Effects of Acute Myocardial Ischemia on Intramyocardial Contraction Heterogeneity
A Study Performed with Ultrasound Integrated Backscatter During Transesophageal Atrial Pacing

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Background—Subendocardial thickening is greater than subepicardial thickening and acute myocardial ischemia mainly impairs the former. Integrated backscatter cyclic variations (IBScv) reflect regional myocardial contractility and are blunted during myocardial ischemia. We hypothesized that stress-induced myocardial ischemia mainly affects subendocardial IBScv.

Methods and Results—Multiplane transesophageal echocardiography and simultaneous atrial pacing were performed in 12 patients without coronary artery disease (CAD) and in 25 with significant CAD. In a transgastric 2-chamber view, we calculated IBScv in subendocardium and subepicardium and a heterogeneity index, both at rest and at peak-pacing. In 27 myocardial segments of patients with normal coronary arteries, and in 16 myocardial segments supplied by coronary artery without significant stenosis in patients with CAD, there was a transmural gradient of IBScv at rest and the heterogeneity index did not change during all the protocol steps. In the 53 myocardial segments related to a significantly narrowed coronary artery, the transmural gradient of IBScv, present at rest, significantly decreased at peak-pacing because of subendocardial blunting, but promptly recovered 5 seconds after pacing interruption. Moreover, the myocardial thickening at rest and peak pacing correlated with the subendocardial IBScv behavior and not with the subepicardial one.

Conclusions—IBScv are greater in the subendocardium than in the subepicardium. Atrial pacing stress test does not affect IBScv in segments supplied by nonstenotic coronary arteries, whereas it affects segments supplied by diseased coronary arteries, blunting exclusively subendocardial IBScv. Heterogeneity of IBScv intramyocardial changes caused by stress-induced ischemia must be taken into account when using IBScv for investigating myocardial ischemia.

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Key Words: coronary disease ■ echocardiography ■ pacing ■ ischemia ■ myocardial contraction

In normal conditions, myocardium has a transmural heterogeneity of contraction: contractility decreases from subendocardium to subepicardium.1–3 During a reduction of myocardial perfusion capable of inducing myocardial ischemia, the subendocardial layer undergoes contractility impairment even in the absence of functional impairment of the subepicardial layer.4 Available data on myocardial contractility heterogeneity during ischemia have been exclusively obtained in animal studies, because techniques to evaluate the transmurality of ischemia are quite complicated and are often invasive.

Experimental studies have shown that integrated backscatter cyclic variations (IBScv), an innovative approach to functional evaluation of myocardium obtainable during cardiac exploration by ultrasound,5 are related to the overall transmural contractility and myocardial thickening.6 Moreover, IBScv have the potential of investigating transmural heterogeneity of systo-diastolic variation of myocardial echo amplitude,7,8 an expression of the contractile performance across the myocardium.

We hypothesized that (1) a transmural gradient of myocardial contractility favoring the subendocardium, as investigated by IBScv, exists in human beings and that (2) during stress-induced acute myocardial ischemia, the transmural myocardial contractility gradient, explored by IBScv, is blunted. To investigate this hypothesis, we evaluated the behavior of cardiac cycle-dependent IBScv in patients with and without coronary artery disease (CAD) in the different transmural layers, at rest and during transesophageal atrial pacing stress test.
Methods

Study Patients
Forty-eight consecutive patients scheduled for coronary angiography for suspected stable angina pectoris, and undergoing pacing stress test during transesophageal echocardiography because of suboptimal transthoracic image quality, were considered eligible for the present study. All patients had normal left ventricular dimension, wall thickness, and wall motion at baseline echocardiogram. Patients were not eligible if they had a previous myocardial infarction, a previous coronary artery bypass graft or other cardiac surgery, left bundle branch block, or valvular heart disease. Eleven patients were excluded: 3 because of intolerance to atrial pacing stimulation, 3 because of an inadequate atrial capture or because of high degree atrioventricular block, and 5 because of inadequate quality of transesophageal echocardiographic images at rest and during atrial pacing (less than half of the total segments valuable). Thus, the final study population consisted of 37 subjects (27 male and 10 female with a mean age of 53.2±9.5 years). All patients gave written informed consent and the Institutional Ethical Committee approved the study protocol.

Transesophageal Echocardiography
In all patients, transesophageal echocardiography during simultaneous atrial pacing was performed at least 48 hours before coronary angiography.

Commercially available echocardiographic equipment (Sonos 2500 and Sonos 5500, Hewlett Packard Inc) with a 5-MHz omniplane transesophageal probe was used in this study. After complete imaging of the heart and great vessels from the different transesophageal views, the omniplane probe was advanced into the stomach to obtain a clear and stable 2-chamber view of the left ventricle. This view allows excellent visualization of the anterior and inferior wall, perpendicularly to the direction of the interrogating ultrasonic beam, and thus it is ideal for myocardial thickening and IBS data measurements. The zoom of the anterior and posterior wall was used to optimize the imaging of the wall in order to obtain a mural thickness adequate to calculate the cyclic behavior in different transmural layers.

Transesophageal Atrial Pacing Stress Test
Cardioactive drugs were withheld for at least 72 hours before transesophageal echocardiography. Atrial pacing was performed by the transesophageal approach using a transesophageal atrial stimulator and a special sheet with 8 electrodes, designed in such a way as to be easily attached to a transesophageal probe. The first electrode was located at the tip of the probe and the remaining 7 at an equal distance of 5 mm.

Atrial pacing was performed according to a previously described protocol. Briefly, pacing was started at 110 beats/min and increased every 3 minutes by 10 beats/min until chest pain occurred or until a heart rate of 150 beats/min was achieved. A 12-lead ECG at the pacing interruption was considered positive for ischemia if the ST heart rate of 150 beats/min was achieved. A 12-lead ECG at the point was then calculated for at least 3 consecutive cardiac cycles; the dia
d borders was obtainable in each moment of the atrial pacing segments in which clear delineation of both endocardial and epicardial borders was possible. A preprocessing value of 1 and a postprocessing gray scale curve setting of A (the most linear one) were kept at these same identical levels for all patients. Because of the densitometric image dependence on the gain setting, we set the time gain compensation controls at a constant value of 140 (similar in both the anterior and the inferior wall); the transmit gain setting was adjusted to eliminate extraneous echoes from the ventricular cavity, and these parameters were kept constant throughout the entire procedure.11

Acoustic densitometric image loops were acquired in digital format at baseline, during the last minute of stimulation at 150 beats/min (or immediately before atrial pacing interruption), and 5 seconds after atrial pacing interruption. Three sets of data were then saved on optical disks. Offline analysis of IBScv was performed without knowledge of coronary angiography results in all myocardial segments properly visualized on the long axis transgastric view.

Within each specific segment, 2 distinct regions of interest were separately analyzed, representing the subepicardium and subendo
cardium. An elliptic region of interest was placed in the subendo
cardial and subepicardial half of left ventricular wall thickness, taking care to exclude the endocardial and epicardial specular reflections. Acoustic densitometric data were analyzed for subendo
cardial and subepicardial layers in all the myocardial segments suitable for evaluation in the 3 different moments of the atrial pacing protocol (baseline, at peak-pacing, and 5 seconds after pacing interruption). During the IBS measurement, in order to increase the signal-to-noise ratio, the region of interest was set at 31 x 31 pixels. The selected region of interest was repositioned on each frame of the cardiac cycle in order to ensure the analysis of the same myocardial segment of the selected transmural myocardial layer. A time
tensity graph expressed in decibels simultaneously showed the videodensitometric value averaged within each region of interest frame by frame. The magnitude of the IBScv was calculated as the average in 3 consecutive cardiac cycles of the difference between the end-diastolic IBS value and the value at end-systole.

After having obtained a mean value of IBScv in both the subendocardium and subepicardium of each segment, a heterogeneity index was calculated as (subendocardial)–(subepicardial IBScv)/ (subendocardial IBScv) x 100.

Coronary Angiography and Myocardial Segments Classification
Two or more angiographic projections were obtained for each coronary artery. Significant CAD was defined as a luminal diameter narrowing of at least 50% in ≥1 major coronary vessels by 2 observers unaware of the stress echo results.

According to coronary angiography results and to a previously described 3-region scheme of coronary perfusion,12 3 groups of myocardial segments from the transgastric 2-chamber view were identified: no-CAD, not at jeopardy, and at jeopardy. A myocardial segment was defined no-CAD if it was supplied by normal coronary arteries in subjects without CAD. A myocardial segment of a patient with CAD was defined not at jeopardy when it was supplied by a coronary artery without significant stenosis (anterior segment with the left anterior descending coronary artery not significantly narrowed or inferior segment with both right and circumflex coronary arteries not significantly narrowed). Finally, a myocardial segment was defined at jeopardy when it was supplied by a significantly narrowed coronary artery (anterior segment with the left anterior descending coronary artery significantly narrowed or inferior segment with both right and circumflex coronary arteries significantly narrowed).

Data Reproducibility
To test reproducibility we evaluated IBScv in 30 randomly selected myocardial segments in the subendocardial and subepicardial layers. These segments were measured by 2 trained observers in order to calculate intraobserver variability and were measured again 15 days
later by 1 of the 2 for the interobserver one. To assess longitudinal reproducibility the same investigator observed the segments from 2 consecutive sets of baseline images acquired 10 minutes apart in 5 patients without CAD.

Statistical Analysis
All data are expressed as mean±SD. After ANOVA for repeated measures, multiple comparisons among the protocol steps within each of the 2 groups (subendocardial and subepicardial segments) were performed with the Student’s t test implemented with Bonferroni’s correction. Comparisons between measurements of different transmural myocardial layers were obtained using the Student’s t test for unpaired data.

Intra- and interobserver variability, as well as longitudinal reproducibility of IBScv, were estimated by calculating the mean absolute differences between observations. Statistical difference between observations was assessed using repeated measure ANOVA with the paired Student’s t test with Bonferroni’s correction.11

Linear correlation was performed between the magnitude of the normalized stress-induced changes in IBScv (value at rest—value at peak pacing) for both subendocardium and subepicardium, versus (1) the percentage coronary artery stenosis at angiography and (2) the normalized stress-induced changes in myocardial thickening.

Results
Of the 37 patients considered in this study, 12 had normal coronary arteries at coronary angiography. The remaining 25 patients had CAD (9 had significant stenosis of the left anterior descending, 3 had stenosis of both right and circumflex coronary artery, and 13 had stenosis of all 3 vessels).

In the 12 patients with normal coronary arteries, 27 myocardial segments could be evaluated: 14 were anterior and 13 inferior. In the 25 patients with CAD, 69 segments could be evaluated: 16 (not at jeopardy segments) were in a territory supplied by a coronary artery without significant narrowing (6 were anterior and 10 inferior), 53 (at jeopardy segments) were in a territory supplied by a significantly narrowed coronary artery (35 were anterior and 18 inferior).

In the group of patients without CAD, heart rate was 68.3±8.1 beats/min at rest, 143±4 beats/min at peak atrial pacing, and 95.8±6.7 beats/min at recovery. In patients with CAD, heart rate was 69.8±8.9 beats/min at baseline, 143.1±8.8 beats/min at peak atrial pacing, and 97.2±8.4 beats/min at recovery.

Transmural Heterogeneity of IBScv at Rest
A transmural gradient in IBScv in 3 myocardial segments. In the group of no-CAD myocardial segments, the average value of IBScv was 6.9±2.2 dB in the subendocardium and 4.3±2.4 dB in the subepicardium (P<0.001), with a heterogeneity index of 34.6±35.6%. A similar behavior was found in the not at jeopardy myocardial segments (7.7±1.9 dB in the subendocardium and 5.5±1.8 dB in the subepicardium, P<0.001, heterogeneity index 34.6±35.6%) and in the at jeopardy myocardial segments (8±2.3 dB in the subendocardium and 5.2±2.4 dB in the subepicardium, P<0.001, heterogeneity index 34.6±35.6%).

Transmural Heterogeneity of IBScv During Atrial Pacing Stress Test
During the atrial pacing stress test, in the group of no-CAD myocardial segments, the transmural gradient of IBScv remained unchanged at the peak of atrial stimulation with 7±2.2 dB in the subendocardium versus 4.9±2.5 dB in the subepicardium (P=NS versus rest) nor at recovery (7±1.9 dB versus 5±2.7 dB; P=NS versus all other protocol steps). In these segments, the heterogeneity index at rest (34.6±35.6%) did not change significantly at peak atrial pacing (28.7±33.1%, P=NS versus rest) and at recovery (28±36.2%, P=NS versus rest or peak atrial pacing).

In patients with CAD, the not at jeopardy myocardial segments (segments supplied by a coronary artery without a significant stenosis) showed a behavior of IBScv similar to that observed in myocardial segments of patients without CAD: at rest, 7.7±1.9 dB in the subendocardium versus 5.5±1.8 dB in the subepicardium (P<0.001); at peak-atrial pacing, 7.2±2.8 dB in the subendocardium versus 5.4±2.5 dB in the subepicardium (P<0.001); and at recovery, 8±3.3 dB in the subendocardium versus 4.6±2.7 dB in the subepicardium (P<0.001). Also, the heterogeneity index of IBScv did not show differences in the magnitude along the stress test (34.6±35.6% at rest; 23.7±24.3% at peak atrial pacing, P=NS versus rest; 40±27% at recovery, P=NS versus rest or peak atrial pacing).

On the contrary, in the group at jeopardy (myocardial segments of CAD patients supplied by a significantly narrowed coronary artery) (Figure 1), the mean value of IBScv in the subendocardial layer significantly decreased from baseline to peak-atrial pacing (from 8±2.3 dB to 5.4±1.8 dB, P<0.001 versus rest). This value returned to baseline immediately after atrial pacing interruption (7.6±3.1 dB, P<0.001 versus peak atrial pacing, P=NS versus baseline) (Figure 2). In the subepicardium of at jeopardy segments, the IBScv did not change significantly during atrial pacing or after atrial pacing interruption. As a consequence, the baseline heterogeneity index (34.6±35.6%) was totally abolished at peak atrial pacing (−1.5±33.1%, P<0.001 versus rest) and recovered 5 seconds after atrial pacing interruption (28±36.2%, P<0.001 versus peak atrial pacing and P=NS versus rest).

An example of the effect of atrial pacing stress test on IBScv in the different layers of a myocardial segment supplied by a significantly narrowed coronary artery is presented in Figure 3.
A significant correlation was found \((P<0.0001, r=0.425)\) between normalized stress-induced reduction in subendocardial IBScv and CAD severity (expressed as percent coronary lumen reduction). On the contrary, there was no correlation \((P=NS, r=-0.097)\) when comparing coronary artery stenosis severity with the reduction in subepicardial IBScv.

A similar behavior was observed between normalized stress-induced reduction in subendocardial IBScv and normalized stress-induced reduction in myocardial thickening \((P<0.005, r=0.358)\). No correlation was found \((P=NS, r=-0.076)\) when observing the IBScv in the subepicardial layer. The myocardial thickening was clearly measurable at rest and peak pacing in 77 of the 96 (80%) segments analyzed for IBScv.

### Interobserver, Intraobserver Variability, and Reproducibility

There were no significant differences between the 3 repeated measurements of IBScv data. The absolute difference between observations was \(0.8 \pm 0.9 \text{ dB (interobserver variability)}\) and \(0.9 \pm 0.7 \text{ dB (interobserver variability)}\). For longitudinal measurements, the absolute difference was \(0.9 \pm 1.1 \text{ dB with no significant difference between observations.}\)

### Discussion

Experimental studies have shown that microvascular flow, metabolic consumption, and strength of contraction do not behave homogeneously in the different transmural layers of the myocardium. Because of a lower vascular tone of subendocardial vessels, the vasodilatory reserve in this territory is limited.\(^{13}\) In the presence of a significant major vessel coronary stenosis, subendocardial flow reserve is further exhausted and subendocardial ischemia occurs when the oxygen demand increases or when the diastolic perfusion time is reduced because of tachycardia.\(^{14}\) Similar to flow, baseline myocardial contractility is also heterogeneous, resulting in greater contractility in the inner layers of the myocardial wall.\(^{1,2}\) Contractility of the subendocardial half accounts for two thirds of the entire transmural contraction.\(^{1,15}\) More recently, thanks to the use of cardiac magnetic resonance tagging, it has been possible to determine the contribution of different transmural layers to myocardial contraction in normal subjects and in patients with acute myocardial infarction.\(^{3}\) However, data on transmural heterogeneity during transient acute myocardial ischemia has been solely limited to experimental studies. As a consequence, there is a complete lack of knowledge concerning this aspect in humans.

### Effects of Myocardial Ischemia on IBScv

Tissue characterization with IB is related to the overall transmural contractility and myocardial thickening.\(^{6,7}\) During acute abolition of coronary blood flow (coronary occlusion, balloon inflation, acute myocardial infarction)\(^{7,16}\) or stress-induced myocardial ischemia (during either supine exercise\(^{17}\) or atrial pacing stress test),\(^{10}\) IBScv are blunted and recover to baseline value after ischemia cessation.\(^{10,17}\) When using atrial pacing stress test in particular, the recovery of IBScv after stress interruption occurred very rapidly (within a few cardiac cycles after atrial pacing interruption), well before the recovery of regional myocardial thickening. This is probably due to the low degree of induced myocardial ischemia.\(^{10}\) In this study, the reduction in subendocardial IBScv was correlated with the one in myocardial thickening; perhaps these reductions would have been more striking with a more intense stress test, like exercise or dobutamine.
Figure 3. IBScv heterogeneity at peak pacing, in different transmural layers of an at jeopardy segment. Time-intensity graphs show the disappearance of transmural gradient of IBScv curves at peak pacing; at baseline in the subendocardial layer of anterior myocardial wall (top left), 8.1 dB; in the subepicardial layer (bottom left), 5.1 dB with a heterogeneity index of 37%; at peak pacing in the subendocardial layer of anterior myocardial wall (top right), 5.2 dB; and in the subepicardial layer (bottom right), 4.7 dB with a heterogeneity index of 9.6%.
quality to place a 31 × 31-pixel region of interest separately in the subendocardium and in the subepicardium. In agreement with experimental physiological knowledge of myocardial contraction and IBS transmural heterogeneity,6,7 our study demonstrates for the first time in human beings that the videodensitometric IBScv are greater in the subendocardium than in the subepicardium. This result confirms the transmural gradient found with epicardial backscatter echocardiography in dogs at rest and during inotropic stimulation6,7 and in human beings with gray level cyclic variations.8 Moreover, our result closely parallels the transmural contractile heterogeneity shown at rest in the tagging magnetic resonance studies.3,18

Effect of Stress-Induced Myocardial Ischemia on Transmural Heterogeneity

The subendocardium is the first layer to suffer from ischemia in the presence of a significant narrowing of a coronary artery.4 Thus far, only one experimental study has investigated the effects of ischemia on IBScv transmural heterogeneity and demonstrated the abolition of the transmural gradient of IBScv following coronary artery occlusion due to blunting of the subendocardial ones.7

Similarly, in this study, the stress-induced myocardial ischemia totally abolished the transmural gradient of IBScv in myocardial segments supplied by a significantly narrowed coronary artery. The IBScv gradient was abolished because of IBScv blunting involving only the subendocardial layer (Figure 2). On the contrary, the transmural contractility gradient was well preserved during the entire stress test both in no-CAD and in not at jeopardy myocardial segments of patients with or without CAD, respectively. The potential of IBScv in identifying intramyocardial contractility impairment during stress-induced increase of oxygen demand in the presence of a significant CAD is further underlined by the significant linear correlation between stress-induced reduction in subendocardial IBScv and CAD severity or myocardial thickening reduction. This correlation was not detected in the subepicardium.

Limitations of the Study

A common limitation to all IBS studies is the necessity to have the ultrasonic beam as perpendicular as possible to the myocardial wall. In this transesophageal study, in order to optimize the evaluation of IBScv, we only used the transgastric 2-chamber view, where the anterior and inferior wall were almost perpendicular to the exploring beam. Our system analyzed the videodensitometry of unprocessed images and did not use the radiofrequency spectrum analysis. Because our method used a comparison of peak and nadir values at end-systole and end-diastole, no information on time delay in cyclic variations waveform is available. This parameter, not currently available in the echocardiographic analysis package, could offer further improvements to this already successful approach.5,16

Moreover, the heterogeneity index is derived from data expressed on a log scale, and the ratio between the transmural difference and the subendocardium can be affected by the use of logarithms. Thus other choices for the definition of a heterogeneity index can also be made.

Although the transesophageal echocardiography is certainly less feasible and tolerated than the transthoracic one,19 it provides echocardiographic images of such high quality that IBScv can be studied in the different transmural layers and allows evaluation of the myocardial thickening. The results of this study, however, can be conceptually extrapolated to other stress test procedures as well as to transthoracic echocardiography.

Clinical Implications

This study has shown for the first time in humans that the transmural contractile heterogeneity is abolished in the presence of a significant coronary artery stenosis, during a stress test procedure capable of inducing myocardial ischemia.

The demonstration of myocardial contractile heterogeneity and the possibility of its noninvasive evaluation in human beings has potential clinical implications, especially in stress test and in the course of myocardial infarction, to evaluate the transmural extent of infarction and/or viability. Transmural extent of myocardial necrosis is, in fact, a major factor responsible for infarct expansion and left ventricular remodeling.20 Also, tagging magnetic resonance has demonstrated that the functional recovery of viable subepicardial regions is a mechanism for the improvement in ejection fraction.18 The evaluation of a transmural extension of myocardial infarction is currently only possible using complex methods like magnetic resonance tagging. For example, in the everyday clinical setting, wall motion evaluation with echocardiography is not useful for this purpose because a progressive percentage of transmurality of necrosis does not correspond to a linear reduction in contractility. In fact, a complete abolition of myocardial thickening is produced by a necrosis that involves just the subendocardial third of the myocardial thickness.21

Our technique could differentiate the subendocardial myocardial infarctions from those concerning the entire thickness of left ventricular wall, thus identifying clinical situations at different risk of infarct expansion. Moreover, during the dobutamine echo test for viability, the measurements of IBScv (and hence contractility) in the subendocardium and subepicardium could permit in-depth exploration of myocardial segments with overall contractile reserve traditionally detected with conventional echocardiography in: (1) those segments showing contractile reserve in both subendocardial and subepicardial layers and (2) those showing contractile reserve only in the subepicardium.

Conclusions

This study explored the potential of IBS in evaluating intramyocardial heterogeneity in humans and, more importantly, described the different effects of stress-induced increased oxygen demand on the contractility of different transmural layers of myocardium in patients with and without CAD. These results highlight the potential of the assessment of IBScv in clinical conditions that, like subendocardial ischemia, differently affect function of myocardium in its various layers.
References


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