Three-dimensional alignment of the aggregated myocytes in the normal and hypertrophic murine heart

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Several observations suggest that the transmission of myocardial forces is influenced in part by the spatial arrangement of the myocytes aggregated together within ventricular mass. Our aim was to assess, using diffusion tensor magnetic resonance imaging (DT-MRI), any differences in the three-dimensional arrangement of these myocytes in the normal heart compared with the hypertrophic murine myocardium. We induced ventricular hypertrophy in seven mice by infusion of angiotensin II through a subcutaneous pump, with seven other mice serving as controls. DT-MRI of explanted hearts was performed at 3.0 Tesla. We used the primary eigenvector in each voxel to determine the three-dimensional orientation of aggregated myocytes in respect to their helical angles and their transmural courses (intruding angles). Compared with controls, the hypertrophic hearts showed significant increases in myocardial mass and the outer radius of the left ventricular chamber (P < 0.05). In both groups, a significant change was noted from positive intruding angles at the base to negative angles at the ventricular apex (P < 0.01). Compared with controls, the hypertrophied hearts had significantly larger intruding angles of the aggregated myocytes, notably in the apical and basal slices (P < 0.001). In both groups, the helical angles were greatest in midventricular sections, albeit with significantly smaller angles in the mice with hypertrophied myocardium (P < 0.01). The use of DT-MRI revealed significant differences in helix and intruding angles of the myocytes in the mice with hypertrophied myocardium.

hypertrophy; orientation; magnetic resonance imaging; diffusion tensor imaging

THE PUMPING FUNCTION of the ventricular myocardium is determined by the three-dimensional arrangement of the individual myocytes aggregated together within their supporting fibrous matrix. The orientation of these aggregated myocytes can be described in terms of their helical and intruding angles, the latter also being called transverse angle (3, 6, 23). Several recent studies have translated these three-dimensional angulations of the aggregated myocytes to mechanical aspects of cardiac function. The importance of the helical angles in producing centripetal forces and constricting actions during ventricular systole is well recognized (5, 11). Recent studies also pay increasing attention to the influence of the intruding angles. Indeed, most earlier investigators have argued that myocytes rarely intrude by angles of >10 degrees. More current investigations, however, using circular knives and diffusion tensor magnetic resonance imaging (DT-MRI) to sample the ventricular walls, have demonstrated the existence of a significant population of myocytes intruding from epicardium to endocardium at even larger angles (20). Additional studies using force probes suggest that these intruding myocytes working in conjunction with the fibrous matrix, have an impact on myocardial mechanics (16, 17).

When assessing the mechanisms producing myocardial function, therefore, it is important to take note of the intruding angulation of the myocytes as well as their helical angles. To the best of our knowledge, this three-dimensional alignment of the myocytes has not been compared in the entirety of the normal as opposed to the hypertrophied ventricular mass. It is our belief that such information would help to improve the understanding of the cardiac response to various pathological conditions, the more so since the arrangement of the myocytes within the supporting fibrous matrix is markedly heterogeneous (1, 8, 9, 15).

The existence of the population of myocytes that are crossing the myocardial wall from epicardium to endocardium has been well demonstrated histologically (7, 16). Histological techniques, however, suffer from their restricted three-dimensional spatial resolution. The advent of DT-MRI now offers the potential to overcome this shortcoming. The method has been shown to be capable of displaying the alignment of the myocytes in nondestructive fashion (4, 19, 20, 22). Thus hoping to improve knowledge about any myocardial realignment produced by mural hypertrophy, we have now used DT-MRI to investigate the three-dimensional angulation of the ventricular myocytes in a normal population of mice, and in a second population with pharmacologically induced global ventricular hypertrophy.

MATERIALS AND METHODS

We studied a total of 14 black mice (C57Bl16J, Charles River, Sulzfeld, Germany). Ventricular hypertrophy was induced in seven
mice by 2-wk infusion of angiotensin II via an osmotic minipump. For implantation of the pump, the mice were anesthetized with pentobarbital sodium (50 mg/kg ip), and minipumps were placed subcutaneously in the intrascapular area to deliver angiotensin II at a dose of 300 μg/day. The control group, also of seven mice, was given saline through the minipump. Prior to being killed, the animals were injected with 50 units of heparin through the tail vein. The hearts were arrested in diastole using a cardioplegic solution containing 15 mM KCl. The hearts were extracted rapidly after death, and immediately placed into 4% formaldehyde solution. Prior to imaging, the hearts were embedded in agar gel, taking care to avoid any air bubbles in and around the heart. We recorded the body weight and the weight of the hearts after removal. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and the animal experiments were authorized by the responsible authorities at our institution.

Imaging. Imaging was performed using a 3.0-Tesla whole body scanner with a dedicated solenoid mouse coil (both from Philips Medical Systems, Best, The Netherlands). Diffusion weighted scans were acquired in the ventricular short axis, with seven slices covering the heart from base to apex (Fig. 1A). The scan parameters were: diffusion sensitized gradients (b = 700 s/mm²) along 15 directions, effective TR/TE = 1.000/67 ms, matrix of 200 × 200 elements, in-and through plane spatial resolution 0.2 × 0.2 × 1.0 mm³, no interslice gap. Imaging resulted in a B0 image and diffusion weighted images encoded in each of the optimized 15 directions. We also acquired conventional T1 weighted images in a short-axis plane with the following sequence parameters: TR/TE of 500/30 ms, matrix of 256 × 256, slice thickness 1.5 mm, and 0.1 mm interslice gap.

Post-processing and data analysis. The myocardial mass, as well as the endocardial and epicardial radius of the cavities and the walls, were determined by drawing the appropriate contours. For myocardial mass, contours were drawn on the T1 weighted images in all slices covering the heart. The inner and outer radii were determined at the equatorial slice of the heart. The measurements were made with View Forum Software (Philips Medical Systems). The length of the left ventricle and the thickness of the ventricular septum and the free walls were also recorded to document the changes with and without infusion of angiotensin. The values were indexed to the weight of the animal.

After completion of the diffusion scans, we extracted the primary eigenvectors in each pixel as described previously; this technique also serves to trace chains of connected myocytes (10, 22, 26). The pixel-to-pixel concatenation of the primary eigenvectors produces sequences of straight segments of equal lengths. These segments, representing the aggregation of the individual myocytes, were computed and analyzed for a 60°-wide segment of the left ventricular free wall and the ventricular septum (Fig. 1C). We excluded the sites of intersection with the wall of the right ventricle because of the known interlacing, and hence criss-crossing, of the aggregated myocytes in these regions (18).

Measurement of intruding and helical angles. In terms of biophysics, the course of the aggregated myocytes can be described in the three-dimensional context of the ventricular walls by measuring two different angles. The helical angle is the one made between the aggregated myocytes and the ventricular equator, while the so-called intruding or transverse angle measures the extent to which the aggregates angulate from the epicardium toward the endocardium as they penetrate the ventricular wall (Fig. 1B and C). We derived both these angles by measuring the primary eigenvectors using a graphical user interface on a MATLAB platform (The Mathworks, Natwick, MA).

The angles were computed in seven ventricular slices for each of the 14 hearts, numbering the basal slice as 1 and the apical slice as 7. The angle of intrusion was then defined as the angle between the primary eigenvector projected in the x-y plane and a tangent that was applied at the epicardium (Fig. 1C and Fig. 2). The helical angle was defined as the angle between projection of the primary eigenvector on the tangential plane at the epicardium and the x-y plane (Fig. 1B and Fig. 2). We determined positive as opposed to negative direction of these angles as shown in Fig. 2, following the precedent of Schmid et al. (20). For the measurements we applied a local elliptically shaped model of the epicardium (Fig. 2). The coordinate system was locally adjusted in each slice for each separate measurement. The x-axis was clockwise oriented parallel to the epicardium and the y-axis was directed toward the endocardium. The z-axis was chosen as the longitudinal direction along the long axis of the left ventricle.

Histology. Histology of the myocardium using hematoxylin and eosin staining was performed on a total of 14 samples from each mouse, taking the samples from seven slices of the left ventricular free wall and seven from the septum. A total of 50 myocytes per sample slice were evaluated for the distribution of high angles at the subendocardial and subepicardial layers.
Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control Mice (n = 7)</th>
<th>Angiotensin-Treated Mice (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>27.4 ± 0.9</td>
<td>26.9 ± 0.8</td>
</tr>
<tr>
<td>Total heart weight, g</td>
<td>0.15 ± 0.01</td>
<td>0.18 ± 0.01*</td>
</tr>
<tr>
<td>MRI derived data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial mass of total LV (g)</td>
<td>0.09 ± 0.01</td>
<td>0.12 ± 0.02*</td>
</tr>
<tr>
<td>Myocardial mass of total LV weight/body wt, mg/g</td>
<td>3.3 ± 0.1</td>
<td>4.5 ± 0.2*</td>
</tr>
<tr>
<td>Wall thickness of septum, mm</td>
<td>1.16 ± 0.02</td>
<td>1.26 ± 0.05*</td>
</tr>
<tr>
<td>Wall thickness of LV free wall, mm</td>
<td>1.15 ± 0.02</td>
<td>1.24 ± 0.04*</td>
</tr>
<tr>
<td>LV length, mm</td>
<td>7.95 ± 0.41</td>
<td>7.32 ± 0.45</td>
</tr>
<tr>
<td>Outer LV radius, epicardial, mm</td>
<td>2.50 ± 0.13</td>
<td>2.87 ± 0.19*</td>
</tr>
<tr>
<td>Inner LV radius, endocardial, mm</td>
<td>1.18 ± 0.12</td>
<td>1.04 ± 0.09</td>
</tr>
<tr>
<td>Echocardiographic data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>53.5 ± 1.4</td>
<td>50.1 ± 1.6</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>591 ± 12</td>
<td>582 ± 14</td>
</tr>
</tbody>
</table>

Values are means ± SE. Radius was derived from short axis images at the equatorial level of the heart. MRI-derived data that was indexed to the weight of the animal did not change significance levels when comparing the 2 experimental groups. Immediately prior to euthanasia, fractional shortening was obtained in conscious mice from transthoracic echocardiograms (Acuson, Somona Health Products. Imaging was performed by M-mode at the midventricular level. LV, left ventricle. *P value < 0.05 was considered significant.

**RESULTS**

The baseline characteristics are summarized in Table 1. Ventricular hypertrophy was noted in all the mice treated with angiotensin II, the measurements obtained using magnetic resonance imaging confirming the increase in myocardial mass normalized to body weight (P < 0.05). For the hypertrophied left ventricles there was a trend toward decreased length (P = 0.061) and reduced endocardial radius (P = 0.067). The epicardial radius and molar thickness was significantly increased (P < 0.05).

**Intruding angles.** Figures 3 and 4 show the transmural course of the angle in basal, midventricular, and apical slices of the left ventricular free wall and the septum, respectively. In the control animals, we noted a change from the mean in prevalence of positive angles at the base to negative angles at the ventricular apex in the control animals that was highly significant. The transmural distribution of the helix and intruding angle in mice with hypertrophic hearts compared with controls in the left ventricular free wall. Endocardial surface is located at a transmural depth of 0%, epicardial surface at transmural depth of 100%. The angles were measured at the base, midventricle, and apex in steps of 10% thickness of the wall. *Significant differences between the control and hypertrophy (P < 0.05).
statistical significance (P < 0.05). At the base and the apex, these differences were overall larger in the hypertrophic hearts compared with controls. In the free wall, difference between the angles reached statistical significance for the majority of regions of the myocardium to the action of angiotensin II is rapid, with increased synthesis of the proteins in the extracellular matrix, either by its hypertensive hemodynamic action and/or by direct action on the myocytes (27). It is also known to produce increased synthesis of the proteins in the extracellular matrix, such as collagen type I (28). In addition, the response of the myocardium to the action of angiotensin II is rapid, with}

**DISCUSSION**

Our findings of the three-dimensional arrangement of the myocytes aggregated together within the walls of the murine heart endorse, in terms of the helical angles, the descriptions of McLean and Prothero (18). For the most part, they are also in keeping with those described by Streeter and colleagues (24) for the canine heart, Geerts et al. (6) for the goat heart, and Greenbaum and associates (8) and Ingels (11) for the human heart. Indeed, this pattern of myocardial architecture was described by Pettigrew as early as 1864. Thus the myocytes are aggregated together in reciprocal helices in the subendocardial and subepicardial parts of the walls, whereas they are aggregated predominantly in circular fashion within the middle parts of the walls and the septum. These circular myocytes were identified by Krehl as “Triebwerkzeug” and were considered by him to provide the driving force for ventricular constriction.

Assessment of the geometric arrangements of the myocytes by DT-MRI has now permitted us to demonstrate substantial alterations in their spatial angulation within the myocardium in hypertrophied compared with normal hearts. The changes in angulation, however, were most marked in respect to the transmural intrusion of chains of myocytes, the so-called intruding or transverse angles, with less marked changes noted in the helical angles.

Recent studies have provided the basis for investigating the entirety of the murine left ventricular mass using DT-MRI, and have indicated the potential of using this technique to assess cardiac architecture at different stages of induced myocardial hypertrophy (2, 12). Other investigators have used DT-MRI to examine porcine and goat hearts, confirming the existence of the population of intruding myocytes, showing that around two-fifths of the myocytes inclined at intruding angles between 10 and 35° relative to the plane of the epicardial surface (20, 21). These studies, however, had examined only selected transmural slices. In our current study, we examined the global distribution of angles of large segments of the left ventricular free wall and of the septum. Our findings confirm that positive intruding angles prevail at the base, but reveal that negative angles exist at the apex (6), with the additional finding that the angles of intrusion were larger in the hypertrophied hearts than in controls.

Angiotensin II is known to mediate cardiac hypertrophy either by its hypertensive hemodynamic action and/or by direct action on the myocytes (27). It is also known to produce increased synthesis of the proteins in the extracellular matrix, such as collagen type I (28). In addition, the response of the myocardium to the action of angiotensin II is rapid, with
growth in size and expression of new myofibrils noted after 48 h in cultured myocytes (27). In our study we measured by histology an increase of the diameter of the myocytes in the angiotensin II-treated mice compared with controls. One might speculate if such an increase in myocytic size has a direct impact on the angle of intrusion. Any increase in angulation will be part of a complex process of remodeling that occurs during hypertrophy. In this respect, the myocardial mesh is three dimensionally netted with superjacent constituents of the mesh turning on a radial axis (24). As the myocytes increase their size, their coupling with their neighbors, in both tangential and the endoepicardial directions, is structurally fixed. For strictly geometrical reasons, changes in ventricular dimensions must go along with changes in the helical angle. This remodeling process will also necessitate progressive changes in connections of the myocytes with their neighbors. However, future research is needed to substantiate this detail of microstructural remodeling. As mentioned, the circular realignment in helical angle is a function of ventricular dimension. This was recently shown on hearts of rats arrested in systole and diastole (4). Accordingly, in the hypertrophic hearts of our study we did not measure significant changes in helical angle in the subendocardial regions because the inner radius had slightly decreased while the outer radius had increased.

Earlier investigations of mice treated with angiotensin II have shown preservation of systolic pump function, with impunity of diastolic function due to reduced compliance (28). In keeping with these findings, we did not observe any alteration in left ventricular systolic function in the mice with myocardial hypertrophy. Any diastolic dysfunction could be the consequence of mural fibrosis, as well as an increase in myocardial mass, and a minor reduction in ventricular volume capacity by a decline in inner diameter as part of myocardial hypertrophy. Systolic constrictive force, in contrast, might increase as a result of the reduction in helical angles as part of outward displacement of the epicardial surface. On the other hand, a more circular alignment of the tangential netting component has been supposed to reduce stroke volume (11). This might apply if the length of the spiraling chains of serial myocytes would not lengthen, which, however, is part of the mechanism of hypertrophy. Another consequence of circular realignment of the subepicardial helical structure was assumed to be an increase in subepicardial torque relative to subendocardial torque, which ultimately should result in enhanced global ventricular twisting (25). The concept only applies when also taking into account the epi-endocardially intruding netting of the myocardial wall, which means that the helical angle alone does not provide the necessary information about the basic mechanism of twisting. Regional distribution of angles of intrusion might be a better key to the understanding of ventricular torque. Previous reports using force probes suggest that the intruding netting component of the myocardial mesh has an impact on myocardial mechanics (14). We speculate that alterations in regional distribution of intruding angles in the base and the apex might influence the torque of the apex relative to the ventricular base. It would be inappropriate at this stage, however, to indulge in far-reaching inference from our observational findings to intramural mechanics. This matter must be investigated systematically, which could involve, among others, micromechanical analysis.

When comparing the septum with the left ventricular free wall, we found that intruding and helical angles had a similar transmural distribution, albeit differences between the control and hypertrophic hearts were less predominant in the septum.
In the septum, angles were more heterogeneously distributed, but our histological investigation failed to show any myocardial disarray, as is typically found in hypertrophic obstructive cardiomyopathy.

Overall, the regional heterogeneities in myocardial alignment, such as we describe them in our study, throughout the ventricular walls imperatively indicate the need of both global and regional analysis of ventricular function. To speculate about ventricular function on the base of morphological data is hazardous. The interaction between the two highly heterogeneous matrices, namely the myocytes and the fibrous tissue, does not allowed one to infer from the alignment of the myocytes to the potential pathway of displacement of any muscular segments.

Limitations. The study was based on one model of hypertrophy. Significant changes in the alignment of aggregated myocytes were noted, although the degree of hypertrophy was only modest. Future studies should compare various degrees and time courses of hypertrophy to shed more light on the functional importance of our noted architectural changes. In our study, we arrested the hearts in diastole and did not study the systolic arrangements. DT-MRI assessment of intruding muscular segments. The three-dimensional arrangement of the myocytes on the basis of the second and tertiary eigenvectors (4). It is beyond the scope of the present study to determine the precise global arrangement of the myocytes on the basis of the second and tertiary eigenvectors. We suggest, nonetheless, that more work is required relative to this important subject. It should also be noted that, due to the extended time required for acquisition of data, cardiac DT-MRI is currently limited to postmortem studies.

In conclusion, we have shown that DT-MRI provides information concerning changes in the alignment of aggregated myocytes mediated by angiotensin II-induced remodeling. The technique is nondestructive and reproducible. It has provided important information about the intramural arrangement of the myocytes aggregated together in the walls of normal and pathologic hearts. The potential implication of these changes on intramural dynamics should be addressed in future research.

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