Early reports

New concept in echocardiography: harmonic imaging of tissue without use of contrast agent

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Summary

Background Endocardial border detection is important for echocardiographic assessment of left-ventricular function. Second harmonic imaging of contrast agents enhances this border detection. We discovered that harmonic imaging improves tissue visualisation even before contrast injection. We therefore sought objectively to demonstrate the degree of enhancement of endocardial and myocardial visualisation.

Methods An ATL HDI-3000 scanner with software for contrast harmonic imaging was used to record short-axis images of the left ventricle in 27 patients with possible myocardial disease and 22 controls, in the fundamental mode and with harmonic imaging. A computer program measured the relative grey-scale values within six segments of the endocardium and myocardium. An Acuson Sequoia scanner equipped with software for tissue harmonic imaging was used to investigate the reproducibility of ejection-fraction calculations in 22 patients with ischaemic heart disease.

Findings Harmonic imaging produced brighter endocardium within each segment. Relative to the mean grey value of the total imaging sector, the values for harmonic and fundamental imaging were 171·5 *vs* 85·6% ($p<0·0001$) in end diastole and 194·1 *vs* 106·7% ($p<0·0001$) in end systole. Results for the myocardial segments were also significantly better for harmonic imaging. Structure enhancement of similar magnitude was seen among patients and healthy controls. Use of harmonic imaging reduced the proportion of unacceptable images by 14–46% in different views and improved the reproducibility of biplane ejection-fraction measurements.

Interpretation In comparison with fundamental imaging, the relative endocardial and myocardial brightness is enhanced by harmonic imaging.

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Introduction

Contrast agents that can pass through the pulmonary circulation have been developed so that the left ventricle can be visualised more effectively in echocardiographic studies of cardiac function.[1–3] Improvements of contrast agents have led to demand for ultrasonographic modalities adapted to the specific use of these agents.[4] The reception of the transmitted ultrasound at its strongest overtone, the second harmonic (double) frequency, substantially enhances the quality of the echocardiographic study, since harmonic imaging augments the visualisation of contrast media with microbubbles resonating in a non-linear way, thereby generating harmonic frequencies.[4–7] In June, 1996, using an early version of harmonic imaging software developed by ATL (Advanced Technology Laboratories), we discovered a subjective improvement in the visualisation of endocardial borders when applying this technique even before injection of contrast, provided that gain was increased to permit the visualisation of the weaker harmonic signals from the structure.[8] We postulated that harmonic imaging without a contrast agent would enhance detection of the endocardial border and myocardial visibility. We undertook a methodological investigation to test this hypothesis in a clinical setting. We studied patients likely to have endocardial or myocardial abnormalities and others unlikely to have such abnormalities, so that we could assess possible clinical advantages of non-contrast (or tissue) harmonic imaging.

Methods

Participants

71 individuals were assessed by two-dimensional echocardiography. For the purpose of measuring grey-scale values, we investigated two groups of patients with possible changes in endocardial-myocardial reflectivity—five patients with ischaemic heart disease and 22 with systemic sclerosis. The latter disorder can be associated with pulmonary fibrosis, but among the patients studied obstructive pulmonary disease was not prevalent; percentage of predicted forced expiratory volume in 1 s was 103·9% (range 96·2–111·6).

We also investigated 22 healthy individuals who were matched to the patients with systemic sclerosis for age and sex. Variability of measurements between investigators was tested in 15 participants, including the five patients with ischaemic heart disease, as well as six randomly chosen controls and four patients with systemic sclerosis. To investigate the variability of ejection-fraction measurements, we examined another 22 patients with ischaemic heart disease who were admitted to the coronary-care unit and were willing to participate in the study. Informed consent was obtained from all the participants, and the study was approved by the scientific ethics committee.

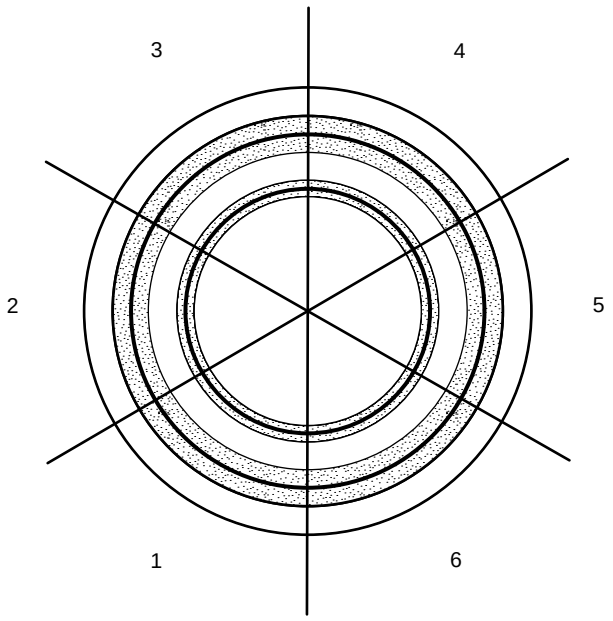

Figure 1: **Segmental subdivision of short-axis view into six segments for measurements of grey-scale values**
Endocardial (inner shaded area, 2 mm width) and myocardial (outer shaded area, 4 mm width) measurement zones indicated.

Echocardiography

One experienced investigator did all the echocardiographic examinations. No changes in output energy were made. Patients were investigated in the left lateral supine position with only a short time between the different recordings and with the patient remaining in the same position. All the participants scheduled for grey-scale evaluation were investigated in June, 1996, by means of an HDI-3000 digital ultrasound scanner (ATL, Bothell, WA, USA) equipped with a 2·5 MHz broadband transducer. It was operated in the fundamental mode, transmitting and receiving at about 2·75 MHz and in the harmonic mode transmitting at 1·67 MHz and receiving at 3·33 MHz. The latter was made possible by an early non-commercial version of harmonic imaging software intended for contrast studies. Depth variable gain was set along a straight line and overall gain was adjusted to produce an optimum image. Super-VHS recordings in the fundamental and harmonic modes were made in the midventricular, parasternal, short-axis view.

The 22 patients who were scheduled for measurement of ejection fraction were investigated by means of a Sequoia digital ultrasound scanner (Acuson, Mountain View, CA, USA) equipped with a 3·5 MHz transducer and software designed for tissue harmonics. It was operated in the fundamental and native tissue harmonic modes, the latter using a centre transmission frequency of 1·75 MHz and receiving at 3·5 MHz. Depths and overall gain settings were optimised for endocardial visualisation. An apical mattress cut-out was used for apical access. Apical four-chamber and two-chamber views in fundamental and harmonic modes were recorded on super-VHS tape, and at least one full beat was stored concomitantly on the hard disk and later on magnet-optical disks.

Measurements and statistics

End-diastolic (ECG R-wave) and end-systolic (minimum dimension) images were chosen in both the echocardiographic modes.

The images for grey-scale evaluation were digitised by a high-resolution frame grabber (VISTA) with a linear gamma correction curve and stored on CD-ROM without distortion or data reduction (PICT format). Dedicated software for grey-scale evaluation was developed and used on a Power Macintosh computer. Grey-scale (16-bit; 0–65 535) average for the image was measured as the mean of all the pixels within the entire sector area, and the program also automatically determined the core of 3×3 pixels (roughly 1 mm^2) with the lowest and highest average grey-scale value. The centre of the endocardium and the centre of the myocardium, as well as the outline of the left-ventricular cavity excluding papillary muscles and other structures, were traced manually. With the geometric centre of the endocardial and myocardial outlines as a reference point, the curves were shrunk and expanded to form bands with the original endocardial and myocardial outline in the centre. The program determined the grey values within 1 mm on each side of the endocardial tracing and within 2 mm on each side of the myocardial tracing, as means around the left-ventricular short axis as well as within six equal segments (figure 1). The grey-scale values along the centre of the endocardium and myocardium, as well as within each endocardial and myocardial segment, were also normalised to the range of grey values within the sector (percentage of maximum−minimum from the 3×3 pixel core) and to the average grey-scale value (percentage of mean value) within the sector. The minimum grey values were found to be zero in every case. Average endocardial and myocardial grey values, with the average grey value of the left-ventricular cavity subtracted as a measure of noise, were also calculated for each group of participants. All the measurements were undertaken in diastolic and systolic frames of fundamental and harmonic mode recordings. To test reproducibility, two investigators remeasured the stored images for 15 participants.

The image sequences for measurement of ejection fraction in stored digital loops were later retrieved and analysed according to the disc sum method for volume estimation by means of software in the Acuson Sequoia. The endocardial border was traced in stop frames and checked for accuracy in motion mode. The same investigator assessed, on separate occasions, loops in the fundamental mode and in the harmonic mode. Both modes were assessed twice, at least 2 days apart, with the patient's identity and previous result concealed from the interpreter.

	Grey-scale measurement parasternal short-axis			Volume measurement apical views
	Healthy controls (n=22)	Systemic sclerosis (n=22)	Ischaemic heart disease (n=5)	Ischaemic heart disease (n=22)
Demography				
Age (years)	52·9 (47·1–58·7)	54·2 (48·2–60·2)	55·2 (43·2–67·2)	63·8 (57·9–69·7)
F/M	19/3	18/4	0/5	14/8
Anthropometry				
Height (cm)	166·9 (163·6–170·2)	166·5 (163·3–169·7)	174·6 (164·2–185·0)	170·0 (165·3–174·8)
Weight (kg)	66·7 (60·7–72·6)	66·0 (62·4–69·7)	82·3 (65·1–99·4)	73·3 (67·0–79·6)
Body-mass index (kg/m^2)	23·8 (22·0–25·7)	23·8 (22·8–24·8)	26·8 (22·9–30·7)	25·3 (23·5–27·1)
Number with body-mass index >27 kg/m^2	4 (18%)	2 (9%)	3 (60%)	7 (32%)
Blood pressure (mm Hg)				
Systolic	133 (125–141)	143 (133–154)	131 (119–143)	132 (124–140)
Diastolic	78 (75–81)	80 (74–86)	82 (75–89)	71 (68–75)

Baseline characteristics of participants (mean and 95% CI or number of participants)

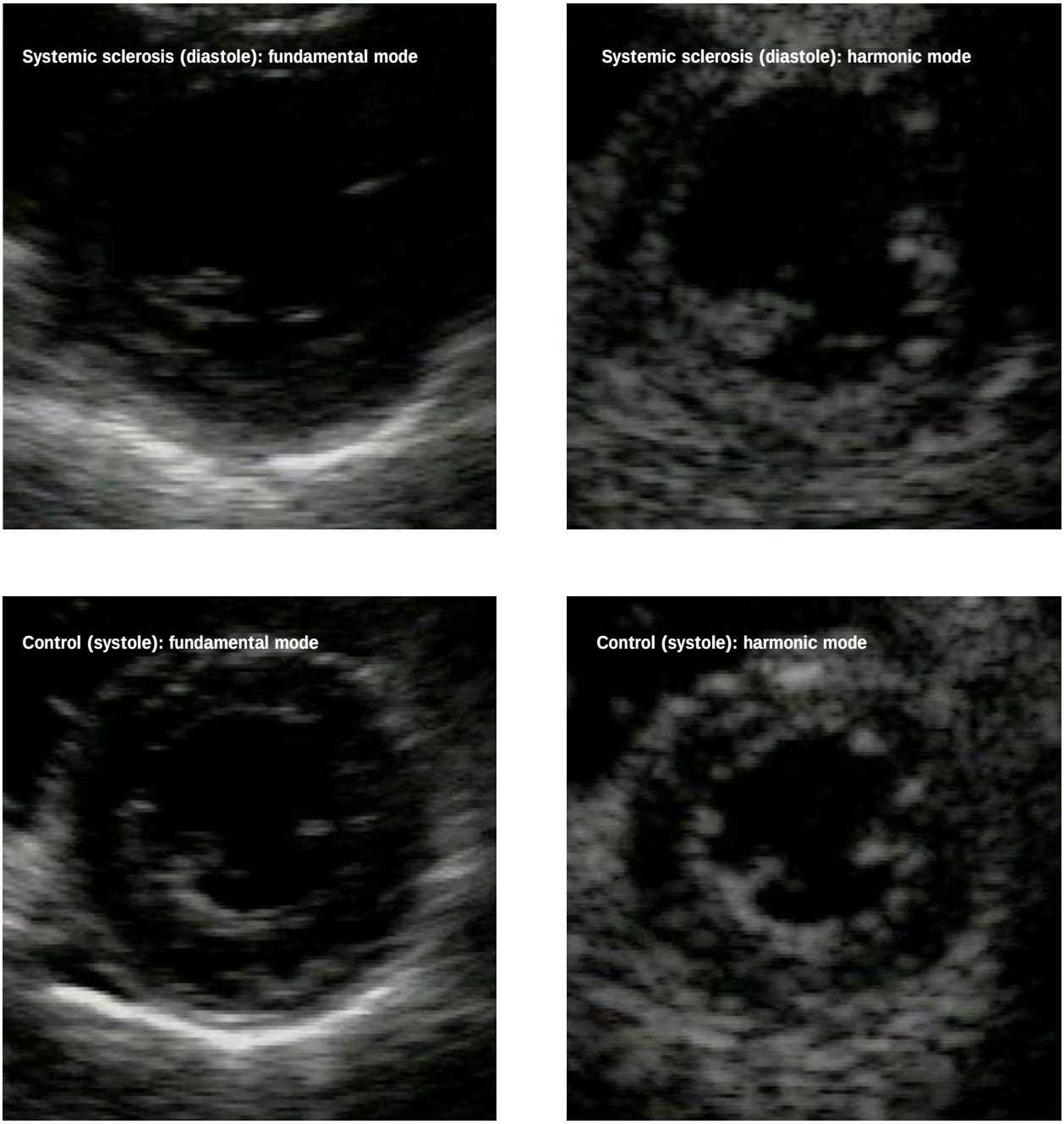

Figure 2: **Short-axis images of left ventricle in fundamental mode (left) and harmonic mode (right)**
Lower panel shows systolic images from a control subject and upper panel diastolic images from a patient with systemic sclerosis.

We recorded the subjective visualisation of the endocardial border in all participants according to the following scale: 1=not visualised; 2=barely visible; 3 and 4=weak; 5 and 6=ordinary; 7 and 8=good. For gradings with two numbers, the lower number represented segments with less than 50% and the higher number segments with 50% or more of segmental length having the respective quality. The highest score applicable for a segment was selected. Image quality review of apical views (four equal-sized segments each) obtained by the Acuson Sequoia was made by an investigator who was unaware of the echocardiographic mode.

For illustrative purposes, we calculated the proportion of acceptable views by fundamental and harmonic imaging with unacceptable images defined as those with more than one segment having an endocardial quality score of 2 or less.

Endocardial grey-scale measurements in the fundamental and harmonic modes, as well as repeated measurements of ejection fraction are given in terms of differences versus average, as suggested by Bland and Altman.[9] The results are presented as mean values with 95% CIs. The Wilcoxon matched-pairs signed-rank test was used to test the significance (with $p<0{\cdot}05$ taken as significant) of differences.

To test the within-mode homogeneity of the endocardial border in the two echocardiographic modes, we calculated the coefficient of variation as the SD of the grey values for the six segments divided by the mean endocardial grey value. ANOVA was used to investigate the difference in endocardial grey-scale values and visual quality, respectively, between fundamental and harmonic modes. The analysis used SAS version 6·12. The model allowed for variation due to patient, segment, phase, and mode.

To test for between-mode homogeneity over segments, the interaction between segment and mode was included. However, if the interaction was found to be non-significant

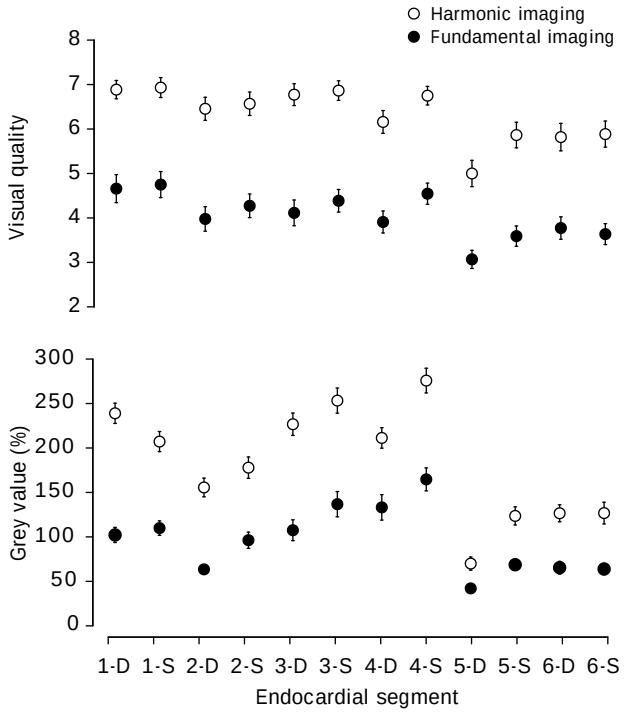

Figure 3: **Relative (endocardial / total sector) grey values in short-axis segments (lower panel) and endocardial visualisation (upper panel)**
Values are mean and SE in 49 participants. D=diastole; S=systole.

(p>0·05), it was removed from the final model. The results are expressed as least-square means, and to estimate the magnitude of the differences between fundamental and harmonic modes, the differences in least-square means are presented together with 95% CIs. The significance of differences and interactions were tested by use of the *F* test. All tests were two-tailed.

Results

The baseline characteristics of the study groups are shown in the table.

Two examples of fundamental and harmonic images are shown (figure 2), one from a control and one from a patient with systemic sclerosis. The improved visualisation of structure with harmonic imaging is clear. With the applied prerelease software for harmonic imaging, the visual impression was that the grey level distribution was more flat—ie, highly reflective tissue such as the pericardium is less intensely white than in the fundamental mode. This phenomenon was obvious despite optimisation of the overall gain level in the respective mode. More recent harmonic mode software releases developed specifically for tissue imaging, as applied in the second part of our study, have reduced the difference in appearance compared with fundamental imaging. Having marked the sector borders manually, our interpretation program automatically scanned the image and calculated the mean and maximum grey values. The maximum and mean grey values of the diastolic and systolic images were significantly higher with the fundamental mode than with harmonic imaging (details available from *The Lancet*'s London office, or the journal's website—http://www.thelancet.com).

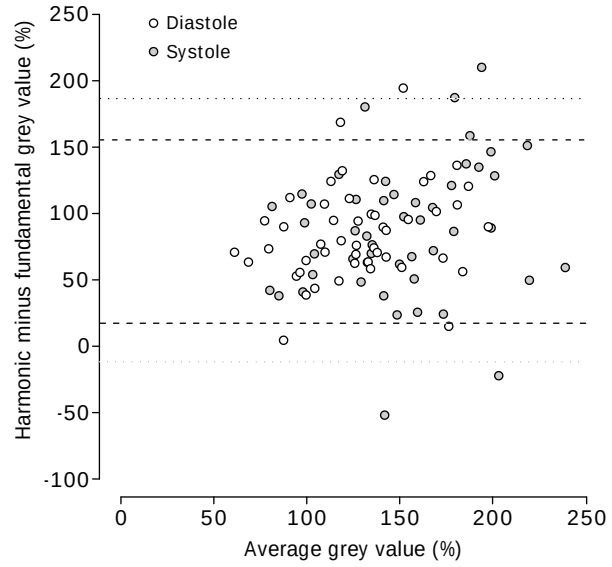

Figure 4: **Difference *vs* average of harmonic and fundamental mode relative (endocardial / total sector) grey values in short-axis view**
95% limits of agreement in diastole (broken lines) and systole (dotted lines) are shown. Values above zero indicate improvement by harmonic mode.

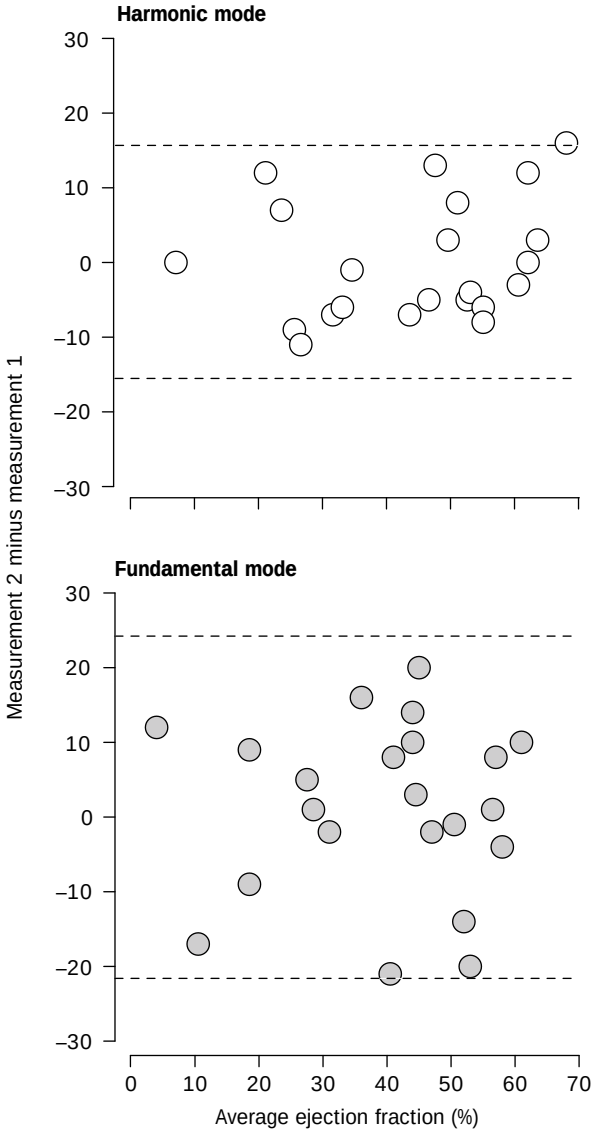

Figure 5: **Difference *vs* average of repeated biplane ejection-fraction measurements**
Broken lines =95% limits of agreement.

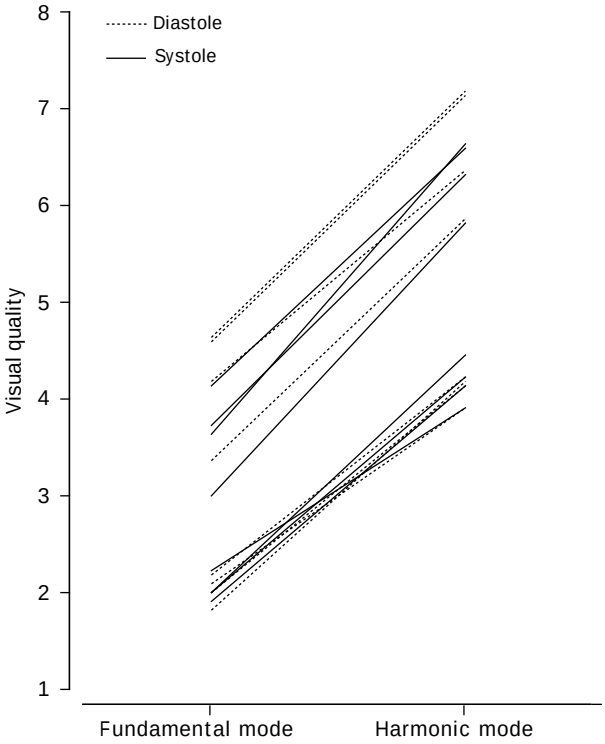

Figure 6: **Endocardial visualisation graded in apical views**
Each line represents an average masked grading (n=22) for one of eight segments (four per view).

Tracings of endocardial and myocardial centres were marked manually and the grey values along these lines were automatically calculated by the interpretation program. Figure 3 illustrates these values with the six left-ventricular endocardial segments as grey values relative to the average grey value in the image. The harmonic mode was significantly better than the fundamental mode in terms of computer-measured endocardial brightness ($p<0·001$) and the visually determined quality of endocardial visualisation ($p<0·001$). The average improvement in endocardial brightness was 87% (79–95) and that in image quality 2·19 (2·04–2·34) grading scores. In the analysis of grey scale, the interaction between segment and the mode was significant. However, analysis of each segment showed a significant difference between harmonic and fundamental modes in all six segments, all in favour of harmonic mode and of magnitude 41% to 117%. There was no significant interaction between the cardiac phase and the mode; the enhancement of endocardial brightness remained throughout the cardiac cycle. Individual endocardial grey values in diastole and systole are shown in figure 4. Only two systolic values were below zero and remaining measurements (98%) indicated enhanced brightness by harmonic imaging. The average endocardial grey values relative to sector mean grey values in diastole were 85·6% (76·2–95·0) and 171·5% (159·9–183·1) for fundamental and harmonic modes ($p<0·0001$), and in systole corresponding values were 106·7% (94·9–118·5) and 194·1% (180·0–208·2) ($p<0·0001$). When endocardial grey values were related to sector maximum grey values, similar differences between fundamental and harmonic modes were found. The increase in relative grey values noted in the harmonic mode was of a similar magnitude for myocardium and for endocardium. Analysis with the left-ventricular cavity subtracted as background noise gave similar results (data not shown).

Variability in endocardial grey values was significantly lower with harmonic than with fundamental imaging. The ratios for the SDs of the endocardial segments and the mean endocardial grey value were significantly lower with harmonic imaging than with fundamental imaging in diastole (0·54 [0·49–0·59] *vs* 0·73 [0·66–0·81], $p<0·0001$) and in systole (0·51 [0·45–0·57] *vs* 0·62 [0·56–0·67], $p=0·0128$). Furthermore, harmonic imaging produced significantly less basis for visibility drop-out. The endocardial minimum segmental grey

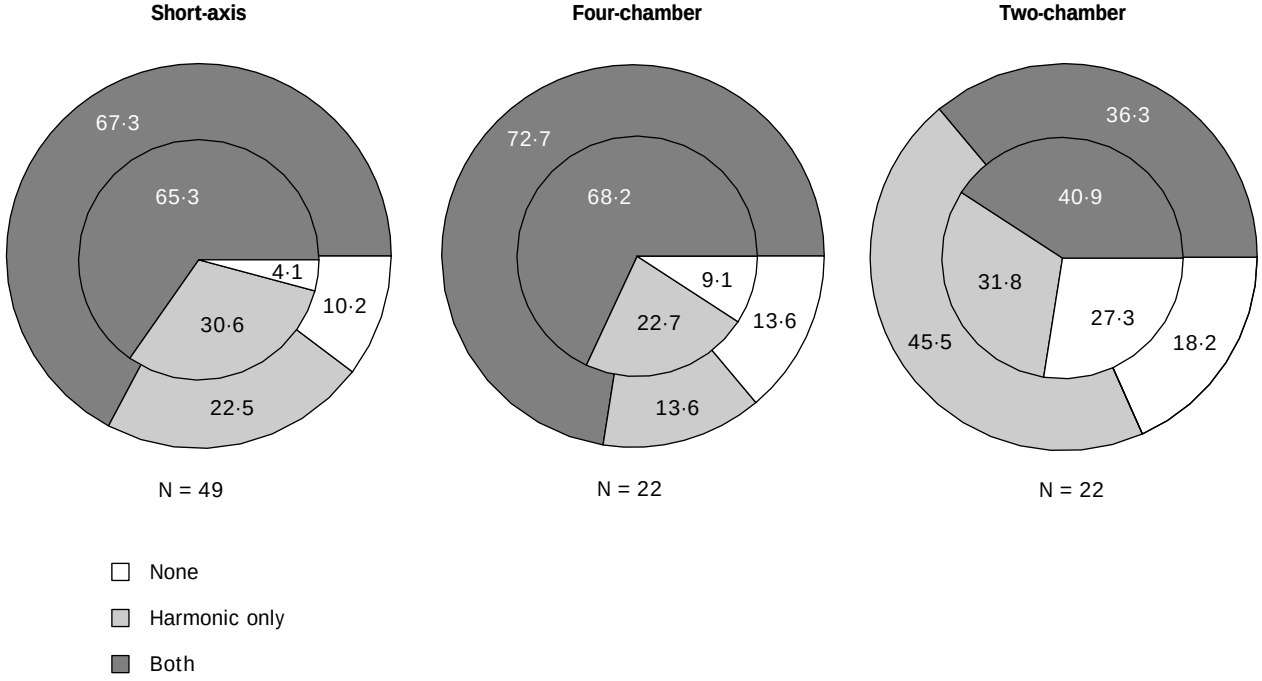

Figure 7: **Proportions of acceptable views in fundamental and harmonic modes (both), harmonic mode only, or none**
Acceptability based on a maximum of one segment with quality grade 2 or lower. Outer circle denotes diastole and inner circle systole.

values (relative to image mean) were 57% (46–69) for harmonic imaging and 23% (17–28) for fundamental imaging in diastole ($p<0·0001$) and 83% (68–98) versus 33% (27–39) in systole ($p<0·0001$). The coefficients of variation between two observers were 5·8/5·0% with fundamental imaging and 3·6/3·9% with harmonic imaging for endocardial diastolic/systolic measurements, and the corresponding values for myocardial measurements were 13·7/8·7% and 7·1/6·8%.

Measurement of ejection fraction was done in 22 patients with ischaemic heart disease, irrespective of image quality, and the results from repeated measurements are shown in figure 5. The variation was less pronounced when measurements were repeated with use of digital loops obtained in the harmonic mode. An obvious reason for this improvement was a clearly better visualisation of the endocardial border (figure 6). The improvement by harmonic imaging was uniform in various segments, as shown by the parallel lines in figure 6. Figure 7 shows the proportions of patients who had interpretable echocardiograms when fundamental or harmonic mode was applied. Harmonic mode increased the number of acceptable echocardiograms by 14% to 46%. The most pronounced benefit was obtained in the two-chamber view.

Discussion

We present objective proof of the enhancement of structures with harmonic imaging in comparison with conventional fundamental imaging. We also found that use of harmonic imaging reduces variations in ultrasound intensity along endocardial and myocardial surfaces, as well as the discrepancy between results when different observers outline these structures.

Echocardiography requires training to carry out investigations as well as interpretations. Since harmonic imaging improves image quality, it may allow acquisition and interpretation of images by less trained personnel. It increases the proportion of interpretable investigations. It is non-invasive and no expertise is needed to convert conventional or fundamental echocardiographic imaging into harmonic imaging when an ultrasonographic scanner is equipped with this capability. With use of harmonic imaging that is adjusted for image generation without contrast, the image appearance is similar to that in fundamental imaging, apart from the beneficial enhancement of structures. New techniques tend to be expensive. However, tissue harmonic imaging is now available from several companies producing echocardiographic equipment, and the extra cost of the technique is low when purchase or exchange of equipment is considered.

We investigated a small number of patients and therefore cannot exclude the possibility that there will be a greater or lesser effect on image quality among patients with other diagnoses. Owing to the appearance of images obtained by the early version of harmonic software in the ATL scanner, we cannot exclude some interpretation bias in the quality grading of the short-axis views. However, the similarity in segmental variation between the subjective quality analysis and the objective grey-scale assessment (figure 3) indicates that any possible bias did not impair results. The masked quality grading of apical images obtained by a later version of harmonic imaging software that allows unbiased interpretation verified improvement in all segments.

In a previous study of another cohort[10] examined solely with fundamental imaging, we found worse parasternal short-axis image quality with 40% visualisation in systemic sclerosis patients and 60% visualisation in controls compared with 82% versus 96% in apical four-chamber views. For this reason we measured grey scale in the parasternal short-axis view in this study. The lateral part of the left ventricle is generally more difficult to visualise owing to the parallel direction of the wall and the ultrasound beam. Consequently, in this investigation, we saw lower grey values for the lateral segments (numbers 5 and 6), but even in these segments harmonic imaging produced significantly higher grey values than did fundamental imaging. We found consistently higher maximum and mean grey values for the diastolic and systolic images in the fundamental mode than with harmonic imaging. However, for the mean grey values, this is a matter of gain setting and reflects the adjustments deemed necessary by the investigator for the optimum visualisation of cardiac structures such as the endocardium.

In this study, patients with possible changes in endocardial/myocardial ultrasonographic properties were chosen in addition to healthy controls, since the usefulness of harmonic imaging might differ. Our group has previously shown cold-induced myocardial perfusion defects in patients with systemic sclerosis,[11] and such patients have a high prevalence of fibrosis in the heart, as do those with ischaemic heart disease. However, the relative mean endocardial grey values were roughly doubled by means of harmonic imaging in patients as well as in controls.

It was therefore not surprising that the reproducibility of ejection-fraction measurements, as well as the visual quality of all apical segments, was improved among unselected patients with ischaemic heart disease. Only around two-thirds of these patients are normally regarded as suitable for echocardiographic ejection-fraction measurements,[12] which explains the scatter shown in figure 5. Tissue harmonic imaging should facilitate echocardiographic analysis of cardiac function including stress echocardiography and automatic border detection, extend interpretability to patients with more difficult characteristics, and reduce the well-known investigator dependence.

Harmonic imaging is likely to make transpulmonary contrast agents clinically useful for delineation of the left-ventricular cavity,[5] as well as for the display of myocardial perfusion and coronary flow.[6,13–15] However, the ultrasound energy destroys microbubbles and the use of contrast is more effective in intermittent or transient-response imaging.[6,16]

New refinements to enhance further the display of harmonic contrast signals include cancelling out the fundamental signal by addition of inverted fundamental pulses. This technique might also prove useful for tissue harmonic imaging. When contrast bubbles are subjected to ultrasound waves, they resonate and generate so-called harmonic frequencies of the transmitted frequency.[7] However, tissue does not resonate like contrast agents, so how are tissue harmonics generated? Transmission of harmonic frequencies is not an explanation since the image improvement observed by

harmonic imaging in poor acoustic windows is not obtained by high-frequency transducers.[17] Moreover, experiments using the transducer and equipment that we used in this study have shown that the second harmonic signals received from tissue are due to non-linear properties of tissue causing distortion of the transmitted signal and are not primarily caused by the transmission of harmonic frequency.[18] Efforts are made to avoid overlap between the transmitted and received frequency ranges in harmonic imaging.[19] Gaussian-shaped signal bursts should be helpful in this respect since they contain less or no energy in the harmonic frequency range. There are several reasons why tissue harmonic imaging increases the signal-to-noise ratio and facilitates interpretation. So-called side lobes cause artefacts in fundamental imaging. Harmonic beams are narrower and generally have lower side-lobe levels than the fundamental ones, thereby making them ideal for imaging purposes.[17,18] Since the harmonic frequencies are generated on sound propagation within tissue, reflections from the chest wall are avoided. Imaging is further improved by the fact that the harmonic signal strength is weak near the transducer, thereby limiting reverberation and scattering artefacts from superficial structures. Furthermore, the combination of transmitting a lower frequency with an inherently higher penetration and receiving a higher frequency with an inherently higher resolution is an attractive concept.

Harmonic imaging seems to constitute an important step in the development of ultrasound imaging and may increase its availability.

Contributors

Kenneth Caidahl conceived the idea, participated in planning and coordination of the study, recruited the patients with ischaemic heart disease, carried out the investigation, analysed the data, and wrote and revised the paper. Anders Waldenström and Elsadig Kazzam proposed the cardiological study of systemic sclerosis on the basis of extensive previous scientific experience and participated in the revision of the paper; Elsadig Kazzam made quality gradings. Solbritt Rantapää Dahlqvist and Grethe Neumann Andersen diagnosed and enrolled the scleroderma patients, took clinical care of them, and planned the rheumatological part of the study. The controls were randomly sampled by Solbritt Rantapää Dahlqvist. Jonas Lidberg and Joakim Nordanstig made the grey-scale measurements and wrote a draft paper. Jonas Lidberg analysed the data and participated in the investigation of coronary patients, and the revision of the paper. Ronny Wikh constructed the measurement program, contributed extensive technical and intellectual support throughout the analysis. All investigators critically reviewed the paper.

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References

1 Geny B, Mettauer B, Muan B et al. Safety and efficacy of a new transpulmonary echo contrast agent in echocardiographic studies in patients. *J Am Coll Cardiol* 1993; **22:** 1193–98.

2 Porter TR, Xie F, Kricsfeld A, Chiou A, Dabestani A. Improved endocardial border resolution during dobutamine stress echocardiography with intravenous sonicated dextrose albumin. *J Am Coll Cardiol* 1994; **23:** 1440–43.

3 Zotz R, Genth S, Waaler A, Erbel R, Meyer J. Left ventricular volume determination using Albunex. *J Am Soc Echo* 1996; **9:** 1–8.

4 Forsberg F, Goldberg BB, Liu JB, Merton DA, Rawool NM. On the feasibility of real-time, in vivo harmonic imaging with proteinaceous microspheres. *J Ultrasound Med* 1996; **15:** 853–60.

5 Porter TR, Li S, Kricsfeld D, Armbruster RW. Detection of myocardial perfusion in multiple echocardiographic windows with one intravenous injection of microbubbles using transient response second harmonic imaging. *J Am Coll Cardiol* 1997; **29:** 791–99.

6 Lindner JR, Dent JM, Moos SP, Jayaweera AR, Kaul S. Enhancement of left ventricular cavity opacification by harmonic imaging after venous injection of Albunex. *Am J Cardiol* 1997; **79:** 1657–62.

7 Schwarz KQ, Chen X, Steinmetz S, Phillips D. Harmonic imaging with Levovist. *J Am Soc Echo* 1997; **10:** 1–10.

8 Caidahl K, Kazzam E, Lidberg J, et al. Harmonic imaging improves the echocardiographic signal to noise ratio without use of a contrast agent [abstract] *Eur Heart J* 1997; **19** (suppl): 146 (abstr).

9 Bland J, Altman D. Comparing methods of measurement: why plotting difference against standard method is misleading. *Lancet* 1995; **346:** 1085–87.

10 Kazzam E, Caidahl K, Gustafsson T, et al. Two-dimensional echocardiographic measurements in systemic sclerosis and a matched reference population. *Am J Noninvas Cardiol* 1991; **5:** 343–52.

11 Gustafsson R, Mannting F, Kazzam E, Waldenström A, Hällgren R. Cold induced reversible myocardial ischaemia in systemic sclerosis. *Lancet* 1989; ii: 475–79.

12 Bonarjee VV, Carstensen S, Caidahl K, Nilsen DW, Edner M, Berning J. Attenuation of left ventricular dilatation after acute myocardial infarction by early initiation of enalapril therapy. CONSENSUS II Multi-Echo Study Group. *Am J Cardiol* 1993; **72:** 1004–09.

13 Beppu S, Matsuda H, Shishido T, Matsumura M, Miyatake K. Prolonged myocardial contrast echocardiography via peripheral venous administration of QW3600 injection (EchoGen®): its efficacy and side effects. *J Am Soc Echo* 1997; **10:** 11–24.

14 Iliceto S, Marangelli V, Marchese A, Amico A, Galiuto L, Rizzon P. Myocardial contrast echocardiography in acute myocardial infarction: pathophysiological background and clinical applications. *Eur Heart J* 1996; **17:** 344–53.

15 Mulvagh SL, Foley DA, Aeschbacher BC, Klarich KK, Seward JB. Second harmonic imaging of an intravenously administered echocardiographic contrast agent: visualization of coronary arteries and measurement of coronary blood flow. *J Am Coll Cardiol* 1996; **27:** 1519–25.

16 Colon PJ, Richards DR, Moreno CA, Murgo JP, Cheirif J. Benefits of reducing the cardiac cycle-triggering frequency of ultrasound imaging to increase myocardial opacification with FSO69 during fundamental and second harmonic imaging. *J Am Soc Echo* 1997; **10:** 602–07.

17 Ward B, Baker AC, Humphrey VF. Nonlinear propagation applied to the improvement of resolution in diagnostic medical ultrasound. *J Acoust Soc Am* 1997; **101:** 143–54.

18 Averkiou M, Roundhill D, Powers J. A new imaging technique based on the nonlinear properties of tissues. In: Schneider S, Levy M, McAvoy B, eds. IEEE Ultrasonics Symposium, Toronto, Ontario, Canada: Institute of Electrical and Electronics Engineers, 1997: 1561–66.

19 Krishnan S, O'Donnell M. Transmit aperture processing for nonlinear contrast agent imaging. *Ultrasonic Imaging* 1996; **18:** 77–105.