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Parametric Harmonic-to-Fundamental Ratio Contrast Echocardiography: A Novel Approach to Identification and Accurate Measurement of Left Ventricular Area Under Variable Levels of Ultrasound Signal Attenuation

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Abstract

Objectives— We introduced a harmonic-to-fundamental ratio (HFR) of the radiofrequency (RF) signals that reduces confounding effects of attenuation. We studied whether HFR analysis of RF signals received from contrast microbubbles allows accurate measurement of the left ventricular (LV) cavity area under varying levels of attenuation.

Background— Attenuation is a fundamental problem in ultrasound imaging and limits the use of clinical echocardiography.

Methods— RF data from short axis systolic and diastolic scans were obtained from 14 open-chest dogs following left-atrial bolus of Optison. Attenuation was induced by interposed silicone pads calibrated to induce 7 dB or 14 dB reductions of the backscattered RF signal. RF images were reconstructed from the RF signals, HFR values calculated for each image pixel for 0 dB, 7 dB and 14 dB attenuation conditions, and LV area obtained by summation of “LV cavity pixels”. A reference LV cavity area was obtained from endocardial border tracings in enhanced scans by experts.

Results— Correlation of the HFR-defined and reference areas at systole was $R = 0.95$, $R = 0.94$, and $R = 0.91$ for 0 dB, 7 dB and 14 dB levels of attenuation, respectively, and at diastole was $R = 0.95$ for 0 dB, 7 dB and 14 dB levels of attenuation. The mean difference from both systolic and diastolic values was $< 1.45 \text{ cm}^2$ (i.e. negligible) in all attenuation settings.

Conclusion— Our novel HFR method supports precise measurement of the LV cavity area in contrast images with simulated high attenuation of ultrasound signals.

Keywords

Contrast echocardiography; ultrasound attenuation; left ventricle cavity area; radiofrequency signal; harmonic signal; fundamental signal

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Contrast microbubbles enhance the detection of blood within the left ventricular (LV) cavity [1,2] and thus extend the ability to echocardiographically assess the LV in many of those clinical cases where attenuation of ultrasound signals would otherwise preclude completion of a diagnostically adequate study [3,4]. However, microbubbles also contribute to variable attenuation of ultrasound signals, particularly microbubbles within the LV cavity where their concentration is 10- to 20-fold higher than in the myocardium [5]. Methods in which the image intensity of the neighboring LV cavity region is used to compensate for attenuation of signals emanating from perfused myocardial tissue [6] may indeed reduce but do not eliminate the spatial heterogeneity in contrast image intensities.

We previously showed in an in vitro study [7] and mathematically [8] that by utilizing both fundamental and harmonic components from a broadband radiofrequency (RF) spectrum (Figure 1), which is received during contrast echocardiography scans, the undesirable effects of signal attenuation, which makes detection of bubbles less reliable, can be minimized. The adjustment for attenuation is practically accomplished by using the harmonic-to-fundamental ratio (HFR) [9], and the fundamental and harmonic spectral components of the ratio are recovered from the broadband spectrum by our custom automatic algorithm [10]. The mathematical model of HFR parameter functioning is reviewed in the Appendix and described in detail together with basic validation in [11].

We found that despite varying echo intensities, the HFR values related to the LV cavity containing contrast microbubbles were consistently different compared to HFR values related to perfused or nonperfused myocardium [12]. This finding suggested that HFR could be used for classification of LV cavity under variable ultrasound signal attenuation. In addition, we speculated that a rapid bolus injection instead of continuing infusion could be used. Continuing infusion maintains a steady state concentration of microbubbles in blood circulation, which is required during the course of variable bubble destruction and replenishment intervals that are necessary for analysis of myocardial perfusion [13]. However, a simple and rapid scanning sequence following the bolus administration of the microbubbles and the corresponding temporary opacification of the LV cavity should be sufficient for the purpose of LV cavity classification and quantitative analysis of its area in systole and diastole for a functional assessment.

Based on this background, we hypothesized that parametric HFR analysis of RF images could identify and accurately measure the LV cavity area under variable levels of attenuation and following a bolus injection of microbubbles.

To test this hypothesis, we designed an experimental study utilizing a canine model in which both the harmonic contrast images and RF images were obtained during systole and diastole. We collected the images not only at baseline but also during acute ischemia induced by occluding the left anterior descending (LAD) or left circumflex (LCX) coronary artery to extend the testing data by variations in LV shape and area.

METHODS

All methods and settings were practiced and optimized in several pilot studies.

Animal preparation

The study protocol was approved by the Mayo Foundation Institutional Animal Care and Use Committee and conformed to the *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 86-23). Measurements were obtained from 14 open-chest adult female hounds, 25 to 31 kg in weight.

Each animal was anesthetized, intubated, and mechanically ventilated with a respirator pump (Model SV900, Siemens Medical Solutions USA, Inc., Malvern, PA). ECG was continuously monitored. Arterial and venous accesses were secured by 8F sheaths for pressure monitoring with high-fidelity catheters (SPC360, Millar Instruments, Inc., Houston, TX) and for injection of fluids and drugs. Following left lateral thoracotomy, the heart was suspended in a pericardial cradle (Figure 2) and the left atrium cannulated to allow administration of contrast microbubbles.

The proximal portions of the LAD and LCX coronary arteries were dissected free from the surrounding tissue. A snare occluder and a flow probe (Transonic Flowprobe, Transonic Systems Inc., Ithaca, NY) were placed around each dissected coronary artery segment. The heart was covered by ultrasound transmission gel (Aquasonic 100, Parker Laboratories Inc, Fairfield, NJ), which was warmed to body temperature to assure appropriate acoustic coupling between the ultrasound transducer and epicardium. At this point, the experimental protocol followed, and upon completion of the protocol, the dog was euthanized with an overdose of sodium pentobarbital.

Myocardial contrast echocardiography

An ultrasonographic scanner (Vivid 5; GE Healthcare, Milwaukee, WI) with a 2.5 MHz transducer was set to the contrast harmonic imaging mode. This scanner was customized to output both conventional and RF signals. Epicardial scans were acquired in a short axis projection by interrogating the testing region during bolus injection of contrast microbubbles. ECG triggering was set so that end-diastolic and end-systolic frames were acquired every 4 cardiac cycles. During scans, the transducer was placed in a fixed holder to keep the same projections during baseline and induced ischemia.

RF images were reconstructed from the RF signals and processed off-line using a dedicated software interface (EchoMat, GE Healthcare, Milwaukee, WI) and in-house applications written in MATLAB (The Math Works, Natick, MA).

Simulation of ultrasonic signal attenuation

Signal attenuation ranges approximately from 5 to 15 dB for the thoracic wall in the clinical setting [14]. To simulate this situation in an open chest model, we used 2 custom-made silicone pads (8 cm in diameter), 3 and 5 mm thick, calibrated to expose a 1.7 MHz ultrasound signal during its round trip to attenuation of 7 dB and 14 dB, respectively.

Injection of contrast microbubbles

The content of a vial of Optison™ (GE Healthcare, Milwaukee, WI) was diluted into a 10 mL syringe containing saline solution, and a 1 mL bolus was injected via the left atrial sheath during each data acquisition sequence.

Experimental Protocol

Conventional harmonic scanning was performed first to optimize orientation of the echocardiographic projection. Appropriate aiming of the transducer was further assured by briefly occluding the testing coronary artery resulting in a visible epicardial discoloration and wall motion abnormalities in the corresponding perfusion territory.

The ultrasound system was then set to the custom RF image mode, which also produces harmonic images; such simultaneous acquisitions facilitated comparisons of conventional and HFR results. Triggered (1:4) acquisition was initiated and was immediately followed by bolus injection of Optison™. In most cases, 8 and no more than 10 triggered images (ie, 32 to 40

cardiac cycles) were required to obtain scans with even opacification. Respiration was suspended during this brief (approximately 30-second) acquisition sequence.

Calculation of HFR

The user approximately outlines the epicardial border (Figure 3A) and custom software calculates HFR for each pixel within the outlined area (Figure 3B), which contains myocardium and the LV cavity. Peak magnitudes of fundamental and harmonic spectral components are recovered by our custom curve fitting automatic algorithm on the basis of a priori known central frequency and bandwidth of the transmitted signal. The algorithm then uses an iterative least squares error approach to subsequently fit modified Gaussian curves to the fundamental and harmonic components of the received broadband power spectrum (Figure 1). The peak magnitude values are obtained from the fundamental and harmonic fit curves.

Histogram of HFR and calculation of the LV cavity area

After a few heart beats following bolus administration, the microbubbles within the myocardium are partially replenished with circulating blood or are not present at all if a myocardial region is not perfused (Figure 3A). As a result, the absolute HFR values become different, even at high levels of attenuation, from those values that represent the LV cavity in which the microbubbles remain and become evenly distributed. This separation is determined by a threshold in the histogram of HFR values (Figure 3B). In the present implementation, the threshold value is identified interactively as a minimum between the values representing the classes of 'cavity' and 'myocardium'. Then, the LV cavity area is calculated by summation of pixels belonging to the cavity (Figure 3B, arrow) in the histogram. Color coding of pixels results in an HFR parametric map of the LV myocardium and cavity (Figure 3C).

Data analysis

LV cavity area based on the HFR method was obtained for each intervention and attenuation setting by interactive determination of the lowest point in the histogram of HFR values, which delimited the values belonging to the cavity, as shown in Figure 3B.

The LV cavity area for reference purposes was obtained for each permutation by manually delineating a harmonic image frame corresponding to the RF image from which the HFR testing area was obtained. Recall that both the "conventional" contrast harmonic and "testing" RF images are obtained simultaneously. Those contrast harmonic images that were obtained with high attenuation were enhanced by adjusting contrast and brightness to allow interactive delineation of LV cavity and calculation of the reference area. We had as many as 117 frames to process by tedious manual delineation, and therefore, asked three experienced colleagues to process, in blinded fashion, a portion of the data.

The results (reference vs. HFR-based areas) were analyzed by a two-sided paired t-test; $P < .05$ was considered statistically significant. Relation of the results was assessed by linear regression and Bland-Altman analyses separately for systolic and diastolic scans. Reproducibility of the method was assessed by interobserver and intraobserver variabilities.

RESULTS

A total of 14 canines were used in the present study. We obtained scans for HFR analysis of the LV cavity at 0 dB, 7 dB, and 14 dB attenuation levels during systole while the LAD or LCX coronary artery was occluded and during systole and diastole prior to occlusion or following reperfusion and recovery of function. Not all canines tolerated all interventions and Tables 1 and 2 provide actual numbers of datasets available for analysis from each setting.

Variability in echogenicities and color coding of HFR values

Figure 4 shows the 1st and 10th (last) frames from the acquisition sequences for the testing levels of attenuation and the resulting color coding of the LV cavity. At no attenuation, the echogenicity of the cavity and some myocardial regions was excessive even at the used low (0.4) mechanical index. At 7 dB attenuation, which simulated an optimal transthoracic scan, the cavity in frame 1 was well defined. The echogenicity of the cavity with microbubbles in frame 10 was similar, by visual inspection, to the echogenicity of the myocardium in the corresponding frame with no attenuation suggesting that a simple gray scale analysis would fail in separation of the myocardium and cavity in contrast scans at variable attenuation levels. At the 14 dB attenuation level, the cavity was difficult to determine in precontrast frame 1. After contrast injection, the cavity was defined (frame 10), but the echogenicities of the cavity and myocardium were different from those at the 7 dB level and at no attenuation.

Color coding in Figure 4 documents that the off-line analysis of HFR values distribution can produce similar delineation of the LV cavity despite the large variability in attenuation of harmonic ultrasound signals.

LV cavity area analysis

Figures 5 and 6 demonstrate close correlation between the LV area based on the HFR analysis and the reference area defined by an experienced user at end-systole and end-diastole, respectively. Figures 7 and 8 show the Bland Altman plots and document good agreement between the HFR-based and reference LV areas at end-systole and end-diastole, respectively. We observed a small systematic overestimation of LV areas by the HFR method, but this bias never exceeded 1.1 cm² for testing areas ranging from 3.5 to 15 cm² at end-systole and 1.45 cm² for the areas ranging from 6.5 to 22 cm² at end-diastole.

Reproducibility

Interobserver variability of 6.5% and intraobserver variability of 4.9% documented good reproducibility of the method.

DISCUSSION

The main contribution of the present study is in demonstrating that ultrasound signal attenuation can be at least partially overcome in contrast echocardiography by a novel approach referred to as parametric harmonic-to-fundamental ratio (ie, HFR) imaging. Our results show that despite the presence of severe attenuation (14 dB) on one hand, which would in many cases render a clinical study uninterpretable, or the complete absence of attenuation on the other hand, which no longer provides “protection” to microbubbles from destruction by ultrasound energy, the HFR method is robust and generates precise and accurate LV cavity under varying levels of attenuation.

Attenuated ultrasound signals cause heterogeneity in contrast harmonic images, and therefore, suboptimal delineation of the endocardial contour for the analysis of LV cavity shape, area, and volume estimation requires experience and is highly subjective. Inclusion of scans during acute ischemia in the LAD and LCX territories not only extended the variability of cavity area and shape in our tests but also broadened the range of received signal intensities, because these ischemic territories were nearly or completely free of contrast microbubbles. Yet, low intraindividual and interindividual variabilities in the analysis of the LV cavity area by the HFR analysis documents that the method has the ability to provide reproducible results under a large variety of scanning conditions.

Contrast-enhanced harmonic echocardiography indeed improves delineation of the LV cavity, and in this way, improves measurement of the LV area and estimation of its volume even under suboptimal scanning conditions [15,16]. However, we propose a method that not only reduces the undesired effects of signal attenuation (ie, heterogeneity in image echogenicities for the same region) but also minimizes the user interaction (and thus subjectivity) to merely picking a threshold on the histogram of HFR image values (Figure 3B). We used gated (triggered) image acquisition to minimize exposure to ultrasound acoustic pressures of microbubbles that were in close vicinity to the transducer; this limited microbubble destruction. In addition, this type of acquisition supports future combination of the cavity detection method with triggered scanning for perfusion analysis.

Color kinesis, which is a method based on acoustic quantification of backscatter data along each scan line, classifies each pixel in gray scale echocardiographic images as either tissue or blood [17] and in this way provides on-line LV cavity border detection and tracking for the preceding period of systole or diastole [18]. Border tracking with color kinesis does not require administration of contrast microbubbles, but becomes less reliable when the image quality is poor due to attenuation or operator factors and, in addition, the accuracy of the method can be hampered by translational motion of the heart [19]. In our method, the LV cavity contour is detected with minimal confounding effect by variable image signal attenuation and once the appropriate point of a minimum in the HFR histogram is defined, the LV area is produced user-independently.

Clinical perspectives

Clinical analysis of LV function by echocardiography could benefit from the proposed method in the following two aspects. Firstly, the HFR approach minimizes the effect of signal loss due to attenuation by utilizing the simultaneously existing fundamental signal as a “normalization” factor for the harmonic signals, which predominantly emanate from the microbubbles in contrast echocardiography. Thus, as Figure 4 demonstrates, mapping of the LV cavity is possible even when the signal loss is 14 dB, ie, an approximately 35-fold loss of signal, compared to a no-attenuation signal magnitude. This promises a robustness of the proposed method for clinical situations in which severe signal attenuation is encountered. Secondly, the HFR method does not require any manual delineation of the LV cavity. The color overlay in Figure 4 is not an enhancement of gray-scale image values for better visual assessment. Rather, the color code represents differentiation of the image into two classes – myocardium and cavity. Once the class “cavity” is defined, calculation of the LV cavity area is straightforward as it is indeed the sum of all pixels belonging to the “cavity” class.

Taken together, the HFR method provides quantitative analysis of LV cavity area irrespective of large variations of signal loss and with no need for manual delineation.

Limitations

The presented method still requires initial user interaction in identifying the region of interest (ie, the LV) within the image and in determining the threshold in the HFR histogram. However, the former is rapid and mainly needed just to reduce processing time by preventing computationally-intense fundamental and harmonic RF spectra recovery in irrelevant regions of the scan frame. The latter interaction is typically straightforward (ie, marking the “valley” in the HFR histogram).

We only tested one mechanical index and one microbubble administration regimen (ie, bolus). This combination was, however, a result of careful pilot optimization tests. We believe that bolus injection and the need to wait for only 10 or less gated frames to approach a frame with proportional LV cavity opacification and thus data best suitable for the HFR analysis of cavity

area is a reasonable and practical approach. If required, a series of frames representing the entire cardiac cycle after the initial 10 or less gated frames pass (and the cavity proportionally opacified) could be recorded and analyzed for dynamic changes in the LV cavity area and its shape.

The experimental setting allowed administration of contrast microbubbles via the left atrium, which was advantageous because it avoided the rather unpredictable loss of bubbles within pulmonary circulation. However, routine use of contrast agents in clinical echocardiography for LV image enhancement documents that effective opacification of the cavity is indeed achievable [20].

We present results from tests with only one contrast agent, ie, Optison. However, our mathematical theory of signal attenuation compensation (see the Appendix) is independent of a specific contrast agent, and our previous work [7,9–12,21] in which we used different contrast microbubbles for HFR analysis supports this claim.

This study was conducted with only one ultrasound system (Vivid 5). Our choice was limited because the HFR analysis requires access to RF data that are not routinely available as an output. However, RF signal and its enveloping for peak value detection is available in ultrasound systems and thus technical implementation of the HFR calculation should be possible.

CONCLUSIONS

The HFR analysis of contrast RF images provides a novel solution for analysis of the LV systolic and diastolic cavity area in suboptimal ultrasound scans. Moreover, we experimentally demonstrate that HFR values have a range that is characteristic for the LV cavity and permits straightforward classification of the cavity, thus providing its parametric map (color overlay) and quantitative analysis of its area.

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ABBREVIATIONS

HFR

Harmonic-to-fundamental ratio

LAD

Left anterior descending coronary artery

LCX

Left circumflex coronary artery

LV

Left ventricle, ventricular

RF

Radiofrequency

APPENDIX

A detailed mathematical model of ultrasound signal propagation through medium containing contrast microbubbles and the mathematical theory of attenuation reduction with HFR can be found in our previous publication [11]. Briefly, consider an ultrasound signal scattered by microbubbles which has magnitudes F_0 and H_0 at the fundamental and second harmonic frequencies, respectively. One can assume that F is proportional to the incident ultrasound pressure A ($F_0 \sim A$), whereas the second order nonlinear response H is proportional to A^2 ($H_0 \sim A^2$). As the signal propagates back towards the transducer, it is attenuated. Attenuation of ultrasound follows the exponential law, $\exp[-\alpha(f) \cdot d]$, where $\alpha(f)$ is the frequency-dependent attenuation coefficient, and d is the distance traveled. In practice, the attenuation coefficient usually changes along the propagation path. In such case, we can write the total attenuation in a piece-wise manner as $\exp[-\sum \alpha(f) \cdot d_i]$, where the distances d_i are small enough so that the attenuation coefficient can be considered as a constant within each d_i .

Therefore, the received echo signal has a fundamental component of

$$F \sim A \cdot \exp \left[- \sum \alpha_f(f_0) \cdot d_i \right], \quad (\text{A1})$$

and a harmonic component of

$$H \sim A^2 \cdot \exp \left[- \sum \alpha_f(2f_0) \cdot d_i \right], \quad (\text{A2})$$

where f_0 is the center frequency of the transmitted ultrasound.

It is evident that both F and H magnitudes of the received ultrasound signal are confounded by the accumulated attenuation along the propagation path. In addition, these magnitudes are also affected by the local intensity of incident ultrasound pulse, which varies within the 2D image.

These confounding effects can be very misleading if one solely depends on pixel brightness in either a fundamental or harmonic image as indicator of the presence or absence of microbubbles. In HFR, the fundamental component is used to normalize the harmonic component in the received signal as follows:

$$HFR = \frac{H}{F} \sim \frac{A^2}{A} \cdot \frac{\exp \left[- \sum \alpha_f(2f_0) \cdot d_i \right]}{\exp \left[- \sum \alpha_f(f_0) \cdot d_i \right]}. \quad (\text{A3})$$

Under the assumption that attenuation coefficients increase linearly with the frequency, we can write: $\alpha_f(2f_0) = 2\alpha_f(f_0)$. Therefore, Equation (A3) becomes

$$HFR \sim A \cdot \exp \left[- \sum \alpha_f(f_0) \cdot d_i \right]. \quad (\text{A4})$$

We can rewrite Equation (A2) as

$$H \sim A^2 \cdot \exp^2 \left[- \sum \alpha_f(f_0) \cdot d_f \right]. \quad (\text{A5})$$

Compared to the harmonic signal in Equation (A5), which is typically utilized in contrast imaging, HFR in Equation (A4) reduces the dependency on both incident ultrasound pressure A and attenuation by a power of 1. This comparison provides a mathematical rationale why analysis of HFR should be a more reliable and robust approach in contrast echocardiography than (conventional) analysis of a harmonic spectrum alone.

Figure 1.

Harmonic-to-fundamental ratio (HFR) analysis of ultrasound spectra. The solid line represents the received broadband radiofrequency power spectrum. The dotted line represents the fundamental and harmonic components recovered by a custom curve fitting algorithm. The dashed line represents the recovered approximation of the entire broadband spectrum. HFR is calculated as the difference of the harmonic and fundamental spectra on a decibel scale using the recovered components and their peak magnitudes. Higher frequencies are attenuated proportionally more than lower frequencies, which explains why the received broadband spectrum (solid line) appears skewed to the left. The recovered components are centered at the transmitted (fundamental), ie, $f_0 = 1.7$ MHz, and harmonic, ie, $2f_0 = 3.4$ MHz frequencies.

Figure 2.

Experimental setup with the heart immersed in transmission gel in a pericardial cradle. A silicone pad with a defined attenuation coefficient is interposed between the transducer and the left ventricle (LV) to simulate attenuation of a chest wall (RV, right ventricle). The transducer holder assures that projections are kept unchanged when different attenuation pads are interposed.

Figure 3.

Left ventricular (LV) cavity analysis using harmonic to fundamental ratio (HFR). **A**, A contrast image following left anterior descending coronary artery occlusion and contrast injection scanned with 14 dB attenuation. The example shown is an 8th triggered (1:4) systolic frame after contrast administration. The LV wall is quickly manually approximated (dotted line) to define the region of interest for histogram analysis. **B**, Histogram of HFR values for each pixel within the region of interest. The histogram has a clear valley (dashed line) and the bottom point defines a threshold (solid line) separating pixels representing LV muscle and cavity. The sum of the latter provides the cavity area. **C**, Color-coding of HFR values in the histogram results in an HFR parametric map that defines LV muscle and cavity. Note the bulging anterior wall corresponding to perfusion deficit (visible as a dark myocardial region in panel A).

Figure 4.

First (frame 1) and last (frame 10) images from gated (1:4) systolic sequences of contrast scans at three testing levels of attenuation. The example is from an animal in which the left anterior descending coronary artery was occluded right before the start of each acquisition sequence and the next sequence started after reperfusion and functional recovery. Harmonic-to-fundamental ratio (HFR) overlay identifies the cavity by blue and cyan color at all levels of attenuation.

Figure 5. Correlation between the end-systolic cavity area calculated based on the harmonic-to-fundamental ratio analysis at no attenuation (**A**), 7 dB attenuation (**B**), and 14 dB attenuation (**C**), respectively, as compared to the area outlined in corresponding conventional harmonic images by an experienced user.

Figure 6.

Correlation between the end-diastolic cavity area calculated based on the harmonic-to-fundamental ratio analysis at no attenuation (**A**), 7 dB attenuation (**B**), and 14 dB attenuation (**C**), respectively, as compared to the area outlined in corresponding conventional harmonic images by an experienced user.

Figure 7.
Bland Altman analysis of the data at end-systole with no attenuation (A), 7 dB attenuation (B), and 14 dB attenuation (C), respectively.

Figure 8. Bland Altman analysis of the data at end-diastole with no attenuation (**A**), 7 dB attenuation (**B**), and 14 dB attenuation (**C**), respectively.

Table 1

The actual numbers of datasets for each testing combination at end-systole

	Systole, LAD occlusion	Systole, LCX occlusion	Systole, no occlusion	Total
0 dB attenuation	8	6	10	24
7 dB attenuation	9	6	9	24
14 dB attenuation	11	9	13	33

LAD = left anterior descending, LCX = left circumflex coronary arteries.

Table 2

The actual numbers of datasets for each testing combination at end-diastole

	Diastole, LAD occlusion	Diastole, LCX occlusion	Diastole, no occlusion	Total
0 dB attenuation	7	1	6	14
7 dB attenuation	4	1	6	11
14 dB attenuation	4	1	6	11

LAD = left anterior descending, LCX = left circumflex coronary arteries.