Left ventricular torsion is equal in mice and humans

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Left ventricular torsion is equal in mice and humans. Am J Physiol Heart Circ Physiol 278: H1117–H1123, 2000.—Global cardiac function has been studied in small animals with methods such as echocardiography, cine-magnetic resonance imaging (MRI), and cardiac catheterization. However, these modalities make little impact on delineation of pathophysiology at the tissue level. The advantage of tagged cine-MRI technique is that the twisting motion of the ventricle, referred to as torsion, can be measured noninvasively, reflecting the underlying shearing motion of individual planes of myofibrils that generate wall thickening and ventricular ejection. Thus we sought to determine whether the mechanism of ventricular ejection, as measured by torsion, was the same in both humans and mice.

Nine mice and ten healthy humans were studied with tagged cine-MRI. The magnitude and systolic time course of ventricular torsion were equivalent in mouse and human hearts, when normalized for heart rate and ventricular length. The end-systolic torsion angle was $12.7 \pm 1.7^\circ$ in humans vs. $2.0 \pm 1.5^\circ$ in mice, normalized and $1.9 \pm 0.3^\circ$/cm vs. $2.7 \pm 2.3^\circ$/cm when normalized for ventricular length. These results support the premise that ventricular torsion may be a uniform measure of normal ventricular ejection across mammalian species and heart sizes.

MURINE MODELS OF CARDIOVASCULAR disease are assuming a prominent role in cardiovascular research, necessitating development of noninvasive methods of characterizing ventricular function in these animals. Many of these models affect structural proteins with consequences for regional myocardial function at a cellular level, and therefore it will be important to develop methods for assessing intramural as well as global mouse heart function. In this work, we use a method for examining myofiber kinetics noninvasively with magnetic resonance imaging (MRI) to show that mouse and human ventricles behave similarly in terms of systolic ventricular torsion.

Echocardiography (13–14, 18, 22, 34, 42), ventriculography (31), and MRI (1, 11–12, 17, 19, 28, 32, 36, 37, 39, 43, 46) are all feasible tools for evaluating mouse heart function. Methods such as echocardiography and traditional cine-MRI allow assessment of wall thickening and ejection fraction but not deformation of small tissue segments transmurally or quantification of torsion and shear, the underlying mechanisms of wall thickening (4). MRI possesses an intrinsic advantage in that it can be used to study intramural wall motion and myofiber kinematics using a method called myocardial tagging (5, 7–9, 27, 30, 45), which allows tracking of noninvasively placed fiducial markers in the myocardium. Before the advent of tagged cine-MRI, radiopaque markers or sonomicrometer crystals implanted in heart tissue were used to study local tissue deformation. This technique is invasive, but these studies have shown that changes in torsion may precede any change in global ventricular function and cardiac output, thereby proving that it is a sensitive indicator of altered myofiber function (15–16, 44).

Lower (25) studied left ventricular torsion in the late 17th century. The twisting motion of the left ventricle (LV) about its long axis results from the contraction of the obliquely oriented epicardial and endocardial fibers. Histologically, the mammalian ventricle is not only composed of radial and longitudinal fibers but also obliquely running sheets of fibers that course in a helical spiral from the apex to base (2). Radial and longitudinal fiber contraction results in radial and longitudinal shortening during systole. Contraction of the helical ventricular fibers results in a twisting motion of the LV about its axis (40). Ventricular twist and subsequent shearing between adjacent sheets of myocytes are thought to represent a primary mechanism of wall thickening and ejection (36). It also has been proposed to be an energy-minimizing mechanism by which the LV reduces wall stress and oxygen demand (6). Armour and Randall (2) evaluated the gross structure of the heart ventricles in nine mammalian species ranging in size from the ground squirrel to the elephant and found similar arrangements of fiber orientations across the wall from endo- to epicardium and also from apex to base.

Therefore, because ventricular architecture is similar across many mammalian species (although it has not been specifically evaluated in the mouse) and because ventricular torsion likely reflects one of the fundamental mechanisms of wall thickening, we sought to determine whether ventricular twist and torsion (twist per unit length) magnitudes and patterns in the mouse are similar to those found in humans, using myocardial tagging with MRI. The establishment of both mouse and human hearts use the same mecha-
nnisms for contraction is one essential step before attempting to interpret the effects of genetic manipulation on mouse heart function and extending them to human disease.

**MATERIALS AND METHODS**

Image acquisition in mice. Nine normal CD-1 white mice were studied. The protocol was approved by the institutional animal care and use committee. Washington University is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. The animals were placed into a halothane anesthesia box and treated with 5% halothane to sedation, typically for less than 1 min. They were then placed supine into a laboratory-built cylindrical mouse holder, and sedation was maintained with 0.75% halothane-1.25% O₂ (2 l/min) by a nose cone. The animals were bedded on gauze for warmth and positioning. With this method, they typically survived 3–4 h of light sedation with minimal decrease in heart rate, promptly awakening after withdrawal of halothane.

Standard solid gel electrodes (Uni-Patch, ECG Supplies; Wabasha, MN) were then placed firmly on the bilateral front paws and the right rear leg. Electrocardiogram (ECG) amplitude was found to be directly proportional to the quality and quantity of surface contact. These ECG electrodes covered the distal half of the forelimbs. We required no other surface preparation to obtain adequate signal to noise for triggering.

The animal was centered in a 12-cm ID Helmholtz transmitter coil. A laboratory-built 1.5-cm OD circular two-turn surface receiver coil was placed on the chest over the apex of the LV. The transmitter and receiver coils were placed orthogonally to achieve 40 dB isolation between them.

Imaging was performed using a 4.7 T MR spectrometer comprised of a 40-cm Oxford Instruments magnet, Varian INOVA console, and 26-cm Oxford Instruments self-shielded gradients. (Oxford Instruments; Oxford, UK and Varian Associates; Palo Alto, CA). To ensure that MR examinations provided data that were comparable between subjects and from examination to examination, a standard protocol was followed for image acquisition (24). The examination began with a sagittal image to locate the position of the heart in the chest. The imaging parameters were then obtained to define the interventricular septum. Images parallel to the interventricular septum in the LV were then acquired, yielding a vertical long-axis view. Images acquired through the long axis of this image resulted in a horizontal long-axis view. From this view, the tricuspid and mitral valve planes were defined in the end-diastolic frame, and true short-axis planes were determined. We have found this method of defining the short-axis view to be reproducible to within a few degrees in each axis.

For tagging, we used an ECG-triggered DANTE (10, 29) gradient echo cine sequence with the following parameters: echo time (TE)/repetition time (TR) 5/10 ms, flip angle 10°, DANTE train of 32 pulses, DANTE pulse width of 768 µs. The tagging gradient was 5.33 mT/m with a tag delay of 0.2 ms. These parameters yielded a grid spacing of 1.2 mm with a temporal resolution between cine frames of 9.4 ms. In-plane resolution was 234 × 234 µm from a field of view of 3 cm² and acquisition matrix of 128 × 128. ECG trigger inhibit was chosen at 500 ms. This sequence was used to acquire images through 110% of the cardiac cycle in the short-axis plane at three levels in three separate acquisitions: 1) a basal level, 2) a midventricular level, and 3) an apical level. The midventricular level was chosen at 50% of the distance between the atrioventricular valve plane and the apex. The base and apex levels were chosen 2 mm in either direction of the midventricular plane. The total murine ventricular length was typically 7–8 mm.

Image acquisition in humans. Ten healthy volunteers (ages 23–41 yr; 5 males, 5 females) with no prior history of heart disease or other chronic disease were recruited for this study. All volunteers were verbally screened for evidence of chronic or acute illness, including heart disease. The local institutional review board approved the study and all subjects gave written informed consent. Imaging was performed using a 1.5 T MRI scanner (Magnetom SP4000, Siemens Medical Systems; Iselin, NJ). Normal cardiac structure and wall motion were confirmed with a standard cine-MRI of the heart before tagging. To ensure that MR examinations provided data that was comparable between subjects and from examination to examination, the same protocol used for the mice (but scaled for the human heart) was followed for image acquisition (23a, 24). An ECG-triggered SPAMM tagged gradient echo cine sequence (TE/TR 14/42 ms, flip angle 20°, grid spacing 7 mm, temporal resolution 42 ms, slice thickness 7 mm, slice gap 3 mm, scan matrix 128 × 256, image acquisition resolution 1.2 × 2.4 mm) was then used to acquire images throughout systole and early diastole in the short-axis plane at basal, mid, and apical levels of the ventricle. The midventricular level was selected at 50% of the ventricular length from valve plane to apex. The basal slice was selected as the highest short-axis level where through-plane motion of the atrium did not contaminate the slice, and the apical level was selected as the most apical level that contained a visible blood pool throughout systole.

Comparison between mouse and human imaging protocols. The imaging protocols described were designed to be equivalent in spatial and temporal resolution accounting for heart size. In the human hearts the tag spacing of 7 mm allowed definition of 1–2 tag lines across the normal thickness myocardium (~1 cm in thickness). In the mouse the tag spacing of 1.2 mm also allowed definition of 1–2 tag lines across the mouse myocardial wall (1–2 mm in thickness). The temporal resolution in the human (42 ms) allowed acquisition of approximately seven frames throughout systole (generally about 300 ms in duration with a heart rate of 60 beats/min), whereas in the mouse the temporal resolution of 9.4 ms allowed acquisition of approximately six to seven frames throughout systole with a heart rate of 300–400 beats/min.

Image analysis of mice and humans. Figure 1 shows a sample image from a mouse and Fig. 2 shows a sample image from a human. Tag spacing relative to wall thickness at end diastole is about 1:1 in both the human and the mouse. The intersections of the grid lines shown in these figures are tracked throughout systole to define the motion of small segments of myocardium as described below.

Analysis software (TAGASIST) developed by the authors (9) was used to analyze all images. The end-diastolic image (the first image in the ECG-triggered sequence) served as a baseline reference, whereas grid intersection points were identified visually and marked manually in each temporal frame of the tagged cine images. The myocardium was divided into triangular tissue elements using sets of adjacent grid intersection points as vertices. The epicardial and endocardial contours were traced manually for each temporal frame. The end-diastolic frame was identified as the first frame of the ECG-triggered sequence, and the end-systolic frame was identified as the frame with the smallest blood pool before the reversal of ventricular twist angle during isovolumic relaxation. The epicardial boundary was used to calculate the center of mass of the LV at each time point, and the epicardial and endocardial borders together were used to exclude grid intersections falling outside the myocardium.
from further analysis. This procedure was repeated for the three short axis levels of the LV.

Twist analysis. The local twist angle in slice $i$ ($\theta_i$), was defined as the angle between radial lines connecting the center of mass of the LV to the centroid of a specific triangular element at end diastole and at any other time during systole (Fig. 3). The centroid of each triangular element was calculated and referenced to the center of mass of the LV for each temporal frame throughout systole. Because the base and apex of the ventricle twist in opposite directions, we also calculated the net twist angle for the ventricle. The net ventricular twist angle ($\theta_{\text{net}}$) was defined as the difference between the twist angle in the apex ($\theta_{\text{apex}}$) and the twist angle in the most basal slice ($\theta_{\text{base}}$) (thus $\theta_{\text{net}} = \theta_{\text{apex}} - \theta_{\text{base}}$). Local twist angle and net ventricular twist angles for each short axis slice were calculated as a function of percent systole to normalize for differences in heart rate. The $\theta_{\text{net}}$ was also expressed as a function of ventricular length (torsion) to account for differences in length between human and murine ventricles. Because of limited image quality in late systole in some subjects, only the first 80% of systole was analyzed for all subjects.

Reproducibility and statistical analysis. Data are presented as the means $\pm$ SD, and where comparisons are made between ventricular slices, single-factor ANOVA was used to determine statistical significance. In all cases a value of $P < 0.05$ was considered to indicate statistical significance.

We previously had reported reproducibility (interstudy as well as inter- and intraobserver) for human data using these methods (23a). To obtain a limited assessment of intraobserver variability for the mouse, one mouse apical images were analyzed twice by the same observer. To assess the interobserver variability a single mouse apical images were manually labeled as described above by two observers.

Fig. 1. Sample tagged image from mouse at end diastole (A) and during systole (B). One centimeter mark is shown for scale. Tag spacing is 1.2 mm. Note tag deformation during systole compared with at end diastole.

Fig. 2. Sample tagged image from human at end diastole (A) and end systole (B). A 1-cm mark is shown for scale. Tag spacing is 7 mm. Note greater deformation of tags in larger human heart compared with mouse (Fig. 1).
whole slice \( \phi_i(t) \) values were compared for differences at each time point in systole.

RESULTS

Systolic twist pattern. Systolic twist was qualitatively similar in both human and mouse as shown in Figs. 4 and 5. The most apical slice in each subject group rotates counterclockwise (positive angle) throughout systole. The midventricular slice rotates counterclockwise in early systole and plateaus for the remainder of systole. The most basal slice rotates counterclockwise in early systole and then turns clockwise in later systole.

Systolic net twist angle. The net twist angles throughout systole (apex-base) are shown in Fig. 6. Although the patterns are similar, the human twist angle magnitude is significantly greater than that of the mouse \( (P < 0.05) \) because of the larger length of the ventricle in humans. Therefore, we also described the ventricular torsion angle, defined as the twist angle per unit length of the ventricle, expressed as degrees per centimeter. The net torsion angle throughout systole (apex-base) is similar in magnitude between mouse and human as shown in Fig. 7. Typical mouse ventricular length was 7–8 mm, whereas typical human ventricular length was 60–70 mm. Standard deviation bars are shown for reference. There was no significant difference between

the mouse and human ventricular torsion angles (degrees/cm) at any point during systole.

Reproducibility. In humans, we have previously found \((23a)\) that the mean interstudy difference for \( \phi_i(t) \) for all segments between serial repeated studies was \( 2.1 \pm 1.6^\circ \). There was no significant difference for all \( \phi_i(t) \) measured in the three repeated studies \( (P = 0.92) \). The mean intraobserver difference in twist in a midventricular slice through seven separate frames of systole was \( <0.05^\circ \). The mean interobserver difference for twist angle in the same slice was \( 0.1 \pm 0.1^\circ \).

For mice the mean intraobserver difference in net twist angle for the apex (7 frames throughout systole) was \( 0.8 \pm 1.7^\circ \). The mean interobserver difference for the apex was \( 0.3 \pm 1.0^\circ \).

DISCUSSION

With the advent of genetically and surgically manipulated murine models of cardiovascular disease, full characterization of mouse cardiovascular performance is key to apply experimentally gained knowledge to our understanding of human heart function. Global cardiac function and cardiac chamber properties have been studied in small animals \((13–14, 18, 22, 31, 34, 42)\) with methods such as echocardiography, cardiac catheteriza-
with fiber angle determinations. Components of strain eras et al. (35), using MR myocardial tagging combined ing and wall thickening has been studied by Rademak-
dium (20). The relationship between cross-fiber shorten-
cleavage planes and cross-fiber strain in the myocar-
wall thickening has been attributed to the existence of
cellular collagen matrix. A large fraction of the observed
in concert but by the twisting or shearing of groups of
not through simple shortening of individual myocytes
torsion (twist normalized to length) (3, 4).

The twisting of the apex with respect to the base,
or midventricular region acting as the transitional
zone. The twisting of the apex with respect to the base,
clockwise in systole, is referred to as torsion, or midventricular
clockwise, with the equatorial
midventricular region acting as the transitional
zone. The twisting of the apex with respect to the base,
clockwise in systole, is referred to as torsion (twist normalized to length) (3, 4).

It is now understood that the myocardium thickens
both parallel and perpendicular to the fiber orientation
were determined. Cross-fiber strain was near zero at
the epicardium but was large at the endocardium and
increased from base to apex. The study concluded that
the primary source of myocardial wall thickening was
the interaction between the different layers of the
myocardium.

In addition to the information provided by global
ventricular measures such as ejection fraction, myocar-
dial mass, and volume, ventricular torsion provides
insight into myocardial function at the cellular level.
For example, in patients with hypertrophic cardiomy-
opathy, diastolic untwisting is prolonged throughout
the course of diastole (26, 33). These findings are
associated with the findings of decreased shortening
velocity and delayed relaxation. Physiologically hyper-
throphied hearts in athletes, however, show normal
rapid systolic twisting and diastolic untwisting (41).
Transplanted human hearts display alterations in sys-
tolic- and diastolic-torsion patterns in early rejection (16,
44). Torsion has also been shown to be altered during
remodeling after myocardial infarction in the pig, sheep,
and rat (21, 23, 38). Ultrastructural alteration of the
extracellular matrix proteins may result in subtle
changes in myocardial function that are not evident
with global ventricular function measurements. There-
fore torsion assessment can be viewed as complement-
tary to the standard array of ventricular function
parameters. Torsion may be measured using tagging as
demonstrated here or with phase contrast MRI to
measure myocardial velocities or displacements (1, 11,
46).

In conclusion, mouse and human systolic twist pat-
tterns are qualitatively similar in pattern but not in
absolute magnitude. Normalization to ventricular
length (torsion), however, results in comparable magni-
ditudes of net ventricular systolic torsion between species
(i.e., torsion angles that are not statistically different)
throughout systole despite a 10-fold difference in heart
length, a much shorter R-R interval(150–200 ms), and a
>200-fold difference in ventricular mass. The fact
that the basic properties of this observation are con-
served from human to mouse supports the hypothesis
that torsion is fundamental to normal ventricular
function. However, the fiber orientation across the wall
of the mouse ventricle has not been studied specifically,
and comparison of torsion between species presented in
this study relies on the assumption that the fiber
orientations are similar. Future work will focus on
characterizing the microstructural and energetic com-
ponents responsible for these similarities, determining
the effect on torsion of diseases that alter the extracel-
lular matrix structure and the effects of altered hemody-
namics on ventricular twist and torsion.

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