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Insights from echocardiography, magnetic resonance imaging and micro-computed tomography relative to the mid-myocardial left ventricular echogenic zone

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Abstract

Background: The anatomic substrate for the mid-mural ventricular hyper-echogenic zone remains uncertain, but it may represent no more than ultrasound reflected from cardiomyocytes orientated orthogonally to the ultrasonic beam. We sought to ascertain the relationship between the echogenic zone and the orientation of the cardiomyocytes.

Methods: We used 3D echocardiography, diffusion tensor imaging, and micro-computed tomography to analyse the location and orientation of cardiomyocytes within the echogenic zone.

Results: We demonstrated that visualisation of the echogenic zone is dependent on the position of the transducer, and is most clearly seen from the apical window. Diffusion tensor imaging and micro-computed tomography show that the echogenic zone seen from the apical window corresponds to the position of the circumferentially orientated cardiomyocytes. An oblique band seen in the parasternal view relates to cardiomyocytes orientated orthogonally to the ultrasonic beam.

Conclusions: The mid-mural ventricular hyper-echogenic zone represents reflected ultrasound from cardiomyocytes aligned orthogonal to the ultrasonic beam. The echogenic zone does not represent a space, a connective tissue sheet, a boundary between ascending and descending limbs of a hypothetical helical ventricular myocardial band or an abrupt change in cardiomyocyte orientation.

Keywords: helical ventricular myocardial band, echocardiography, diffusion tensor magnetic resonance imaging, computed tomography, cardiomyocyte orientation (m*aximum 6 keywords)*

Introduction

A mid-mural hyper-echogenic zone within the muscular ventricular septum was initially described by Feigenbaum in 1981 in a patient with a hypertrophied left ventricle.[1] At that time, the phenomenon was described as an "echo of unknown origin". For several decades thereafter, the nature of the echogenic zone remained unresolved, gaining very little scientific attention. In 2005, however, Boettler and associates studied 30 healthy humans using standard echocardiography and proposed two main hypotheses for the origin of the echogenic zone.[2] Demonstrating an echogenic zone in all subjects examined, they suggested that either an abrupt change in cardiomyocyte direction, or the presence of coronary arteries in the middle of the septum, may explain the hyper-echogenic properties of the area. Since the echogenic zone was present in both B- and M- mode images, and noting that it was best visualized in four chamber views, they considered the ventricular septum a bilayered structure.[2]

A recent study in *Echocardiography* has now suggested that the echogenic zone may represent a boundary line between ascending and descending segments of the putative helical ventricular myocardial band[3] originally proposed by Torrent-Guasp.[4] Torrent-Guasp had dissected the ventricular mass following prolonged boiling of the heart. Using his dissection, and following alleged cleavage planes, he was able to produce a single band of muscle running from the pulmonary to the aortic roots. When reformed, it twisted into a rope-like structure with a helical arrangement.[4, 5] As has already been pointed out by Boettler and colleagues, the dissection performed by Torrent-Guasp disrupts the interconnected arrangement of the cardiomyocytes, and creates artificial cleavage planes that provide the impression of a bilayered ventricular septum.[2]

We have, in several recent scientific contributions, pointed towards the misleading nature of the concept of the helical ventricular myocardial band. It is our opinion that the notion of the myocardial band, in which the orientation of myocytes follows the long axis of this hypothetical structure from pulmonary to aortic root, is not supported by any independent anatomical studies[6-9]. The

conclusions of Hayabuchi and colleagues prompted us to engage in correspondence relating to their account. In our letter, we suggested that the echogenic zone, rather than representing a thin boundary between parts of the ventricular myocardial band, could be a consequence of a higher reflectivity of ultrasound from aggregates of cardiomyocytes orientated in an orthogonal direction to the ultrasound beam.[10]

In the midwall of the left ventricle the cardiomyocytes are aligned so as to encircle the ventricular cavity, and approach a perpendicular orientation relative to the left ventricular long axis.[8, 11, 12] The circumferentially orientated myocytes have a long axis at right-angles to the ultrasonic beam when viewed from the cardiac apex in a four-chamber view. The echogenic zone, therefore, should be present in the entire circumference of the left ventricle. It is well worth noting that, to date, no imaging modalities other than ultrasound have demonstrated the presence of any well-defined anatomical structure in the left ventricular midwall in the position of the echogenic zone.

Three-dimensional echocardiography allows one to exploit the differences in any particular planar view when it is reconstructed either from a data set obtained from an apical transducer position or from the parasternal window. When the beam is delivered from the parasternal window, all the cardiomyocytes encountered in the mural segment have similar orientation to the beam, as the principal orientation of each aggregate of cells is in or near to the tangential plane, irrespective of its helical angle. When the beam is delivered from the apex, however, only the circumferentially oriented cells in the midzone will be perpendicular to angle of incidence. The helical angle of the inner and outer zones will progressively take the orientation of the cardiomyocytes away from the perpendicular. If orientation of the cardiomyocytes to the beam is a major factor in its reflectance, then images reconstructed from parasternal insonation will show relatively even texture of reflected echoes, but those reconstructed from the apical view will be expected to show greater reflectance in the mid-zone. This mid zone accentuation, moreover, would be expected to be visible in any reconstruction from apical insonation, as it is a phenomenon related to the delivery of the beam,

and not to the process of reconstruction. In order to investigate the hypothesis that the mid-mural ventricular hyper-echogenic zone arises from the circumferentially orientated cardiomyocytes, we studied the presence and extent of the echogenic zone in three-dimensional reconstructed echocardiographic views. We then compared the images with the predominant orientation of cardiomyocytes derived from both high-resolution micro-computed tomography and diffusion tensor magnetic resonance imaging.

Materials and methods

Echocardiography

Illustrative clinical echocardiographic images were obtained in a 73-year-old asymptomatic hypertensive woman using GE Vivid E9 equipment (GE Healthcare, Copenhagen, Denmark.) equipped with Vivid E9 BT 11 M5S and 4V transducers. Left ventricular short axis slices were reconstructed from 3D images obtained from both the apical and parasternal windows using EchoPAC software analysis (EchoPac PC 110.1.2 software for Vivid 7 and Vivid E9 GE Healthcare, Copenhagen, Denmark.).

Diffusion tensor imaging

Five neonate lambs were intubated after establishment of an intravenous access and administration of Propofol (1-5 $mg \cdot kg^{-1} \cdot h^{-1}$) and Fentanyl (2–4 $\mu g \cdot kg^{-1} \cdot h^{-1}$) by continuous infusion. The animals were ventilated (Servo300 ventilator, Siemens, Mississauga, ON, Canada) aiming at a $PaCO_2$ between 35 and 50 mmHg. The lambs were sacrificed with an overdose (100 $mg \cdot kg^{-1}$) of thiopental sodium, and the hearts were harvested through a median sternotomy. The coronary arteries were flushed with cardioplegic solution (20 mM potassium supplemented Ringer's Lactate) and subsequently fixed with 10% formalin administered manually slowly at sub-physiological pressure. The hearts were stored in formalin for at least two days, then rinsed and stored in phosphate buffered solution at 4°C until scanning.

Diffusion tensor magnetic resonance imaging was performed on all ovine hearts using a 9.4T pre-clinical MRI scanner (Agilent, Santa Clara, CA), equipped with a 400 mT gradients and the 'vnmrJ 4.0' operating system. In the scanner room, temperature was kept constant at 22.0±1.5 °C and air humidity at 50±10%. A diffusion weighted multi-slice 2D spin-echo sequence was employed with the following parameters: Isotropic voxel resolution of 310 microns, repetition time: 7000 milliseconds, echo time: 30 milliseconds. With one averaging diffusion sensitive images, with 30 predefined isotropically distributed diffusion encoded directions, were acquired[13] with diffusion sensitivity b-values of 1000 s/mm^2 and one image with a b-value of 0 s/mm^2. Total scan duration per heart was approximately 16 hours. The ovine hearts were harvested at the University at Buffalo, Buffalo NY, USA, and obtained under conditions approved by the local Laboratory Animal Care Committee.

Micro computed tomography

A fresh human heart of a 60 year old female, which was deemed not viable for transplantion (cause of death from bronchiogenic metastatic cancer) was cardiopleged, recovered, and transported to the University of Minnesota via LifeSource (Minneapolis, MN): all appropriate consents were obtained for donation of this specimen for research (HH074 on http://www.vhlab.umn.edu/atlas/histories/). This heart was considered to have no known pathology and was initially flushed with saline solution to remove residing clotted blood. Next, it was perfusion-fixed to maintain an end-diastolic state, as previously described.[14] and transported to the University of Manchester: A Materials Transfer Agreement was executed and shipping methods followed the guidelines of the University's Environmental Health & Safety Department. Then the specimen was stained with 7.5% I_2KI for 14 days. The contrast agent was replaced at day 7, as previously described.[15, 16] The heart was then rinsed with distilled water to remove excess contrast agent. To maintain the chambers in an inflated state, the heart was filled with warm agarose solution, which becomes a stable gel at room temperature. The heart was scanned using the Nikon Metris Custom Bay 225/320 kV CT scanner at the Manchester X-ray Imaging Facility (MXIF, University

of Manchester). During image acquisition, 3142 radiographic projections were acquired over a 360° of rotation, using an accelerating voltage of 155 keV and a current of 55 µA. Total scan time was approximately 50 minutes. Data was reconstructed using filtered back-projection, resulting in tomographic image data with an isotropic voxel size of 73 x 73 x 73 μm^3.

Data analysis

The diffusion tensor imaging raw data was imported using custom-made software[17] and the diffusion tensors representing the orientation of the cardiomyocytes were calculated. Original 2D image series from micro computed tomography were imported into MatLab Release 2011b (The MathWorks Inc., Natick, Massachusetts, USA 2011) for further processing. Structure tensors representing the orientation of the cardiomyocytes were calculated from the raw grey scale image files as described previously by using the primary eigenvector at each voxel.[18, 19] Subsequently, the tensor-based data sets from both modalities were imported into Mathematica (version 9, Wolfram Research Inc., Champaign, IL, USA 2012). Appropriate short axis and apical four chamber views were identified to match the scan planes of conventional echocardiography. Within the planes, a fan-shaped vector field was defined mimicking the ultrasonic beam. This was done by calculating a 'beam vector' for each voxel in the plane of interest, spanning from the narrow end of the virtual beam, where the scanner probe would be positioned, to the individual voxel (Figure 1). For every voxel, the angle between the primary eigenvector of the tensor, equal to the long axis of the cardiomyocytes, and the beam vector was calculated. All angles above 75 degrees were subsequently mapped in 5-degree intervals by grey scale colour coding. Furthermore, using the micro computed tomography data, the 3-dimensional arrangement of the coronary vasculature within the interventricular septum was analysed. Using Amira 5.33 (FEI), vessel luminal space was segmented using semi-automatic segmentation techniques, as previously described,[20] from which 3-dimensional volume renderings were created.

Results

Echocardiography

Conventional echocardiographic images are shown in Figure 2. The echogenic zone is absent in most parasternal views, but present in all apical views. The zone is seen in the inferolateral and inferior regions, and is not confined to the septum. In Figure 3, left ventricular short axis images are reconstructed from three-dimensional data sets obtained from the parasternal and apical windows. In the images obtained from the parasternal window, it is evident that the echogenic zone is absent from both the original long axis images and the reconstructed short axis images. In contrast, in the short axis images reconstructed from long axis images obtained from an apical window, an obvious echogenic zone is seen in the expected mid-septal position. Here, the echogenic zone assumes the shape of a ring continuous throughout the entire left ventricle. The online supplemental video images of the reconstructed short-axis views, derived from the apical data set, show that the diameter of circumferential echogenic zone decreases during systole, and probably represents shortening of the midwall cardiomyocytes. An oblique echogenic zone can be found in the left ventricular free wall, when originating from the usual short-axis view from the parasternal window, but with either some angulation towards the apex or slipping more laterally between the parasternal and the apical views (Figure 4).

Diffusion tensor imaging

The results of the diffusion tensor imaging analyses are shown in Figure 5. The location of the echogenic zone is reproduced in both the septum and left ventricular free wall when mapping the orientation of the cardiomyocytes using diffusion tensor imaging in the four-chamber view. Anatomical photographs matching the regions are provided showing no evidence of tissue planes visible to the naked eye. In the short axis representations from the parasternal view, no echogenic zone is visible in either modality. There is a striking resemblance between the seemingly random scatter on the ultrasonic images and the grey tones in the diffusion tensor imaging data set. The oblique echogenic zone was also replicated using diffusion tensor imaging (Figure 4).

Micro Computed Tomography

The results from the cardiomyocyte orientation analyses in the human micro computed tomography data is compared with that of the ovine diffusion tensor data in Figure 6. The micro-computed tomography produces a zone similar to that of echocardiography. It is most pronounced in the septum when reproduced in the equivalent of the four-chamber view. The short axis views of the two modalities in Figure 6, reproduced in the equivalent parasternal view, are also similar in terms of the pattern of white colours and the absence of a mid-mural ventricular hyper-echogenic zone. Figure 7 shows short axis images reconstructed from data sets in which the angles have been calculated using the apical viewpoint. Similar to the findings in Figure 3, the echogenic zone is present in the entire circumference of the left ventricle, as shown in the diffusion tensor data. A similar white line is seen in the right ventricular free wall. The mid septal zone is particularly evident in the micro-computed tomographic data although not as clearly visible in the free walls of the left and right ventricles (Figure 7). We have also visualised the intramural coronary vessels in three dimensions by segmentation of the iodine-deficient vascular lumens (Figure 8). The location of the mid-septal vessels indicated in red in figure 8 matches the location of the echogenic zone in the midwall component of the septum.

Discussion

As far as we are aware, this is first study to investigate the origin of the mid-mural ventricular hyper-echogenic zone of the left ventricle using a range of imaging modalities and image processing. Our investigation has combined the modalities of echocardiography, computed tomography, and magnetic resonance imaging. We have confirmed that, when using standard and three-dimensional echocardiography, the echogenic zone is best visualised in the apical views, but not well seen in the parasternal views. The diffusion tensor imaging studies, however, have shown that the position of the mid mural hyper-echogenic zones coincides with the orthogonal orientation of the cardiomyocytes to the ultrasound beam. This latter finding, obtained using ovine hearts, was then

confirmed by high-resolution microcomputed tomography of the human heart. The oblique echogenic zone in the left ventricular free wall, shown by oblique parasternal insonation, was also reproduced by diffusion tensor magnetic resonance imaging. Using the microcomputed tomographic images, we also showed that the position of the mid-mural ventricular hyper-echogenic zone corresponds with the circumferentially running intramural coronary vessels within the mid-septum. It is possible, therefore, that these intramural vessels may also contribute to the formation of the mid-mural ventricular hyper-echogenic zone. The absence of the vessels at other sites where the echogenic zone is visible makes it more likely that the circumferential cardiomyocytes make the major contribution to the genesis of the echogenic zone. Taken overall, our findings are consistent with both histology and pneumatographic findings of a continuum of myocardial structure, as previously demonstrated by Lunkenheimer and Niederer[21].

Discussion on the nature of the echogenic zone is currently ongoing. Boettler and colleagues had already considered the possibility that the purported helical ventricular myocardial band could provide an explanation for their findings. They had emphasised that the bilayered structure disclosed by blunt dissections could only be produced by disrupting the connections between the aggregates of the cardiomyocytes.[2] Despite this obvious caveat, Hayabuchi and associates, in their recent echocardiographic study, considered that the very presence of the echogenic zone provided supporting evidence for the concept of the myocardial band.[3] They speculated that the hyper-echogeneity is caused by an abrupt change in the orientation of the cardiomyocytes, despite the fact that many earlier studies have shown that the change in transmural orientation of all the myocytes within the left ventricle occurs gradually.[8, 12, 22, 23]

Using short axis reconstructions from three-dimensional echocardiography, we have now shown that the echogenic zone is not confined to the septum. When viewed from the apex, it can also be found around most of circumference of the left ventricle. Other studies have previously pointed towards the appearance of the echogenic zone in other parts of the left ventricle than the septum.[3,

[24] To the best of our knowledge, we are the first fully to show its extent. Furthermore, we have illustrated, using diffusion tensor imaging and micro-computed tomography, that the appearance of the echogenic zone coincides with areas in the myocardium where the orientation of the cardiomyocytes is perpendicular to the direction of the ultrasonic beam. Crosby and colleagues have elegantly shown that the ultrasonic backscatter in myocardium is highly dependent upon the orientation of the myocytes.[25] Their work is in keeping with our notion that the intensity of the backscatter is increased when the cardiomyocytes are perpendicular to the direction of the ultrasonic beam[25]. Their results also endorsed the findings of several earlier studies of both the heart [26, 27] and human tendons.[28] The similarity between echocardiography, and structure tensor analysis from micro-computed tomography, and diffusion tensor imaging in our study, reinforces this viewpoint. Since it has been known for decades that the amount of backscatter in echocardiography is dependent upon fibrous orientation in the tissue, it is surprising that so many alternative explanations of the origin of the echogenic zone have been proposed. For example, it has been stated that the echogenic zone represents a space approximately 100 µm wide, dividing the septum into two functionally different halves.[29] Such a space has not been demonstrated using histology or any other imaging modality other than ultrasound. It is striking, furthermore, that a transmural myocardial septal biopsy does not readily fall into two pieces when removed from the heart. In our view, it is evident that the concept of the helical ventricular myocardial band is inadequate for explaining the nature of the echogenic zone.

Evidence against the helical ventricular myocardial band

One of the main premises for the notions of Torrent-Guasp is that the strip of oriented myocytes represented by the unwrapped heart acts like a skeletal muscle, with an origin at the pulmonary root and an insertion at the aortic root. [30] It should be noted, however, that when viewing the unfolded helical ventricular myocardial band, the grain of the tissue runs in multiple directions, rather than along its length.[31] In our opinion, the concept of a myocardial band is an oversimplification. It does

not exist as an anatomical reality in which behaviour within the band is somehow separated from behaviour outside or across it. As early as 1864, Pettigrew described a myocardial continuum in seven layers of cardiomyocytes throughout the ventricular wall, showing spirals of helical grain, and a midwall layer with the myocytes oriented circumferentially.[32] Based on pneumatic distension, the more recent study by Lunkenheimer and Niederer reformats Pettigrew's notion of seven layers into five approximate zones: namely sub-epicardial, outer, middle, inner and sub-endocardial zones, with the latter including the trabeculations.[21] The zones described are of an arbitrary nature, without abrupt transition from one zone to the next, and without any discrete planes of connective tissue interposed between the zones. The zones, therefore, are not true layers as such, but gradual complicated regions of transition. In the short axis view, the inner, middle and outer zones make up the bulk of myocardium, and in some regions form multiple chevron-like shapes (Figure 9), giving an appearance similar to a feather, and representing arrays of lamellar units.[6] The central zone of the left ventricular wall consists of cardiomyocytes orientated in a circumferential direction, and is made up of the apices of the chevrons.[11] Within this arrangement is a continuous mesh of cardiomyocytes surrounded by an extracellular matrix, made up mostly of a collagen framework. The pneumatographic studies of Lunkenheimer and colleagues showed similar chevron-like structures, suggesting major aggregates of cardiomyocytes are separated by extracellular matrix, thus forming fan-like tissue planes or clefts running in the outer and inner zones along each wing of the chevron (Figure 9). Between these major tissue planes are interconnecting cardiomyocytes. In the mid zone, the cardiomyocytes merge into a circumferential orientation (Figure 9).[21, 33] The concept of the helical ventricular myocardial band does not allow for the circumferential orientation of the cardiomyocytes in the ventricular midwall. The position of the echogenic zone confirms that the signal originates from uniformly oriented cardiomyocytes, rather than from a distinct space or tissue interface between them.

Support for the concept of the myocardial band is also lacking from studies investigating the development of the embryonic cardiac loop.[34, 35] Neither does data from ventricular activation

support the notion of a myocardial band. The propagation of electrical waves within the ventricles normally originates in the endocardium near the apical septum, and proceeds from endocardium to the epicardium and towards the base.[36] The progression of the wave-front of depolarisation is not consistent with activation along the alleged myocardial band, although it may well explain the delayed activation of different left ventricular segments recently found by Hayabuchi and colleagues.[3] Taken overall, therefore, evidence remains lacking to support the concept of a single helical ventricular myocardial band. Our findings do not provide support for the notion of connective tissue planes between its alleged segments, a space between them or an abrupt change in the orientation of the cardiomyocytes within in the ventricular midwall. The dissection of the heat-denatured bovine heart, in our opinion, was the consequence of producing an apparent cleavage plane by entering the open side of the chevrons, splitting the apex of the chevrons to separate their inner and outer wings, and thus destroying the midwall circumferential cardiomyocyte aggregates.

Implications for a structure-function relationship

Our findings have a crucial impact in understanding the relationship between left ventricular structure and function. The motion of the left ventricle is a consequence of cardiomyocytic shortening and thickening. During systole, the epicardium shows only slight inward motion (Figure 9), whilst the base moves towards the apex.[37] There is little reduction in myocardial volume during systole, and therefore the wall thickens in a radial direction during contraction, displacing luminal volume and ejecting the stroke volume.[38] Cardiomyocyte shortening of approximately one-fifth results in an ejection fraction of around 65%, given a normal left ventricular mural thickness.[39] Absolute mural thickening is a major determinant of ejection fraction (Figure 9).[38] Any increase in mural thickness, including hypertrophy or infiltration, leads to a relative increase in absolute wall thickening and ejection fraction for a given myocardial contractile strain.[39, 40] Because a reduced contractile strain is seen almost universally in thick-walled ventricles, the ejection fraction is often normal or only mildly reduced.[41] Approximately two-thirds of the ejection fraction is attributable to

midwall circumferential shortening, and one-third to longitudinal shortening.[37] The three-dimensional arrangement of cardiomyocytes and extracellular matrix must allow the actin-myosin interaction with cell shortening, shear strain, reorientation, sliding between aggregates to minimise shear stress, compression and thickening of long chains of cardiomyocytes, accompanied by torsion of the base relative to the apex during contraction, and a reversal of this process during relaxation (Figure 9).

Any interpretation of cardiodynamics needs to be consistent with continuum mechanics. Inroads are now being made so that the ejection fraction can be mathematically derived from myocardial strain and wall thickness.[42] Normal and abnormal left ventricular mechanics, such as twist, as well as systolic and diastolic function, furthermore, can readily be explained without invoking the concept of a helical ventricular myocardial band.[40, 43] The twist of the left ventricle is explained by the known anatomical arrangements of cardiomyocytes.[40] Abnormalities of calcium homeostasis and collagen deposition may both result in abnormal relaxation and compliance.[43] Abnormal ventricular suction during early diastole is related to the combination of higher diastolic calcium levels[43], and a loss of compression energy stored during systole resulting in reduced elastic recoil.[44] Abnormalities of left ventricular structure have important and quantifiable effects on myocardial function.[45-47] A proper understanding of myocardial function has vital implications for comprehending the processes of cardiovascular disease such as heart failure[48, 49] and can be achieved without resort to a hypothetical helical ventricular myocardial band.

Limitations

For the diffusion tensor imaging studies, we used ovine neonatal hearts. In contrast to human specimens, ovine hearts are readily available and small enough to fit inside the small bore of the magnet. The anatomical differences between sheep and humans have not been investigated scientifically using diffusion tensor imaging, but when comparing studies of sheep [50] and humans, [22] the differences are small. To support this viewpoint, we conducted identical analyses using human

micro computed tomography data, and found very similar results to those obtained using the ovine hearts.

The technique of diffusion tensor imaging possesses the intrinsic limitation of averaging data. Because the myocardium is subdivided into numerous voxels each with a volume of 30 nanolitres, within each voxel the average orientation of approximately 900 cardiomyocytes is assessed. We note that the myocardium is a very heterogeneous mesh, and thus such averaging could potentially skew the data towards an unnatural degree of order. To obviate this difficulty, we also provided high-resolution computed tomography data. In the computed tomographic dataset, each voxel contains only approximately 9 myocytes, hence smoothing is less of an issue. This higher resolution probably explains why the data obtained using computed tomography is apparently noisier than that provided by diffusion tensor imaging. It may also be argued that structure tensor analysis of the computed tomography data is not as accurate as diffusion tensor analysis, but this remains to be investigated. The overall consistency between all three used modalities, nonetheless, indicates that the above limitations are of minor significance.

Conclusion

We have shown that the mid-mural ventricular hyper-echogenic zone is anatomically related to the circumferentially orientated cardiomyocytes, and that the oblique echogenic zone arises from orthogonally orientated cardiomyocytes. We suggest that both echogenic zones arise as a consequence of increased reflectivity of structures such as cardiomyocytes lying perpendicular to the ultrasound beam. Our conclusions are supported by new three-dimensional data from echocardiography, diffusion tensor imaging, and micro computed tomography, as well as a wealth of previous anatomical studies. The echogenic zone does not represent an interface between the segments of a hypothetical helical ventricular myocardial band. It is, however, consistent with an intricate mesh-like structure of the myocardium.

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Figure legends

Figure 1:

Schematic of the vector field emulating the ultra sound beam. For each voxel (blue) a vector is calculated originating from the virtual tip of the echo probe and ending in each voxel. Subsequently, the angle between the primary eigenvector within each voxel and the corresponding echo probe vector is calculated and plotted.

Figure 2:

Note the absence of the echogenic band in the parasternal long-axis view (top left). The echogenic zone is easily seen in all the apical long axis views particularly the ventricular septum, inferolateral and inferior regions (arrows) giving an apparent 3 layer structure. The left ventricle appears to have two layers in the anterolateral and anterior walls.

Figure 3:

Top image: Images attained from parasternal long axis derived images. Note the absence of an obvious echogenic zone. Bottom image: Images attained from apical long axis derived images. Note the presence of a near circumferential midwall echogenic zone. Left panels are the original images with dotted lines delineating the reconstruction areas. Remaining images are reconstructed slices from this area.

Figure 4:

An oblique echogenic zone can sometimes be found in the left ventricular free wall, when angulating the ultrasound beam towards the apex from the parasternal window (left panel). When reproducing this viewpoint using diffusion tensor imaging a similar oblique zone is seen (right panel).

Figure 5:

Left panels: Echocardiographic images from the apical four chamber view (top) and the parasternal short-axis view (bottom). Note the echogenic zone in the septum in the four chamber view (arrow)

and the complete absence of any hyper echogenic areas in the short axis view. Centre panels: Fixed human heart specimens for anatomical comparison. No macroscopic structures are to be found aiding in the explanation of the hyper echogeneity of the mid septum. Right panels: Diffusion tensor imaging representations. The four chamber image is flipped vertically for improved comparison with the conventional apex-up four chamber view in echocardiography. White and gray areas indicate cardiomyocytes with an orientation perpendicular to the direction of the ultra sound beam as outlined by the dotted triangle (see methods). Note the resemblance between the short axis echogardiography and diffusion tensor images. LV: Left ventricle. RV: Right ventricle.

Figure 6:

Diffusion tensor imaging (left panels) and micro CT (right panels) representations of the conventional echocardiografic views of the heart. White and gray areas indicate cardiomyocytes with an orientation perpendicular to the direction of the ultra sound beam (see methods). The view- point of the virtual echo probe is depicted in each panel by black arrows. In the top panels the orientation of the cardiomyocytes outline an area similar to the echogenic zone in echocardiography (grey arrows). The zone is absent in the virtual parasternal views in both techniques (bottom panels).

Figure 7:

Short axis reconstructions of diffusion tensor imaging (top) and micro CT data (bottom). White and gray areas indicate cardiomyocytes with an orientation perpendicular to the direction of the ultra sound beam (see methods). Angles assessed relative to the apical viewpoint thus comparable to the bottom image in figure 3. Arrows mark the septal echogenic zone.

Figure 8:

Coronary vessels segmented from the mid-wall of the interventricular septum in the human heart that is the same region populated by the circumferentially orientated myocytes or the triebwerkzeug. Figure shows intramural coronary vessels visualised by segmentation of the iodine deficient vascular lumens. The location of the mid-septal vessels indicated in red matches the

location of the echogenic zone in the midwall component of the septum. The vessels here assume a circumferential orientation similar to that of the cardiomyocytes in this area. (A) four chamber view, (B) right lateral view, (C) enlarged right lateral view with myocardium removed.

Figure 9:

Multiple chevrons form a feather like appearance with a gradual change in the dominant orientation of cardiomyocyte aggregates (left panel). The change in aggregate orientation is illustrated by the colour gradient in each image. In diastole (middle panel) the apex of the chevron represents the circumferential orientated cardiomyocytes. During systole the wall thickens flattening the chevrons (right panel). There is a minor reduction in external dimension of the left ventricle during systole (dash-dot line). The difference between end-diastolic wall and end-systolic wall thickness is the absolute wall thickening (AWT, between dashed line and endocardial border) and is an important determinant of ejection fraction. Note the sub-endocardium thickens to a greater extent than the sub-endocardium during systole.

Supplemental material

Video 1 - "Band Apical SAX sweep.avi"

Parasternal apical sweep. Note the presence of an echogenic zone towards the apex

Video 2 - "Banded Myocardium 38 Apical.wmv"

Short axis slices reconstructed from reconstructed views from three-dimensional images derived from the apical view. Note the presence of an echogenic zone.

Video 3 - "Banded Myocardium 36 Parasternal.wmv"

Short axis slices reconstructed from reconstructed views from three-dimensional images derived from the parasternal view. Note the absence of an echogenic zone.