IS THERE A CHANGE IN MYOCARDIAL NONLINEARITY DURING THE CARDIAC CYCLE?

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Abstract—The distortion of a sound wave during propagation results in progressive transfer of the energy from fundamental to higher harmonics, and is dependent on the nonlinearity of the medium. We studied if relative changes in acoustical nonlinearity occur in healthy myocardium during the cardiac cycle. Radiofrequency data were acquired from transthoracic echocardiography (2.5 and 3.5 MHz), parasternal long axis view, from five dogs and nine healthy volunteers. Integrated backscatter was calculated after filtering for fundamental (FIB) and second harmonic frequencies (SHIB), from a region in the posterior myocardial wall. The results suggest that there is little difference between the SHIB and FIB, although there were large variations between individuals. The maximal changes in nonlinearity, as estimated by SHIB/FIB ratio, mostly occurred during systole. SHIB presented similar cyclic variation with FIB (p NS). Further studies are necessary to separate the role of myocardial nonlinearity, attenuation, propagating distance, or acoustical properties of the blood. The results are important in further tissue characterization studies employing second harmonic data. (E-mail: Pislaru.Cristina@mayo.edu) © 2001 World Federation for Ultrasound in Medicine & Biology.

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INTRODUCTION

The interaction of sound with tissue structures is a complex and still not fully understood process. It is believed that each specific tissue has its own “ultrasonic signature,” and changes in textural appearance and reflected spectra would be associated with specific pathologic features. Several experimental and clinical studies have demonstrated that alterations in myocardial structure and composition, such as ischemia (Mimbs et al. 1981; Barzilai et al. 1984; Vandenberg et al. 1991; Ayward et al. 1998; Bijnen et al. 2000), myocardial viability (Neskovic et al. 1998; Takiuchi et al. 1998), stunning (O’Brien et al. 1995), hypertrophy (Masuyama et al. 1989), acute cardiac rejection (Stempfle et al. 1993; Angermann et al. 1997), diabetes mellitus (Di Bello et al. 1995), hemochromatosis (Lattanzi et al. 1993), or apoptosis (Czarnota et al. 1997) lead to changes in acoustical properties of the myocardium.

Recent studies have demonstrated the superiority of the second harmonic imaging mode over the fundamental mode, in terms of endocardial border definition, wall motion scoring and in delineation of intramyocardial vessels during contrast echocardiographic studies (Thomas and Rubin 1998; Kornbluth et al. 1998). Increased myocardial echogenicity in harmonic imaging has also been observed in patients with recent myocardial infarction, which was not obvious in fundamental imaging. Hence, tissue characterization using second harmonic integrated backscatter might better characterize ischemia (Colonna et al. 1998; Kaul 1998). This increase in echogenicity may be related to either changes in nonlinear acoustic properties of the abnormal vs. normal tissue (Christopher 1997), or to an improved signal-to-noise ratio (SNR) (Burns et al. 1996).

The propagation of the ultrasound (US) waves used in medical instruments, through media such as water or...
soft tissues, is a nonlinear process (“finite amplitude waves,” Wells 1969). The ultrasonic waveform is progressively distorted nonlinearly as the wave propagates through the medium. In the frequency domain, this translates into a progressive addition of harmonic frequencies to the fundamental spectrum, with a nonlinear change in waveform shape in the amplitude domain. The occurrence of the waveform distortion has been demonstrated in different biologic media using focused or plane ultrasonic beams. The degree of nonlinearity of a medium depends on the nonlinearity parameter of the medium (B/A), which is defined as the ratio of the coefficients of the quadratic term to linear term in the Taylor expansion of the equation of state (Beyer 1960). B/A has been measured for various biologic media and tissues (Goss et al. 1978; Law et al. 1985; Bjorno 1986), and was proposed as a parameter for US tissue characterization. In vitro and in vivo studies have demonstrated that normal and abnormal tissues can be differentiated based on their nonlinear parameters (Sehgal et al. 1984; Starritt et al. 1985, 1986; Fatemi and Greenleaf 1996). Little is known about the in vivo waveform distortion during passage through the myocardium or how this distortion might change over the cardiac cycle.

Therefore, the aim of our study was to estimate if there is a change in the nonlinearity of the myocardium during the cardiac cycle. To achieve this, we extracted the high frame rate integrated backscatter (IB) of the healthy myocardium calculated from the fundamental (FIB) and second harmonic (SHIB) frequencies of the backscattered signal. The amplitude of the cyclic variation of the SHIB/FIB ratio was used as a measure of the relative change in myocardial nonlinearity during the cardiac cycle.

METHODS

Experimental setup

Data were collected from two sources, dogs and humans. The initial pilot study was performed in five closed-chest dogs. Mongrel dogs of 18 to 25 kg were sedated with xylazine (Rompun®, Bayer AG, Leverkusen, Germany, 50 mg IM), and anesthetized with sodium pentobarbital (Nembutal®, Sanofi, Libourne, France; 15 mg/kg IV for induction and 0.1 mg/kg/min for maintenance). Each dog was intubated and mechanically ventilated with a mixture of oxygen and air. The ventilation was adjusted to maintain the pH and arterial blood gases within physiologic range. Body temperature was kept constant with a heating pad. After dissection of one carotid and one jugular vein, the dogs received heparin (100 IU/kg bolus IV and continuous infusion 20 IU/kg/h Heparine Novo®, Novo Nordisk Bagsvaerd, Denmark) and aspirin (5 mg/kg IV bolus; Aspegic®, Synthélabo, Paris, France). ECG, heart rate, and blood pressure were monitored throughout the experiment.

The second study was performed in 9 healthy volunteers. All subjects were young, with no previous cardiac history, and a normal physical and echocardiographic examination. Informed consent was obtained from all subjects before entry into the study.

All animal experiments conformed to the “Position of American Heart Association on Research Animal Use” and were conducted with the approval of the Ethics Committee of the University of Leuven.

Radiofrequency (RF) data acquisition

A high-resolution US scanner (GE Vingmed, System V, Horten, Norway) was used to acquire continuous IQ data (the “in-phase quadrature” sampled RF data), using 3.5-MHz and 2.5-MHz broadband phased-array transducers in second harmonic mode. Two-dimensional (2-D) echocardiography was performed from a standard parasternal long-axis view, and IQ data from 2 to 4 consecutive heart cycles were acquired from a 20°to 30° sector angle, in 2.2/4.4-MHz and 1.7/3.3-MHz second harmonic mode. To avoid undersampling of the reflectivity curves, IQ data were acquired with high frame rate (>100 Hz) (D’hooge et al. 2000). The ECG signals were digitized by the scanner simultaneously with the RF signals. Data were stored on the hard disk of the System V, and downloaded to a PC for off-line analysis. Transmit power was set to maximum (equivalent to a mechanical index of 1.7), and the focus was positioned at the level of the myocardial posterior wall (at end-diastole) and kept constant for all acquisitions. To avoid respiratory motion artefacts, all acquisitions were performed in apnea (in animals by switching off the respirator for 20 to 30 s).

RF processing and integrated backscatter measurements

The raw RF signals were analyzed with the dedicated software EXTRACT (Brandt 1998). This package enables the reconstruction of the 2-D scan-converted images from the IQ data, and allows semiautomatic delineation of the endocardial and epicardial borders of the posterior myocardial wall. To avoid specular reflections from the endocardium and epicardium, the evaluated region was reduced on each side by approximately 20% of the wall thickness. In end-diastole, the region of interest (ROI) measured approximately 1.5 to 2 cm²; therefore it differed during the cardiac cycle, being smaller in diastole and larger in systole. The distance from the transducer to ROI varied slightly with cardiac cycle, as the heart moved inward and outward. A second circular ROI of approximately the same size as the one set into the myocardium was placed into the left ventric-
ular (LV) cavity, at a fixed depth, right above the LV posterior wall.

The same software calculates the IB frame-by-frame based on IQ data as the sum of the squares of the magnitude of all data points in the ROI, normalized by the number of points (Hart et al. 1987; Rijsterborgh et al. 1993). In this way, the calculated IB represented the frequency-averaged energy reflected from the selected ROI, and compensated for the variation in the size of the region over the cardiac cycle. End-diastole was selected as the frame corresponding to the peak R-wave on the ECG, and end-systole as the frame at the end of the ECG T-wave and with the smallest LV cavity size. Each data set contained 300 to 600 frames from 2 to 4 consecutive heart cycles.

To extract the energy from the fundamental and second harmonic band of the spectrum of the received signal from the ROI, we used two bandpass Gaussian-type filters, selected according to the central frequency of the transmitted pulse (2.2 MHz and 1.67 MHz). Figure 1 is a schematic representation of the received signal and the 2 filters used. One filter was chosen to have its central frequency at the fundamental transmitted frequency, filtering out the second harmonic information (bandwidth: 1.4 to 3.4 MHz for the 3.5-MHz transducer, and 0.8 to 2.4 MHz for the 2.5-MHz transducer). The second filter was chosen with the central frequency at twice the fundamental frequency (4.4 MHz and 3.3 MHz, respectively), filtering out the fundamental frequencies (bandwidth: 3.2 to 5.4 MHz for the 3.5-MHz transducer, and 2.0 to 4.2 MHz for the 2.5-MHz transducer). According to the chosen filters, the two IB traces were calculated from the two bandpass-filtered signals: fundamental IB (FIB), and second harmonic IB (SHIB), respectively.

**Data analysis**

The extracted FIB and SHIB traces were resampled using linear interpolation to give all heart cycles from each subject the same number of sample points (200). Mean traces for one cardiac cycle were then obtained for each subject by averaging the 2 to 4 resampled traces between the ECG R-R intervals. A median filter was applied to reduce noise. The cyclic variation of FIB and SHIB was computed as the difference between the end-diastolic and end-systolic values, averaged for all cardiac cycles. Mean FIB and SHIB traces for each subject were calculated for one heart cycle by averaging the re-sampled traces. Mean FIB and mean SHIB were calculated as the average value over the cardiac cycles. For a relative analysis, individual FIB and SHIB curves for one cardiac cycle, from each data set, were first normalized to the total mean energy (mean FIB + mean SHIB); thus, obtaining for each individual one normalized FIB and one normalized SHIB trace, respectively. For visual comparison, both traces were then displayed as zero mean curves by subtracting their mean values.

The SHIB/FIB ratio was calculated as the ratio in linear domain between the normalized SHIB and FIB traces, then converted to a logarithmic scale. From these ratio traces, the peak value during the end-diastole, end-systole, and minimum systolic (irrespective of the time of the occurrence: early systole, mid systole, or late systole) was measured in each data set.

Finally, for both species and both transducer frequencies, each normalized FIB, SHIB, and SHIB/FIB trace from each data set was averaged to calculate overall mean FIB, SHIB, and SHIB/FIB ratio, respectively.

The data from the region set into the LV cavity was analyzed identically. The extracted values were compared with corresponding data from LV posterior wall.

End-diastolic wall thickness, systolic wall thickening, and the distance from the transducer to posterior pericardium (at end-diastole) were measured on the M-mode images reconstructed from the IQ data.

**Statistical analysis**

Statistical analysis was performed with the SAS software (SAS/STAT User’s Guide, 1988). The comparison of the cyclic variations of FIB and SHIB, mean SHIB/FIB ratio in the posterior wall and LV cavity, and of the end-diastolic to end-systolic, and end-diastolic to minimum systolic values of the SHIB/FIB ratio were performed with paired t-tests. The influence of various factors (FIB, distance to posterior pericardium, end-diastolic wall thickness, systolic wall thickening, heart rate, and age) on SHIB was evaluated for the volunteers (from the 3.5-MHz data set) with a stepwise multiple regression analysis. A p value less than 0.05 was considered significant. The normal distribution was tested with the
Shapiro-Wilk statistic. All calculations were performed with the SAS procedures PROC TTEST, PROC REG, and PROC UNIVARIATE. Results are presented as mean ± SD.

RESULTS

All volunteers were men, with a mean age of 30.4 ± 6.7 years, and mean heart rate of 68.7 ± 7.7 bpm. The dogs had a mean heart rate of 112.2 ± 8.0 bpm. The distance to posterior pericardium was significantly larger in humans than in dogs (11.0 ± 0.7 cm vs. 7.5 ± 0.3 cm, respectively; p < 0.05).

The cyclic variations of FIB and SHIB, and SHIB/FIB ratio from the posterior wall from all acquisitions are summarized in Table 1. As expected, FIB had higher values than SHIB. No significant differences were observed between the cyclic variations of FIB and SHIB. The SHIB/FIB ratios were only slightly higher (p = NS) with the high-frequency transducer, in both dogs and humans.

The normalized FIB and SHIB traces (logarithmic scale) from two representative volunteers are shown in Fig. 2. Both SHIB and FIB curves are plotted as a zero mean curve to facilitate visual assessment of the relationship between the two. These curves were obtained by subtracting their mean values. It can be observed that FIB and SHIB were very similar in diastole, but different in systole (Fig. 2A, C). The SHIB/FIB ratio clearly changed during the cardiac cycle; the maximal change occurring in the systolic phase, with minimal changes in the diastolic phase (Fig. 2B, D). Generally, two patterns could be observed: one of a systolic decrease in SHIB/FIB ratio (8 of 9 volunteers and in 2 of 5 dogs; see volunteer No. 1 in Fig. 2B), and the second of a systolic increase in SHIB/FIB ratio (one volunteer and the remaining 3 dogs; see volunteer No. 2 in Fig. 2D).

The overall mean FIB, SHIB, and SHIB/FIB ratio of all volunteers (3.5 MHz) is shown in Fig. 3. No significant variation could be observed in the overall mean SHIB/FIB ratio during the cardiac cycle. However, when individual peak end-diastolic and minimum systolic

| Table 1. Cyclic variations of FIB and SHIB, and the SHIB/FIB ratio for all acquisitions |
|-----------------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|----------------------------------------|
|                                         | Dogs (n = 5) (dB)                        | Humans (n = 9) (dB)                     |
|                                         | 2.5 MHz                                 | 3.5 MHz                                 |
|                                         | 6.0 ± 1.7                               | 5.7 ± 1.3                               |
|                                         | 6.6 ± 3.3                               | 5.9 ± 2.4                               |
|                                         | 5.5 ± 0.6                               | 6.2 ± 2.0                               |
|                                         | 7.5 ± 2.9                               | 7.1 ± 2.3                               |
|                                          |                                          |                                          |
| SHIB/FIB ratio                          |                                        |                                          |
| mean                                    | −27.1 ± 3.1                             | −24.4 ± 4.3                             |
| end-diastolic                           | −28.3 ± 2.7                             | −25.6 ± 3.6                             |
| end-systolic                            | −30.3 ± 6.8                             | −26.1 ± 5.2                             |
| minimum systolic                       | −30.7 ± 6.8                             | −25.8 ± 4.6                             |
| Left ventricular cavity                | −36.8 ± 1.3†                            | −29.8 ± 2.2†                            |
| SHIB/FIB ratio                         | −39.1 ± 1.5‡                            | −33.1 ± 2.1‡                            |

FIB = fundamental integrated backscatter; SHIB = second harmonic integrated backscatter; * Significantly lower values in volunteers than in dogs (at corresponding frequencies); † significantly different from the end-diastolic values (at corresponding frequencies); ‡ Significantly lower values in left ventricular cavity when compared with LV posterior wall. All data are presented as mean ± SD.
were compared (irrespective of the occurrence time of the peak during systole), the end-diastolic SHIB/FIB values were statistically significant different from minimum systolic values ($p < 0.05$), but not different from end-systolic values ($p = NS$).

Examples of normalized FIB and SHIB from the LV cavity are shown in Fig. 4. Both normalized SHIB and FIB curves are plotted as a zero mean curve by subtracting their mean values. On the individual traces, the cyclic variation in SHIB/FIB ratio in LV cavity were smaller when compared with the LV posterior wall. Mean values of SHIB/FIB ratio were significantly smaller when compared with values from the LV posterior wall, in both dogs and humans, and with both frequencies used (Table 1).

At the multiple regression analysis (3.5-MHz data set in humans), SHIB was taken as a dependent variable, and FIB, distance to the pericardium at end-diastole, end-diastolic wall thickness, systolic wall thickening, heart rate, and age as independent variables. The most powerful regressors were FIB and end-diastolic wall thickness ($r^2 = 0.66$ and 0.78 after inclusion into the model, respectively; $p < 0.05$). The distance to posterior pericardium, systolic wall thickening, heart rate, and age did not have a significant effect on SHIB ($p = NS$ for all).

**DISCUSSION**

In this study, we have compared the high frame rate integrated backscatter calculated from the fundamental
and second harmonic frequencies of the RF signal from the normal myocardium. FIB and SHIB traces had a maximum value near end-diastole, a minimum value roughly near end-systole, and similar magnitude of cyclic variation (Table 1). We could not observe a statistically significant difference between the cyclic variations of FIB and SHIB, a finding in agreement with other studies in open-chest animal model (Schreckenberger 1998 et al.) or patients (Colonna et al. 1998). Overall, there was no statistically significant variation in SHIB/FIB ratio. However, the temporal behavior of FIB and SHIB was different in a majority of subjects, resulting in a cyclic variation of the individual SHIB/FIB curves.

Since, theoretically, no second harmonic frequencies are emitted in second harmonic imaging mode, and because tissue scatters linearly, all energy in the second harmonic band can be attributed to nonlinear wave propagation. This nonlinearity is known to be closely related to the amplitude of the wave and can thus assumed to be negligible during propagation back to the transducer. As a consequence, the ratio of the energy in the second harmonic band to the energy in the fundamental band measured from the reflected signal from a region within the image (i.e., the SHIB/FIB ratio) gives a relative measure of the amount of nonlinear distortion of the waveform along its path of propagation toward the region. When imaging the posterior wall in a parasternal long axis view, this path consists of the chest wall, right ventricular wall and cavity, the anteroseptal myocardium, the blood from LV cavity and, finally, the posterior myocardium.

The overall mean SHIB/FIB curve (Fig. 3) shows a large standard deviation of the ratio, which resulted in no statistical significant change in the overall ratio during the cardiac cycle. This might be attributed to the fact that this curve is the ratio of two noisy curves (i.e., the FIB and SHIB). However, a more thorough analysis showed that these large standard deviations can be explained, for example, by the summation of traces with 2 different patterns in different individuals, and by the fact that the peak SHIB/FIB during the cardiac cycle occurred at different time-points in different individuals. On the traces of most of individual subjects, the cyclic variation of the ratio could clearly be identified (Fig. 2B, D).

Thus, our study showed a variation of the SHIB/FIB ratio during the cardiac cycle, with a maximal change during the systole. This would imply a change in nonlinearity of one of the media traversed by the wave during the cardiac cycle. We assume that myocardium would generate most of this nonlinearity because it has the highest B/A ratio (Law et al. 1985). However, other factors could have influenced the observed change in SHIB/FIB ratio, such as propagating distance, myocardial thickness, attenuation, acoustic properties of the blood, or difference in beam volumes. We will discuss further the potential influence of each of these factors.

Propagating distance

According to the finite amplitude theory, the distortion of the wave is influenced by the propagating distance, type of transducer (frequency and amplitude of the wave), and medium (nonlinearity parameter of the medium B/A, attenuation). In the frequency domain, this is translated by the addition of harmonic frequencies to the original fundamental spectrum, as the sound wave travels deeper into the medium. Because the harmonics are increasing with propagating distance, less energy is expected to occur in the second harmonic band during systole due to the systolic inward motion of the posterior wall. However, there is a distance at which a maximum of harmonic content occurs, which depends on the interaction of two opposing effects: the generation of harmonics due to finite-amplitude propagation and attenuation (Wells 1969; Shung 1993). This was demonstrated in beef liver by Starritt et al. (1986), who showed the distortion of the ultrasonic waveform as a function of depth by comparing the relative amplitude of the second harmonic (f2) to the fundamental component (f1). They observed that the point of maximal distortion of the waveform occurs at 6 cm (f2/f1 = −10.5 to 15 dB) using 2.5-MHz transducer (compared with 7.5 cm in water, f2/f1 = −7 dB). Beyond that point, the level of harmonics decreased slightly due to attenuation and beam divergence. The authors found that the relative magnitude of fundamental and second harmonic components of the received pulse varies nonlinearly with distance and frequency of the transducer. Therefore, for identical transmitted pulses in media with different attenuation properties, the position of this maximum will shift: the lower the attenuation, the farther away the maximum. Since no two subjects are identical, we can presume that, in our experiments, this maximal distortion point occurred at different depths in different subjects and using different frequencies. Our finding of systolic increases or decreases of the SHIB/FIB ratio in different subjects may be influenced by the relative position of the posterior wall (and, hence, the ROI) to the maximal distortion point (see Fig. 5). One could anticipate that, if the ROI lays between the transducer and the maximal distortion point during diastole, the anterior displacement during systole (from A to B on Fig. 5) would be associated with a decrease in SHIB/FIB ratio, and the opposite behavior would occur when the region moves beyond that point (from C to D in Fig. 5). The slightly lower SHIB/FIB values obtained from the LV cavity than in the posterior wall in our study suggest that propagating distance would be an important factor in generation of the cyclic variation of SHIB/FIB ratio obtained. On the other hand,
no correlation was found between the SHIB or SHIB/FIB ratio and distance from the transducer to the posterior pericardium. Finally, if propagating distance would appear to explain the opposite behaviors encountered in the change of the ratio during systole, this factor alone would probably not account for the magnitude of the change in ratio observed in each individual subject (up to 8dB in one volunteer).

Change in attenuation

Attenuation is caused by the absorption of energy in the intervening tissues and the omnidirectional scattering (Morse and Ingard 1968). Within the current range of diagnostic US frequencies, the frequency-dependence of sound attenuation in soft tissue is approximately linear, higher frequencies being absorbed more than lower frequencies.

As the heart thickens in systole, the absorption of energy varies as more myocardial tissue (anteroseptal) is entering into the pathway of the sound. Glueck et al. (1985) reported a change in slope of the attenuation from 0.78 dB/cm/MHz to 0.58 dB/cm/MHz between contracted and relaxed muscle in a study on frog gastrocnemius muscle. Van der Steen et al. (1991, 1995) reported a decrease in attenuation from end-diastole to end-systole in healthy subjects, in parasternal long axis view, and concluded that this change was not related to myocardial thickness but, more probably, to the tension. Even if previously reported studies do not offer a solid conclusion, the change in attenuation during the cardiac cycle will, theoretically, affect the amount of second harmonics generated.

Acoustical properties of the blood

Because the ROI was located in the posterior wall in our study and the ultrasonic pulse traveled through the blood to reach it, changes in acoustic properties of the blood (such as attenuation and nonlinearity) may have influenced the SHIB/FIB ratio from the posterior wall. From in vitro studies, it is known that the B/A of blood is slightly lower than that of myocardial tissue (Law et al. 1985). Because the distance traveled by the US wave through the LV cavity is larger than across the myocardial tissue, and the attenuation in the blood is lower than in the myocardial tissue (Goss et al. 1978), more harmonics could be generated in the blood than in the anteroseptal myocardium.

Interestingly, we have also found a cyclic variation in SHIB/FIB ratio in the LV cavity (Fig. 4). This was mostly due to variations in FIB, and only minimal variations in SHIB. Variations in FIB in the blood pool cavity may be due to different mechanisms (Shung 1993). The LV cavity region was kept at the same distance from the transducer; therefore, SHIB was not affected by the variations in distance from end-diastole to end-systole. No consistent cyclic variation was observed in SHIB in the LV cavity, which may suggest a decrease in nonlinearity during systole, or to reflect the reduced noise. We believe that the broadband transducer used in this study facilitated the use of more information; therefore, we consider that our measurements do not simply reflect the noise floor level. The ratio in the LV cavity did not always vary in the same direction with the ratio in the posterior myocardium (in three animals and two volunteers). We conclude that the variation in SHIB/FIB ratio in the LV cavity will influence only in part the variation in the SHIB/FIB ratio in the posterior myocardium.

According to the literature, the decrease of B/A values with the decrease in pressure (from 3 to 1 atm) has been reported in liquids and liquid-like-media (Endoh et al. 1993). Our preliminary in vitro measurements suggest that the SHIB/FIB ratio in blood does not change within the range of clinically relevant pressure (from 20 to 300 mmHg; data not shown) and, consequently, cannot explain our finding of cyclic variation of the SHIB/FIB ratio from the posterior wall.

The difference in beam volumes

The difference in beam volumes between the fundamental and second harmonic frequencies may have resulted in different sizes of ROIs analyzed, making one component more sensitive to small changes than the other.

Taking into account all these influencing factors, it is obvious that our results cannot be interpreted in a simplistic way. Many factors could explain our observa-
tions. The rationale in favoring the changes in nonlinearity as the possible cause of our observations is that, in nonlinear acoustics, it is known that the nonlinearity of a medium depends on the applied stress on the medium (Ostrovsky 1992). Moreover, the measurement of the acoustic nonlinearity parameter has been proposed as a diagnostic method to assess the strength properties in metals (Korotkov et al. 1993), and it has been shown to increase with the presence of microcracks that accumulate under increased loading conditions (pressure). Therefore, we hypothesize that changes in myocardial acoustic nonlinearity could, presumably, reflect changes in wall stress, but this cannot be answered from our study. Our finding that maximal changes in the SHIB/FIB ratio occurred in systole concurs with this hypothesis.

Clinical implications

The SHIB/FIB ratio (as a measure of nonlinearity) could be used as a supplementary tool for ultrasonic tissue characterization. Addressing a specific part of the myocardium, the estimation of myocardial nonlinearity may better distinguish healthy from injured myocardial segments. Previous reported work has demonstrated much higher sensitivity to changes in structure (between healthy and pathologic tissues) of the nonlinear acoustic parameters when compared with linear parameters (velocity, density, attenuation) (Zhang et al. 1996). Therefore, this method may allow extraction of more information on the structural changes occurring in the myocardium during pathologic states.

Although we did not observe a significant difference in the cyclic variation of the FIB and SHIB, their maxima and minima did not always correspond. This different behavior of the two IB curves may be explained by their different mechanisms, as the FIB reflects the backscattered energy and SHIB the cumulative nonlinearity along the path. Although the cyclic variations of FIB and SHIB as an average were not statistically significant, the individual variations in SHIB and FIB were significant enough to be taken into account. Because the second harmonic mode has almost completely replaced fundamental imaging, the investigators have to be aware of this potential influence when tissue characterization studies are conducted in second harmonic mode.

Study limitations

A more precise approach to our hypothesis would have been to directly measure the B/A parameter. However, this would require analyzing two single-frequency components of the backscattered signal (a fundamental f_0 and its harmonic 2f_0), which is extremely noise sensitive as was shown by O’Donnell et al. (1979). For this reason, the authors felt that a better approach would be to define a frequency-averaged value for the fundamental and second harmonic frequency components (i.e., a fundamental and second harmonic IB value). A more reliable estimate of the nonlinearity would require the use of the transmission method. However, because transmission experiments are not clinically feasible, we performed our in vivo measurements with standard echocardiographic equipment.

A well-known limitation when working with the IB calculated from the RF data, is the large variation due to the stochastical nature of the backscatter signals. Minor change in distribution of the scatterers could give rise to up to 20% deviations in the mean IB (Bijnens et al. 1999). To reduce the noise in the curves, we have applied a median filter of 3:1 to each individual IB trace (as a compromise between the noise reduction and the temporal resolution).

It has been shown that the attenuation estimation itself depends on the nonlinearity of the medium (D’hooge et al. 1999). Because the attenuation will affect the amount of nonlinearity generated, the change in the slope of the attenuation with the angle of insonation (Verdonk et al. 1996) may influence the SHIB/FIB ratio. In our study, care was taken to acquire and analyze data from the same myocardial wall segment, as perpendicular as possible to the ultrasonic beams. However, this is not always technically feasible, due to the selection of the best echocardiographic window, and differences between individuals in the orientation of the heart and cardiac fibers.

Other factors, such as the potential overlap between the fundamental and second harmonic frequencies, the sensitivity of the transducer at different frequencies, or possible generation of a harmonic field by the transducer, should be constant during the cardiac cycle and, therefore, we reason that their effects would cancel out. However, it could influence the mean value of SHIB/FIB ratio measured from a heart.

CONCLUSIONS

This study represents the first attempt at determining whether relative changes in myocardial nonlinearity occur during the cardiac cycle, using in vivo experimental data. The analysis of the IB calculated from fundamental and second harmonic frequencies of the backscatter signal, using parasternal long-axis imaging, showed that, overall, SHIB presented similar axis imaging, showed that, overall, SHIB presented similar cyclic variation with FIB, and their ratio did not differ significantly during the cardiac cycle. However, the large variation between individuals suggests that there is a cyclic variation in SHIB/FIB ratio in some individuals, with a maximal change occurring during systole. Further studies are necessary to separate the role of myocardial nonlinearity, attenuation, propagating distance, or acous-
tical properties of the blood. These results have to be taken into account in further US tissue characterization studies employing second harmonic data.

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