The quantitative evaluation of cardiac structures and major thoracic vessels dimensions by means of lateral contrast radiography in Wistar albino rats (*Rattus norvegicus*)

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**Article Info**

**Article History**
Received: 14.02.2022
Accepted: 27.09.2022
DOI: 10.33988/auvfd.895668

**Keywords**
Cardiac structures
Contrast radiography
Major thoracic vessels
Wistar albino rat

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The aim of this study was to define reference values for vertebral heart score (VHS) and modified left atrium (LA)-VHS, cardiac structures, and major thoracic vessels measurements and ratios obtained from thoracic contrast radiography Wistar albino rats. VHS, modified LA-VHS, left (LV) and right (RV) ventricles, interventricular septum (IVS), aorta (Ao), caudal vena cava (CaVC), and fourth thoracic vertebrae (v) length (T4) were measured from left lateral thoracic contrast radiographs of 50 young, healthy adult male Wistar albino rats. LV/T4, RV/T4, IVS/T4, Ao/T4, CaVC/T4, and CaVC/Ao ratios were calculated from these values. Measurements were performed by two observers unaware of the signalment data for interobserver repeatability analysis. Median values and references ranges were 8.2v (7.4-9.1) for VHS, 1.2 (1.0-1.5) for modified LA-VHS, 7.8 mm (6.2-9.1) for LV, 3.4 mm (2.8-4.5) for RV, 2.1 mm (1.8-3.0) for IVS, 2.1 mm (1.8-2.8) for Ao, 2.2 mm (1.7-3.0) for CaVC, 4 mm (3.5-4.5) for T4, 2 (1.5-2.3) for LV/T4, 0.85 (0.68-1.22) for RV/T4, 0.52 (0.42-0.83) for IVS/T4, 0.53 (0.42-0.75) for Ao/T4, 0.55 (0.45-0.7) for CaVC/T4 and 1.05 (0.74-1.13) for CaVC/Ao. Further studies are now needed to determine whether measurement values obtained from contrast radiography in rats are useful in the diagnosis of cardiomyopathy and heart failure. The radiographic measurement values presented in this study can be used as a reference baseline for both pet and laboratory rats.

**Introduction**

Cardiac diseases are seen in both pet (12) and laboratory (36) rats. Spontaneous cardiomyopathy is reported to be relatively common in elderly rats, and the presence of left atrial and ventricular thrombosis is associated with this cardiac disease (30). Rat models are frequently used in cardiovascular research. These models include coronary ligation-induced cardiac hypertrophy, aortic banding (overload-induced cardiac hypertrophy), diabetic cardiomyopathy, the desoxycorticosterone acetate/salt model of hypertension, renal ischemia via renal artery stenosis, and aortocaval fistula (3, 10). Imaging methods are important for the diagnosis and follow-up of the progression of cardiac diseases in both pet rats and experimental cardiovascular rat models. Although echocardiography is the primary diagnostic method in cardiac diseases, it is not routinely used in rats. The size and shape of the heart and thoracic vessels can be evaluated using thoracic radiography (21), which is easier to perform and less expensive than echocardiography.

Since 1995, a quantitative assessment of the size of the cardiac silhouette from thoracic radiography has been performed via the vertebral heart score (VHS) system, which is simple, objective, and repeatable (8). Using this system, cardiac axes measured from thoracic radiographic views are compared with the length of specific thoracic vertebrae, thus minimizing the error associated with subjective interpretations and interpatient variability (37).
Normal VHS values have been established for Sprague-Dawley and Wistar albino rats (13, 15), as well as for various other animal species (5, 11, 14, 16, 17, 26, 32, 37, 38).

Radiographic indices that permit evaluation of the left atrium (LA) at lateral thoracic radiography are available. Vertebral left atrial size (29) and radiographic left atrial dimension (34) are widely recognized indices for dogs. LA-VHS, modified from VHS, was defined by Schober et al. (35) for cats. However, these indices have not been reported for rats.

Obtaining thoracic radiography at the peak of inspiration in rats is problematic due to their high respiration rate (13). The cranial border of the cardiac silhouette on lateral radiographs is also insufficiently clear due to soft tissue opacity of the cranial and ventral mediastinum. The apex of the heart may be indistinct on radiographs obtained during expiration as it may be located beyond the diaphragm. These factors can all adversely affect VHS measurements in rats. Our study hypothesis was that the sizes of the heart and LA can be directly evaluated with their distinct borders and that the widths of the left (LV) and right (RV) ventricles, the thickness of the interventricular septum (IVS), the widths of the aorta (Ao) and caudal vena cava (CaVC) can be measured using contrast radiography, independently of the cardiac silhouette.

The aim of this study was to define normal values for radiographic indices (VHS and modified LA-VHS), measurements (LV, RV, IVS, Ao, CaVC, fourth thoracic vertebrae length (T4)), and ratios (LV/T4, RV/T4, IVS/T4, Ao/T4, CaVC/T4, CaVC/Ao) obtained from left lateral thoracic contrast radiography of 50 healthy, adult male Wistar albino rats.

Materials and Methods

Animals: The experimental protocol was approved by the Akdeniz University animal care ethics committee on the use of animals, Türkiye (No: B.30.2.AKD.0.05.07.00/27). Fifty healthy, young adult, intact male rats (Rattus Norvegicus, Albinus, Wistar) were housed in the Akdeniz University Experimental Research and Application Center (Türkiye), in groups of four to six animals (of the same sex) at 50-60% humidity and 20-21°C in a 12-h dark/light cycle. Standard rat chow and free access to water were provided.

Rats underwent daily physical examinations during the study. Neither cardiovascular nor pulmonary abnormalities (murmur, arrhythmia, abnormal respiratory sounds, etc.) were detected in any of the rats included in the study, and their hydration status was normal.

Anesthesia protocol: Radiographs were obtained from all rats under general anesthesia. The anesthesia protocol was applied as previously described by Dias et al. (13). Briefly, the rats were placed in an induction chamber, and anesthesia was induced using 5% isoflurane (Aerrane Volatili®, Eczacibas-Baxter, Istanbul, Türkiye) plus oxygen (2 L/min). Anesthesia was maintained using 1–3% isoflurane delivered in oxygen at 1 L/min, by means of a small face mask and non-rebreathing circuit. All animals maintained spontaneous breathing throughout the entire procedure, except for the exposure time. Intravenous access was established in the tail vein of each animal under anesthesia for the administration of contrast medium.

Thoracic contrast radiography: Each animal was imaged in the left lateral projection (X-ray tube: ORIX-65, Ardel®, Istanbul, Türkiye. Parameters: 65 kVp, 8 mA, 0.1 s, 30-cm film-focus distance). The animal was placed lying on its left side on the X-ray cassette. The thoracic legs were then pulled cranially, and the beam was centered at the level of the thorax between the scapulohumeral joint and the last rib. Contrast radiography was performed after plain radiography (Figure 1A). However, radiographic measurements were performed only from contrast radiography. Contrast radiographs were obtained by bolus injection of 0.75 ml of non-ionic opaque contrast agent (300 mg I/ml, Iohexol, Omnipaque®, Opakim, Türkiye) from the tail vein. Exposure was performed as soon as the contrast agent injection was completed (Figure 2B). A positive pressure breath-hold was performed at the time of radiographic exposure in order to obtain radiographic images at the inspiration peak. While positive pressure breath-hold was being employed, care was taken not to cause pulmonary hyperinflation, to preserve the opacity of the CaVC, and not to cause excessive caudal displacement of the diaphragm. Otherwise, all animals continued breathing spontaneously throughout the entire procedure.

Radiographs with substantial symmetry/superposition of the ribs and scapulae and widely accessible cardiothoracic structures with adequate contrast and pulmonary inflation were considered to be of sufficient diagnostic quality.

Radiographic images obtained using a computed radiography reader (FCR Prima T2, FujiFilm®, Tokyo, Japan) were stored. These radiographic images were anonymized and randomized and then evaluated by two observers using commercially available computer software (Image Intelligence™, FujiFilm®, Tokyo, Japan). The observers were blinded to the age and body weight of each rat. They were able to manipulate the images as needed, including by changing the window width, window level, and magnification, and measurements were made of the radiographic cardiac indices and ratios. Corresponding mean radiographic cardiac indices (VHS and modified LA-VHS), measurements (LV, RV, IVS, Ao, CaVC, T4), and ratios (LV/T4, RV/T4, IVS/T4, Ao/T4, CaVC/T4, CaVC/Ao) were calculated for each observer, these being used for statistical purposes.
Measurements of radiographic cardiac indices and structures and major thoracic vessel: The heart was assessed by measuring the VHS, as described by Buchanan (9). On left lateral contrast rat radiographs, the cardiac long axis (L) was measured from the ventral border of the left mainstem bronchus (carina) to the cardiac apex. The cardiac short axis (S) was determined by measuring the widest distance between the cranial and caudal borders of the heart. Commercially available computer software was used to apply a 90-degree rotation between L and S. These two axes were then repositioned over the thoracic vertebrae from the cranial edge of T4, parallel to the vertebral column, and each length was then expressed in terms of the number of thoracic vertebrae (v) to the nearest 0.1v. The sum of the two values was used as VHS (Figure 2A).

The LA-VHS method previously described by Schober et al. (35) was subjected to some modification. A line was drawn between the carina and cardiac apex. The distance between this line and the intersection of the dorsal wall of the CaVC and the caudal border of the heart was measured perpendicular to this line. This measurement was divided by the T4 and used as modified LA-VHS (Figure 2B).

The widest parts of the LV and RV were measured perpendicular to IVS (Fig 2C). IVS thickness was measured from the same level. The widths of the Ao and CaVC were obtained by measuring the distance between the dorsal and ventral borders of each vessel. The Ao was measured caudally to the carina and dorsocaudally to the base of the heart. CaVC was measured immediately caudally to the intersection of the caudal border of the heart with this vessel. T4 was obtained by measuring the distance between the cranial and caudal edges of the vertebral body (21, 22) (Figure 2C). The measurements were performed digitally using the software. Except for VHS and modified LA-VHS, all the data obtained were expressed in mm. LV/T4, RV/T4, IVS/T4, Ao/T4,
CaVC/T4, and CaVC/Ao ratios were calculated from these measurements.

Statistical analysis: All statistical analyses were performed on Statistical Package for the Social Sciences software version 22.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were generated. The normality of data distribution was assessed using the Shapiro-Wilk test. None of the variables were normally distributed, and results were reported as the median and interquartile range (IQR). Correlation between radiographic cardiac indices, measurements, and ratios was calculated using Spearman’s rank-order correlation coefficient. Interobserver variabilities were assessed for radiographic cardiac indices, measurements, and ratios via intraclass correlation coefficient (ICC) estimates and their 95% confidence intervals (CI) based on a single rater, absolute agreement, and a two-way random (interobserver) effect. An ICC value >0.9 was considered excellent, 0.75 to 0.9 good, 0.5 to 0.75 moderate, and <0.5 poor (23).

Results
The study population consisted of 50 healthy, male Wistar albino rats with median age and weight of 12 weeks (10-16) and 370 g (275-460), respectively.

No anesthesia or contrast agent-related complications or death occurred in any animals. Measurements of radiographic cardiac indices and structures and major thoracic vessels were performed uneventfully from all contrast radiographs.

The radiographic cardiac indices and measurements and ratios for cardiac structures, major thoracic vessels, and T4 reported by the observers are summarized in Table 1-3.

Median values and references ranges were 8.2 v (7.4-9.1) for VHS, 1.2 (1.0-1.5) for modified LA-VHS, 7.8 mm (6.2-9.1) for LV, 3.4 mm (2.8-4.5) for RV, 2.1 mm (1.8-3.0) for IVS, 2.1 mm (1.8-2.8) for Ao, 2.2 mm (1.7-3.0) for CaVC, 4 mm (3.5-4.5) for T4, 2 (1.5-2.3) for LV/T4, 0.85 (0.68-1.22) for RV/T4, 0.52 (0.42-0.83) for IVS/T4, 0.53 (0.42-0.75) for Ao/T4, 0.55 (0.45-0.7) for CaVC/T4 and 1.05 (0.74-1.13) for CaVC/Ao.

Table 1. Mean and standard deviation (SD) and 95% confidence interval values (95% CI) for vertebral heart score (VHS) and modified left atrium-vertebral heart score (LA-VHS) recorded by two observers in healthy male Wistar albino rats.

<table>
<thead>
<tr>
<th>Observer</th>
<th>VHS</th>
<th>Modified LA-VHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observer1</td>
<td>Mean±SD 8.21±0.36</td>
<td>1.15±0.12</td>
</tr>
<tr>
<td></td>
<td>95% CI 8.12-8.32</td>
<td>1.11-1.18</td>
</tr>
<tr>
<td>Observer2</td>
<td>Mean±SD 8.25±0.4</td>
<td>1.17±0.13</td>
</tr>
<tr>
<td></td>
<td>95% CI 8.14-8.36</td>
<td>1.13-1.21</td>
</tr>
</tbody>
</table>

Table 2. Mean and standard deviation (SD) and 95% confidence interval values (95% CI) for measurements of radiographic cardiac structures, major thoracic vessels, and fourth thoracic vertebrae recorded by two observers in healthy male Wistar albino rats.

<table>
<thead>
<tr>
<th>Observer</th>
<th>Measurements (mm)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LV</td>
<td>RV</td>
<td>IVS</td>
<td>Ao</td>
<td>CaVC</td>
<td>T4</td>
</tr>
<tr>
<td>Observer1</td>
<td>Mean±SD</td>
<td>7.69±0.69</td>
<td>3.51±0.39</td>
<td>2.09±0.29</td>
<td>2.13±0.29</td>
<td>2.19±0.26</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>7.49-7.88</td>
<td>3.39-3.62</td>
<td>2.0-2.17</td>
<td>2.05-2.21</td>
<td>2.05-2.21</td>
</tr>
<tr>
<td>Observer2</td>
<td>Mean±SD</td>
<td>7.88±0.72</td>
<td>3.48±0.43</td>
<td>2.15±0.26</td>
<td>2.13±0.21</td>
<td>2.26±0.26</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>7.68-8.09</td>
<td>3.35-3.60</td>
<td>2.08-2.23</td>
<td>2.1-2.22</td>
<td>2.18-2.34</td>
</tr>
</tbody>
</table>


Table 3. Mean and standard deviation (SD) and 95% confidence interval values (95% CI) for ratios of cardiac structures, major thoracic vessels, and fourth thoracic vertebrae recorded by two observers in male Wistar albino rats.

<table>
<thead>
<tr>
<th>Observer</th>
<th>LV/T4</th>
<th>RV/T4</th>
<th>IVS/T4</th>
<th>Ao/T4</th>
<th>CaVC/T4</th>
<th>CaVC/Ao</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observer1</td>
<td>Mean±SD</td>
<td>1.94±0.20</td>
<td>0.89±0.12</td>
<td>0.53±0.08</td>
<td>0.54±0.08</td>
<td>0.55±0.06</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>1.89-2.0</td>
<td>0.85-0.92</td>
<td>0.50-0.55</td>
<td>0.52-0.56</td>
<td>0.54-0.57</td>
</tr>
<tr>
<td>Observer2</td>
<td>Mean±SD</td>
<td>1.99±0.21</td>
<td>0.88±0.13</td>
<td>0.55±0.07</td>
<td>0.55±0.06</td>
<td>0.56±0.07</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>1.93-2.05</td>
<td>0.84-0.92</td>
<td>0.52-0.57</td>
<td>0.53-0.56</td>
<td>0.54-0.57</td>
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DOI: 10.33988/aufvd.895668
Positive correlation was observed between VHS and modified LA-VHS ($r = 0.327$, $P < 0.021$), between Ao and CaCV ($r = 0.471$, $P < 0.001$), between LV/T4 and RV/T4 ($r = 0.505$, $P < 0.000$), between RV/T4 and IVS/T4 ($r = 0.321$, $P < 0.023$) between Ao/T4 and CaCV/T4 ($r = 0.472$, $P < 0.001$), and between CaCV/Ao and CaCV/T4 ($r = 0.307$, $P < 0.03$). Negative correlation was observed between CaCV/Ao and Ao/T4 ($r = -0.681$, $P < 0.000$).

ICC evaluation revealed an agreement of 0.91 (95% CI: 0.85-0.95) for VHS, 0.76 (95% CI: 0.66-0.89) for modified LA-VHS, 0.93 (95% CI: 0.89-0.96) for LV, of 0.78 (95% CI: 0.64-0.87) for RV, 0.68 (95% CI: 0.49-0.80) for IVS, 0.78 (95% CI: 0.64-0.87) for Ao, 0.77 (95% CI: 0.63-0.89) for CaVC, 0.92 (95% CI: 0.88-0.97) for T4, 0.95 (95% CI: 0.92-0.97) for LV/T4, 0.86 (95% CI: 0.77-0.92) for RV/T4, 0.75 (95% CI: 0.60-0.85) for IVS/T4, 0.81 (95% CI: 0.69-0.89) for Ao/T4, 0.71 (95% CI: 0.54-0.83) for CaVC/T4, and 0.53 (95% CI: 0.29-0.70) for CaVC/Ao. Agreement between the observers may therefore be considered excellent for VHS, LV, T4, and LV/T4, good for modified LA-VHS, RV, Ao, CaCV, RV/T4, IVS/T4, and Ao/T4, and moderate for IVS, CaVC/T4, and CaVC/Ao.

**Discussion and Conclusion**

VHS, derived from measurements of the cardiac silhouette from thoracic radiographs, is based on a good correlation between silhouette size and the length of the thoracic vertebral body (34). This radiographic cardiac index is affected by respiratory phases and the cardiac cycle (7, 31). While the effect of the cardiac cycle on this index cannot be eliminated, the effect of respiration can be minimized by radiographs obtained at the inspiratory peak. Rats have a high respiration rate and cardiac cycle. Unfortunately, it is not possible to obtain thoracic radiographs in the inspiratory phase in non-intubated rats because intubation is both difficult and not routinely performed (13). In the present study, respiratory movement-related motion artifact was minimized with the radiographs obtained by positive pressure breath-hold at the time of radiographic exposure. The soft tissue opacity of the rat cranial ventral mediastinum due to soft tissues such as the thymus and intrathoracic fat often causes insufficient prominence of the cranial border of the heart (Figure 1A), especially if pulmonary inflation is poor. False VHS values may therefore be obtained from plain radiographs. Moreover, since only the cardiac silhouette is evaluated via this index, high VHS values can be obtained in the presence of pericardial effusion or pericardial fat. VHS is a reliable index for evaluating cardiac enlargement associated with eccentric hypertrophic cardiomyopathy (HCM) in patients with suspected heart disease, particularly due to volume overload (8, 9). However, the cardiac silhouette may be normal in size in concentric HCM, and VHS values can thus be obtained within the reference range. Contrast radiography made it possible to define all the cardiac borders, LV, RV, IVS, Ao, CaCV and the heart was also evaluated directly rather than using the cardiac silhouette in this study (Figure 2A).

VHS values obtained from healthy rats have been reported in previous studies (13, 15). The VHS values presented in the present study were higher than those obtained elsewhere from Sprague-Dawley rats (13). The difference between our values and those reported by Dias et al. (13) can be attributed to VHS being breed-specific in rats as well as dogs (4, 6, 33). Although Dogan et al. (15) also measured VHS in Wistar albino rats, the difference between the VHS values may be due to the fact that they used a different VHS measurement method (27) and obtained VHS values from elderly rats (10 months of age). Further studies are now needed to evaluate whether breed-associated variations and age would affect VHS in rats.

Quantitative evaluation of LA size is important in both pet and laboratory rats with suspected heart disease, similar to cats (19, 35) and dogs (29, 34). LA was subsequently further quantified by applying a VHS system for radiographic measurement of the LA, known as LA-VHS, in cats (35). Rats are comparable to cats due to their relatively standard thoracic conformation and size. The present study, therefore, evaluated the left atrium using the modified LA-VHS. Previous studies involving healthy cats have reported 1.0v (range: 0.72-1.30) (35) and 0.87±0.21v (19) values for LA-VHS. Schober et al. (35) explained that the portion of LA measured on thoracic radiographs is small, that it is difficult to divide a thoracic vertebra into 10 equal segments in cats, and that this is a potential cause of the error. We, therefore, modified the LA-VHS using the LA/T4 ratio. The LA can be evaluated more objectively in rats with this modification.

The cardiac cycle is a factor that directly affects ventricle and IVS measurement values. However, these measurement data may be useful as baseline values in the radiographic differential diagnosis of different forms of cardiomyopathy. Further studies are now needed to determine whether the differential diagnosis of cardiomyopathies (dilated vs hypertrophic, and eccentric vs concentric hypertrophic cardiomyopathy) can be established with these measurement values.

CaVC is visible on lateral thoracic radiographs as it runs from the diaphragm to the caudal border of the cardiac silhouette. Right heart function can be assessed by measuring the CaVC (2). In dogs and cats, dilatation of the CaVC is often used as an indicator of right-sided congestive heart failure (18, 24). In addition, dilatation of these vessels is also useful as a radiographic manifestation of heartworm disease (1, 25, 28), pericardial disease (18), pulmonic stenosis (20), tricuspid valve regurgitation, and dilated cardiomyopathy (24). The ratio of CaVC to other...
anatomic structures may permit quantitative analysis of the CaVC. Quantitative evaluation is therefore performed to compare the average diameter of the CaVC with the diameter of the Ao, and the length of the T4 (20, 24). The CaVC/Ao ratio is sensitive in cats with right heart failure compared to healthy animals (39). The CaVC/T4 ratio is less than 1 in healthy dogs and cats (24, 39). In the present study, the median CaVC/T4 ratio was 0.53. Similar to our results, the CaVC/Ao ratio is approximately equal to 1 in dogs (21).

This study has several limitations. The cardiac cycle, phase of respiration (despite our efforts to obtain radiographs at the inspiratory peak), hydration status, and intra-thoracic pressure may have interfered in the radiographic cardiac indices, measurements, and ratios obtained from this study.

The radiographs generated in this study were diagnostic, permitting accurate radiographic cardiac indices, measurements, and ratios in all animals.

In conclusion, this study established reference values for VHS, modified LA-VHS, and ventricle, IVS, Ao, and CaVC measurements on left lateral contrast thoracic radiographs from healthy, male Wistar albino rats. The results show that lateral thoracic contrast radiography is a simple, uncomplicated, and effective imaging technique for the quantitative evaluation of cardiac size and chambers and major thoracic vessels in healthy rats. Further studies are now needed to determine whether radiographic indices and ratios obtained from contrast radiography in rats are useful in the diagnosis of cardiomyopathy and heart failure. The radiographic measurement values presented in this study can be useful as a reference baseline for both pet and laboratory rats.

Financial Support
This research received no grant from any funding agency/sector.

Conflict of Interest
We declare that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Author Contributions
MAÇ and MK conceived and planned the experiments. MK and MAÇ carried out the experiments. DB helped with experimental protocols. MK and MAÇ contributed to the interpretation of the results. MK and MAÇ wrote the original draft and contributed to reviewing and editing.

Data Availability Statement
The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement
This study was approved by the Animal Care Ethics Committee of Akdeniz University (no: B.30.2.AKD.0.05.07.00/27).

Animal Welfare
The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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