interest from the same images.

**Data analysis.** Data derived from long-axis images were analyzed separately for the first six subjects (video camera digitization) and for the next 10 subjects (direct digital data acquisition). Data derived from short-axis images of the 10 subjects from whom digital images were acquired were also analyzed separately. For each group, the significance of differences found between the gray level of the posterior left ventricular wall at end-diastole and end-systole and of the ventricular septum at end-diastole and end-systole were determined with a paired t test. Interobserver variability was evaluated by linear regression (least squares) analysis, comparing the data of observer 1 with those of observer 2. Intraobserver variability was evaluated by linear regression analysis in which the data derived by one observer on 2 different days were compared. These reproducibility analyses were performed for posterior wall gray level at end-diastole and at end-systole and for the change in posterior wall gray level from end-diastole to end-systole.

**Results**

Long-axis images from all 16 subjects were judged to be adequate for analysis in this study and short-axis images from seven of the 10 subjects in whom they were obtained by digital data acquisition were judged adequate for analysis; three short-axis images were excluded due to artifacts that precluded evaluation of the posterior wall and ventricular septum at either end-diastole or end-systole.

Qualitatively (figure 1) the posterior wall and ventricular septal myocardium exhibited a decrease in echocardiographic intensity at end-systole compared with end-diastole and this was visible both in long-axis and short-axis orientations.

As shown in figure 3, the mean gray level decreased from end-diastole to end-systole on long-axis images. This decrease was observed in the data obtained directly from the freeze-frame memory of the scanner and that from digitized photographs of videotape images. The individual echo amplitude values derived from long-axis images at end-diastole and end-systole for all 16 of our subjects are illustrated in figure 4. The posterior wall gray level decreased from end-diastole to end-systole in 15 of our 16 subjects. In figure 5 the gray level changes observed in the short-axis views are il-
Mean posterior left ventricular wall gray level values from long-axis images for all individuals studied; data acquired from videotape are shown on the left and data acquired from the freeze-frame memory are on the right. In 15 of the 16 subjects there was a decrease in gray level from end-diastole (ED) to end-systole (ES).

The echocardiographic data acquired by photographic digitization indicated that the gray level of the posterior wall decreased from 175 ± 5.8(SEM) at end-diastole to 167 ± 5.1 at end-systole, a mean change of 7.3 (4.2%) (p < .05). The data acquired directly in digital form showed a decrease in gray level in the long-axis view from 71 ± 3.4 at end-diastole to 59 ± 2.5 at end-systole, a mean change of 12 (17%) (p < .005). On directly digitized short-axis images gray level decreased from 70 ± 5.2 at end-diastole to 59 ± 4.0 at end-systole, a mean change of 11 (16%) (p < .05). As a result of use of different methods of digitizing the echocardiographic image data, the absolute gray levels or change in gray levels during contraction obtained with the two methods could not be directly compared.

The data from the ventricular septum were more variable. One observer found a significant difference in septal gray level from end-diastole (69 ± 4.4) to end-systole (60 ± 5.3, a mean change of 9 or 13%) (p < .05). The other observer found a trend toward change from end-diastole to end-systole that was not illustrated; these also demonstrate a decrease from end-diastole to end-systole.

FIGURE 4. Mean posterior left ventricular wall gray level values from long-axis images for all individuals studied; data acquired from videotape are shown on the left and data acquired from the freeze-frame memory are on the right. In 15 of the 16 subjects there was a decrease in gray level from end-diastole (ED) to end-systole (ES).

Discussion

In the present study we have determined that there is a variation in average left ventricular posterior wall and ventricular septal gray level in two-dimensional echocardiographic images from normal men that is related to cardiac contraction: the average gray level decreased from end-diastole to end-systole. The data we obtained confirms findings from previous investigations in which radio frequency data were used and extends these findings to image (video) data obtained from a standard echocardiographic scanner.

Comparison with previous studies. Echo amplitude as measured in our study is not precisely the same thing as integrated ultrasonic backscatter calculated from radio frequency data. This is due in part to alteration of video ("detected") data that occurs when it is processed in the echocardiographic scanner. Nonetheless, the directional change in echo amplitude that we demonstrated (decreasing from end-diastole to end-systole) is similar to that noted by Madaras et al., who used radio frequency data obtained in open-chest dogs. These investigators have also demonstrated in dogs that the degree of contraction-related variability in backscatter varies in different portions of the ventricle. We have also noted regional differences in contraction-dependent variation in gray level, with data from the posterior wall showing more consistent changes than data from the ventricular septum. It should be noted that we measured gray level alterations within a single cycle, whereas in the previous study cited above, backscatter data were acquired over several cardiac cycles.

Mechanisms of contraction-dependent gray level changes. The mechanisms for normal and abnormal

Diag nostic Methods—Echocardiography

FIGURE 5. Mean posterior left ventricular wall gray level values from short-axis images. Grouped data are shown at the left and individual data points are shown at the right. ED = end-diastole; ES = end-systole.

Vol. 70, No. 6, December 1984

statistically significant (79 ± 7.2 vs 69 ± 5.2, mean change of 10 or 13%) (p = NS).

Data for interobserver and intraobserver variability are listed in table 1. Interobserver correlation coefficients were .84 for end-diastolic gray level, .63 for end-systolic gray level, and .72 for the change in gray level with contraction. Analogous values for intraobserver correlation coefficients were .97 (diastole), .97 (systole), and .80 (change).

TABLE 1

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<th>Interobserver&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Intraobserver&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td>Diastolic</td>
<td>.84</td>
<td>.63</td>
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<td>Gray level</td>
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<td>Systolic gray level</td>
<td>.97</td>
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<td>Gray level change</td>
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<sup>a</sup>Observer 1 vs observer 2.
<sup>b</sup>Observer 1 on two occasions.
changes in myocardial gray level during the cardiac cycle have not yet been defined. Echocardiographic reflections from normal myocardium are of lower amplitude than those from specular reflectors and arise from many small interfaces (small compared with the wavelength of the echocardiographic signal). These numerous reflections produce a complex interference pattern known as acoustic speckle. The echocardiographic image data that we studied and those described by previous investigators are, in large part, the result of acoustic speckle. The precise relationship between acoustic speckle and underlying anatomic structures is very difficult to characterize and depends in part on the scanning instrument used.

We have postulated four mechanisms that may have contributed to the cardiac cycle–dependent changes in mean gray level we observed. These include (1) changes in structure or geometry of individual muscle fibers, (2) changes in relative muscle fiber orientation, (3) changes in material properties of the myocardium, and (4) variation in myocardial blood flow. In the present study we did not determine the degree to which these various factors contribute to the changes in gray level during cardiac contraction.

Previous investigators have determined that cardiac contraction–dependent backscatter variation of the left ventricle is more prominent near the apex than the base in the dog. Local myocardial fiber orientation and shortening are known to vary in different portions of the intact left ventricle. The extent of regional contraction and regional fiber architecture may therefore be determinants of fluctuations in local echo amplitude with the cardiac cycle. The fact that contraction-related changes in integrated ultrasonic backscatter are blunted with ischemia may in turn be related to decreased regional fiber shortening or decreased local perfusion. This leads one to speculate that evaluation of these changes with standard two-dimensional echocardiography may help increase the sensitivity of diagnosis of a variety of cardiac diseases associated with alterations in regional contractile function, including atherosclerotic heart disease with myocardial infarction or ischemia as well as hypertrophic or infiltrative cardiomyopathy.

Several factors not related to alterations in myocardial acoustic properties may have contributed to an apparent decrease in echo amplitude with contraction. It is noteworthy that as the specular endocardial and epicardial reflections separated during the cardiac cycle (i.e., as the wall thickened), the mean gray level of the myocardium decreased. This suggests that our observations may have been secondary in part to "blooming" of the specular interfaces that may have been included as myocardial reflections during diastole, when these specular reflections were more closely apposed. We took pains in our adjustment of the video echocardiographic image to prevent blooming of endocardial and epicardial specular reflections. If there were blooming, then the average myocardial gray levels as we measured them would have been greater at end-diastole. Another possibility is that a specular interface, in particular the endocardium, may have changed its attenuation characteristics during the cardiac cycle. If the attenuation increased with cardiac contraction, then less myocardial signal would be returned to the echocardiographic transducer, producing an artifactual decrease in gray level. There is probably little biologic or morphologic change in the endocardium during cardiac contraction, making this mechanism for changes in myocardial gray levels unlikely. The attenuation properties of the myocardium itself may have altered with contraction, and thus may have been responsible in part for apparent changes in gray level.

Another possible reason for an artifactual change in myocardial echo amplitude is that during systole the myocardium may have moved into a different portion of the two-dimensional echocardiographic gain compensation profile. In our scans we did not note a significant change in the distance of the posterior wall from the transducer with contraction, making this mechanism unlikely also. Changes in the portion of the posterior wall myocardium sampled during the cardiac contraction may have caused changes in gray levels. For example, the heart rotates and translates during cardiac contraction and it is possible that a different aspect of the posterior wall was sampled at the two points in the cardiac cycle. However, we believe it somewhat unlikely that this would cause a consistent decrease in average gray level in the subjects we studied.

**Clinical implications.** We have demonstrated that myocardial echocardiographic amplitude in humans decreases with cardiac contraction. If previous findings from studies of integrated ultrasonic backscatter can be applied to our approach, then a reduction in gray level variation due to decreased cardiac contraction may be demonstrable by our technique in patients with cardiac disorders such as ischemia. Areas with abnormal wall thickening or decreased wall motion may demonstrate a different degree of gray level change than is apparent in normal myocardium. This technique may thus help differentiate normal from abnormal myocardium in conditions such as in infiltr-
Advantages of our method of echo amplitude analysis include the use of standard echocardiographic instrumentation and the ability to evaluate data acquired during a single cardiac cycle. However, before further clinical applications are considered for this investigative technique, measurement of cycle-dependent changes in other areas of the myocardium must be made. In addition, as a result of the configuration of our data acquisition system, we were not able to evaluate the beat-to-beat reproducibility of contraction-related gray level variation. It is our opinion that the placement of the region of interest is a principal contributor to interobserver variability. Thus, in an individual patient the placement of the region of interest could affect the results of gray level analysis. It is likely that analysis of myocardial echo gray levels and possibly the pattern ("texture") of these gray levels within the myocardium will prove to be a powerful technique in the analysis of pathologic alterations in tissue structure as long as proper data acquisition and analysis techniques are used. While the measurement of integrated ultrasonic backscatter is difficult clinically, echo gray level data appear to provide similar information and may allow a type of ultrasound-based tissue analysis.

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