Reference values from M-mode and Doppler echocardiography for normal Syrian hamsters

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Abstract Aims Echocardiography has recently been introduced to small animal research, allowing serial measurements of cardiac diseases. In addition, the hamster model has been increasingly used, as it mimics many human heart conditions. However, no reference range of echocardiographic values reflecting normal left ventricular (LV) function exists for hamsters. The purpose of this study was to provide one.

Methods and Results The study group consisted of 118 10-week-old, female, Syrian golden hamsters, which underwent high-resolution echocardiography. LV mass was calculated using the corrected cube formula, and LV systolic and diastolic function were assessed by fractional shortening and mitral inflow pulsed-wave Doppler, respectively. The myocardial performance index (MPI) measured the time spent in isovolumic activity and reflected both systolic and diastolic function. The mean ± SD LV mass, fractional shortening, and MPI were 0.19 ± 0.04 g, 44.7 ± 6.6%, and 0.39 ± 0.1, respectively. E and A waves were differentiated in 52% of all animals. Logistic regression adjusted with a cutoff of 378 bpm revealed that the risk of E/A wave fusion was 35 times greater (95% CI: 12.6; 98.4) in animals with a heart rate > 378 bpm.
Conclusion

This study documents echocardiographic characteristics in normal Syrian hamsters, which can be used as control values for future studies.
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Introduction

The hamster has become a popular model for many human heart diseases and has been used to test a variety of therapies.\(^1\)\(^-\)\(^4\) Traditional methods for quantifying left ventricular (LV) systolic and diastolic function in hamsters have been based on invasive measurements and on autopsy findings. However, the use of echocardiography in small animal research was introduced over the last decade.\(^5\)\(^-\)\(^7\) With the development of more powerful machines and software, echocardiography has emerged as a dominant non-invasive method of evaluation, allowing serial measurements of indicators of cardiac diseases\(^8\) as well as the response to various therapeutic interventions.

Both bidimensional and M-mode echocardiography are useful in small rodent models, particularly for the serial assessment of LV chamber size, mass, and systolic function.\(^9\) In addition, pulsed-wave Doppler (PWD) echocardiography has been used in these models for the in vivo assessment of diastolic function\(^10,11\) and global LV performance.\(^12,13\) However, a reference range of echocardiographic values reflecting normal LV function in hamsters is still lacking. Therefore, the purpose of this study was to use echocardiography to evaluate systolic and diastolic myocardial function in normal Syrian hamsters, producing a reference range of normal echocardiographic values that can be used as control values for future studies.

Methods

Study group

The study group consisted of 118 10-week-old, female, outbred Syrian golden hamsters (Mesocricetus auratus), weighing 73–133 g. The Syrian hamsters were obtained from the University of São Paulo Medical School Central Facility and maintained with food and water ad libitum. They were handled according to local regulations and research protocols approved by our internal review board, which conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996).

Echocardiographic studies

Transthoracic echocardiography was performed using a Sequoia 512 machine (Acuson, Mountain View, CA) equipped with a 13-MHz linear-array transducer, which was gently placed on the animals’ shaved left hemithorax. Care was taken to avoid applying excessive pressure, which can induce bradycardia and cardiac arrest. Digital DICOM storage was performed; in addition, the images were recorded on videotape, and measurements were made off-line. Hamsters were anesthetized with pentobarbital (50 mg/kg) administered by intraperitoneal injection. A single channel electrocardiogram also was obtained; electrodes were placed on the upper and lower limbs of the animal and heart rate was recorded for at least 3 consecutive R-R intervals.

A 1-cm region of interest was expanded, allowing high frame rates, and the gain and compression were set for optimal imaging. The heart was first imaged in the bidimensional (2-D) mode, in the parasternal long-axis view followed by the short-axis and apical 4-chamber views. Two-dimensional guided M-mode imaging\(^8,9\) was used to measure the LV end-systolic (LVESD) and end-diastolic (LVEDD) diameters, interventricular septal thickness (IVST) during diastole and posterior wall thickness (PWT) during diastole, all in the short-axis view at the level of the papillary muscles. The angle of interrogation of the M-mode beam was carefully aligned to be perpendicular to the LV walls at the anteroposterior axis; LVEDD, IVST and PWT were measured by the leading edge method, and the LVESD was measured at the posterior wall’s time of maximum anterior motion. All measurements were done according to the American Society of Echocardiography recommendations\(^14\) by the same experienced observer. Three representative cardiac cycles were analyzed and averaged for each measurement. LV systolic function was assessed by fractional shortening (FS), which was calculated from the M-mode echocardiogram\(^15\) using the equation:

\[
FS(\%) = \left( \frac{LVEDD - LVESD}{LVEDD} \right) \times 100
\]
LV mass was calculated with the corrected cube function formula from the M-mode echocardiogram, assuming a spherical LV geometry:

$$\text{LV Mass}(g) = 1.04 \left[ \left( \text{LVEDD} + \text{PWT} + \text{IVST} \right)^3 - \text{LVEDD}^3 \right] 0.8,$$

where 1.04 is the specific gravity of the myocardium.

Doppler studies were performed to assess LV inflow and outflow; a 4-chamber view of the heart was acquired to obtain flow parallel to the sample volume (usually within 15 degrees). Color Doppler flow images were obtained by centering the sampling area to the region of interest, thus making it possible to evaluate potential valvular dysfunction. Pulsed-wave Doppler spectra of mitral inflow were recorded with the sample volume set to the smallest available size (1 mm) and placed at the position on the mitral valve leaflets at which the velocity was maximal.

The peak velocity of early (E) and late (A) diastolic filling, E/A, deceleration time of the E wave (DT), and isovolumic relaxation time (IVRT) were obtained from the mitral inflow recordings, as previously described. The DT and IVRT were obtained in animals when the E and A waves were not fused. The myocardial performance index (MPI) is the ratio of total time spent in isovolumic activity (isovolumic contraction time and isovolumic relaxation time) to the ejection time (ET). The isovolumic contraction time, isovolumic relaxation time and ejection time were measured from mitral inflow and LV outflow time intervals (Fig. 1). Both M-mode and PWD tracings were recorded at a sweep speed of 200 mm/s. Doppler time indices (DT, IVRT and ET) were corrected for heart rate by dividing them by the square root of the R-R interval (in seconds).

The experimental protocol was approved by the Animal Research Committee of the Heart Institute (InCor), University of São Paulo Medical School, São Paulo, Brazil.

**Statistical analysis**

For each echocardiographic parameter assessed, reference limits for the following percentiles were obtained from M-mode and PWD echocardiograms: 99th, 95th, 90th, 75th, 50th, 25th, 10th, 5th, and 1st; the mean, standard deviation, range, 95% confidence intervals, and coefficient of variation of each assessed parameter were also determined. The Pearson correlation coefficient was used to examine relationships between both the heart rate and weight and the LVEDD, LVESD, IVST, PWT, and LV mass. The Student t test and logistic regression were used to examine the relationship between the frequency of E/A fusion and heart rate. Statistical differences were considered significant at a $P$ value <0.05. Data were analyzed with the statistical software SAS V.6.11 (SAS Institute Inc, Cary, NC).

**Results**

None of the animals died during the study. Tables 1—3 summarize the percentiles and other reference echocardiographic values determined from M-mode and PWD echocardiography. Doppler flow studies were successfully performed in all animals in which they were attempted. Color flow imaging clearly demonstrated laminar LV inflow and outflow, without evidence of valvular dysfunction.

No significant relationship was found between heart rate and the LVEDD, LVESD, IVST, PWT, and LV mass ($P = 0.544$); however, these parameters correlated with body weight ($P = 0.0295$). The heart rate was related to the occurrence of E/A fusion, as the greater the heart rate, the greater the frequency of E and A wave fusion ($P = 0.0001$). The E and A waves could be...
differentiated in 52% of all of the hamsters, and the two waves could always be identified when the heart rate was below 378 bpm. Logistic regression adjusted with a cutoff of 378 bpm revealed that the risk of E/A fusion was 35 times greater (95%CI: 12.6; 98.4) in animals with a heart rate >378 bpm.

**Discussion**

Hamsters have been used in cardiac research for several reasons: their brief life span, the low cost of purchasing and housing them, the ability to perform repeated measurements in the same animal, and their similarity to humans in regard to cardiac anatomy, physiology, and age-related changes.\(^1\)\(^-\)\(^4\)

We chose to analyze the cardiac function of young (10-week-old) hamsters in the present study, as echocardiographic data from animals of this age will be useful as control (reference) values for future studies.

Quantitative high-frame-rate echocardiography has become well established as a non-invasive means of carrying out in vivo dynamic assessment of LV anatomy and function in humans as well as in animals. In order to obtain a clear spectral display and a true velocity profile, it is mandatory that the sample volume be positioned close to parallel to the direction of blood flow. However, it is often difficult to obtain a typical apical 4-chamber view in small animals. In addition, the high heart rate in small animals complicates the task of assessing diastolic function, as it leads to a higher frequency of the E and A wave fusion. In normal adult humans, the average E/A ratio ranges from 1 to 2.\(^{21}\) Masuyama et al.\(^{22}\) reported that the normal E/A value in rats is about 2.0, whereas Pollick et al.\(^6\) reported a normal E/A ratio of 2.4±0.66 in mice. The E/A ratio was greater than 1 in all of the hamsters in our study. Prunier et al.\(^{23}\) studied diastolic function after myocardial infarction in rats; these authors could distinguish E and A waves at a maximum heart rate of 300-340 bpm. In our study, the A wave could be differentiated in 52.5% of the animals and had a wide coefficient of variation, even with the use of high frame rates. As expected, higher heart rates were associated with higher rates of E/A fusion (\(P=0.0001\)). In analyzing this relationship, we found that the risk of E/A fusion was 35 times greater in animals with a heart rate greater than 378 bpm than in animals with a heart rate of less.

<table>
<thead>
<tr>
<th>Percentile</th>
<th>99th</th>
<th>95th</th>
<th>90th</th>
<th>75th</th>
<th>50th</th>
<th>25th</th>
<th>10th</th>
<th>5th</th>
<th>1st</th>
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<tbody>
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<td>BW (g)</td>
<td>127</td>
<td>120</td>
<td>116</td>
<td>107</td>
<td>101</td>
<td>96</td>
<td>91</td>
<td>89</td>
<td>81</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>526</td>
<td>508</td>
<td>491</td>
<td>417</td>
<td>368</td>
<td>327</td>
<td>290</td>
<td>271</td>
<td>243</td>
</tr>
<tr>
<td>LVEDD (cm)</td>
<td>0.48</td>
<td>0.47</td>
<td>0.46</td>
<td>0.43</td>
<td>0.41</td>
<td>0.39</td>
<td>0.36</td>
<td>0.35</td>
<td>0.34</td>
</tr>
<tr>
<td>LVEDD/weight (cm/g)</td>
<td>0.52</td>
<td>0.48</td>
<td>0.47</td>
<td>0.43</td>
<td>0.40</td>
<td>0.37</td>
<td>0.35</td>
<td>0.32</td>
<td>0.29</td>
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<tr>
<td>LVESD (cm)</td>
<td>0.30</td>
<td>0.30</td>
<td>0.28</td>
<td>0.26</td>
<td>0.22</td>
<td>0.20</td>
<td>0.18</td>
<td>0.16</td>
<td>0.14</td>
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<tr>
<td>LVESD/weight (cm/g)</td>
<td>0.32</td>
<td>0.30</td>
<td>0.28</td>
<td>0.25</td>
<td>0.22</td>
<td>0.19</td>
<td>0.17</td>
<td>0.16</td>
<td>0.15</td>
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<tr>
<td>IVST (cm)</td>
<td>0.11</td>
<td>0.11</td>
<td>0.11</td>
<td>0.11</td>
<td>0.10</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
<td>0.08</td>
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<td>IVST/weight (cm/g)</td>
<td>0.12</td>
<td>0.12</td>
<td>0.11</td>
<td>0.11</td>
<td>0.10</td>
<td>0.09</td>
<td>0.09</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>PWT (cm)</td>
<td>0.12</td>
<td>0.11</td>
<td>0.11</td>
<td>0.11</td>
<td>0.10</td>
<td>0.10</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>PWT/weight (cm/g)</td>
<td>0.13</td>
<td>0.12</td>
<td>0.11</td>
<td>0.11</td>
<td>0.10</td>
<td>0.09</td>
<td>0.09</td>
<td>0.08</td>
<td>0.08</td>
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<tr>
<td>LV mass (g)</td>
<td>0.27</td>
<td>0.25</td>
<td>0.24</td>
<td>0.21</td>
<td>0.18</td>
<td>0.16</td>
<td>0.14</td>
<td>0.13</td>
<td>0.11</td>
</tr>
<tr>
<td>LV mass/weight</td>
<td>0.26</td>
<td>0.25</td>
<td>0.23</td>
<td>0.21</td>
<td>0.18</td>
<td>0.15</td>
<td>0.14</td>
<td>0.12</td>
<td>0.11</td>
</tr>
<tr>
<td>FS (%)</td>
<td>59.2</td>
<td>56.3</td>
<td>52.8</td>
<td>49.5</td>
<td>44.2</td>
<td>39.7</td>
<td>36.2</td>
<td>35.3</td>
<td>32.2</td>
</tr>
<tr>
<td>E (cm/s)</td>
<td>1.07</td>
<td>1.02</td>
<td>0.96</td>
<td>0.83</td>
<td>0.76</td>
<td>0.68</td>
<td>0.62</td>
<td>0.56</td>
<td>0.48</td>
</tr>
<tr>
<td>A (cm/s)</td>
<td>0.87</td>
<td>0.60</td>
<td>0.56</td>
<td>0.44</td>
<td>0.37</td>
<td>0.32</td>
<td>0.28</td>
<td>0.27</td>
<td>0.19</td>
</tr>
<tr>
<td>E/A</td>
<td>3.29</td>
<td>2.87</td>
<td>2.49</td>
<td>2.23</td>
<td>1.91</td>
<td>1.53</td>
<td>1.28</td>
<td>1.25</td>
<td>1.17</td>
</tr>
<tr>
<td>DT (ms)</td>
<td>71</td>
<td>64</td>
<td>57</td>
<td>51</td>
<td>45</td>
<td>39</td>
<td>38</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td>IVRT (ms)</td>
<td>34</td>
<td>30</td>
<td>29</td>
<td>27</td>
<td>24</td>
<td>22</td>
<td>19</td>
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<td>16</td>
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<tr>
<td>a (ms)</td>
<td>133</td>
<td>120</td>
<td>114</td>
<td>99</td>
<td>92</td>
<td>83</td>
<td>72</td>
<td>67</td>
<td>64</td>
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<tr>
<td>ET (ms)</td>
<td>92</td>
<td>89</td>
<td>84</td>
<td>72</td>
<td>66</td>
<td>59</td>
<td>52</td>
<td>50</td>
<td>46</td>
</tr>
<tr>
<td>MPI</td>
<td>0.64</td>
<td>0.57</td>
<td>0.54</td>
<td>0.46</td>
<td>0.38</td>
<td>0.32</td>
<td>0.26</td>
<td>0.23</td>
<td>0.20</td>
</tr>
</tbody>
</table>

BW, body weight; HR, heart rate; LVEDD, left-ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; IVST, interventricular septum thickness; PWT, posterior wall thickness; FS, fractional shortening; E, peak velocity of early diastolic filling; A, peak velocity of late diastolic filling, DT, deceleration time of E wave; IVRT, isovolumic relaxation time; a, interval between cessation and onset of atrioventricular valve flow; ET, ejection time; and MPI, myocardial performance index.
than 378 bpm. Numerous factors influence the Doppler indices of LV filling, including contractility, loading conditions, ischemia, mitral regurgitation, LV mass, aging, and particularly the heart rate in these animals. Thus, the evaluation of diastolic function remains a challenge in small animals.

The myocardial performance index is a relatively new measurement of Doppler time intervals, which is simple and easily recordable. It appears to correlate well with invasive measures of systolic and diastolic function in both humans and animals and has been described as a more effective measure of global cardiac function than systolic or diastolic indices alone. Furthermore, this index is of prognostic value after myocardial infarction and allows differentiation between a normal and pseudonormal mitral flow pattern.

In our study, calculation of the LV mass was based on measurements from the parasternal view, which was easily obtained in all of the animals; in addition, all parameters that contribute to the calculation of LV mass, such as the LVEDD, IVST, and PWT, had a low coefficient of variation. The use of anesthesia (either injectable or inhaled), which causes slowing of the heart rate, increases one’s ability to differentiate transmitral Doppler E and A waves. Masuyama et al. and Tanaka et al. showed that the use of ketamine-xylazine anesthesia decreased the heart rate by about 40%, which could have influenced cardiac performance in the animals studied. Conversely, studies under chloral hydrate yield heart rates closer to those in conscious animals, with better reproducibility. However, this agent increases the velocity of circumferential fiber shortening, suggesting an associated increase in the release of endogenous catecholamines. In our study, the heart rate of our animals was similar to that observed in other small animal experiments, suggesting that our anesthetic regimen only minimally affected the physiological state.

Table 2  Values from M-mode echocardiography in normal Syrian hamsters

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Absolute range</th>
<th>Range corrected for weight</th>
<th>Lower and upper 95% confidence intervals</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDD (cm)</td>
<td>0.41 ± 0.04</td>
<td>0.31–0.51</td>
<td>0.28–0.59</td>
<td>0.404–0.417</td>
<td>8.74</td>
</tr>
<tr>
<td>LVEDD (cm)</td>
<td>0.23 ± 0.04</td>
<td>0.13–0.31</td>
<td>0.12–0.38</td>
<td>0.221–0.236</td>
<td>17.63</td>
</tr>
<tr>
<td>IVST (cm)</td>
<td>0.10 ± 0.01</td>
<td>0.08–0.11</td>
<td>0.06–0.13</td>
<td>0.099–0.102</td>
<td>8.19</td>
</tr>
<tr>
<td>PWT (cm)</td>
<td>0.10 ± 0.01</td>
<td>0.80–0.12</td>
<td>0.80–0.13</td>
<td>0.100–0.104</td>
<td>8.70</td>
</tr>
<tr>
<td>LV mass (g)</td>
<td>0.19 ± 0.04</td>
<td>0.11–0.29</td>
<td>0.11–0.29</td>
<td>0.179–0.192</td>
<td>20.15</td>
</tr>
<tr>
<td>FS (%)</td>
<td>44.7 ± 6.6</td>
<td>28.3–57.6</td>
<td>—</td>
<td>43.48–45.90</td>
<td>14.86</td>
</tr>
</tbody>
</table>

 LVEDD, left ventricular end-diastolic dimension; LVEDD, left ventricular end-systolic dimension; IVST, interventricular septum thickness; PWT, posterior wall thickness; and FS, fractional shortening.

Table 3  Mitral inflow velocities and diastolic parameters in normal Syrian hamsters

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Absolute range</th>
<th>Mean ± SD of time intervals corrected by heart rate</th>
<th>Lower and upper 95% confidence intervals</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak E (m/s)</td>
<td>0.77 ± 0.13</td>
<td>0.45–1.08</td>
<td>—</td>
<td>0.74–0.79</td>
<td>16.54</td>
</tr>
<tr>
<td>Peak A (m/s)</td>
<td>0.40 ± 0.12</td>
<td>0.19–0.60</td>
<td>—</td>
<td>0.37–0.43</td>
<td>30.23</td>
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<tr>
<td>E/A</td>
<td>1.92 ± 0.47</td>
<td>1.2–3.3</td>
<td>—</td>
<td>1.80–2.04</td>
<td>24.58</td>
</tr>
<tr>
<td>DT (ms)</td>
<td>45.8 ± 8.7</td>
<td>30–71</td>
<td>104.9 ± 16.3</td>
<td>43.7–47.9</td>
<td>18.62</td>
</tr>
<tr>
<td>IVRT (ms)</td>
<td>24.4 ± 3.7</td>
<td>16–34</td>
<td>59.9 ± 6.4</td>
<td>23.7–25.1</td>
<td>15.33</td>
</tr>
<tr>
<td>a (ms)</td>
<td>92.4 ± 15.0</td>
<td>63–133</td>
<td>—</td>
<td>89.6–95.1</td>
<td>16.27</td>
</tr>
<tr>
<td>ET (ms)</td>
<td>66.4 ± 10.8</td>
<td>44.7–92.7</td>
<td>163.8 ± 13.9</td>
<td>64.5–68.4</td>
<td>16.29</td>
</tr>
<tr>
<td>MPI</td>
<td>0.39 ± 0.10</td>
<td>0.18–0.68</td>
<td>—</td>
<td>0.37–0.41</td>
<td>26.26</td>
</tr>
</tbody>
</table>

E, early rapid filling wave; A, filling wave due to atrial contraction; DT, deceleration time of E wave; IVRT, isovolumic relaxation time; a, interval between mitral valve closure and opening; ET, ejection time; and MPI, myocardial performance index.
Conclusions

The present study documents, in detail, the echocardiographic characteristics of LV structure and function in normal Syrian hamsters. The assessed echocardiographic parameters represent a range of normal (reference) values that can be used as controls for comparisons in future investigations.

Acknowledgements

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References