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# Echocardiographic Parameters of Clinically Normal Geriatric Rhesus Macaques (*Macaca mulatta*)

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**The goal of this study was to generate reference intervals for echocardiographic variables in a population of clinically normal geriatric rhesus macaques (*Macaca mulatta*). To do this, we studied 51 animals (age, 18–29 y; weight, 5.24–17.04 kg). The normal values for cardiac indices, including geometry and systolic and diastolic function, were determined by 2D, M-mode, spectral Doppler, and tissue Doppler echocardiography under ketamine hydrochloride sedation. Statistical correlations between the echocardiographic parameters and age, body weight, sex, and heart rate were investigated. All echocardiographic indices were acquired, and their reference intervals were established. Multiple weak to strong correlations emerged between variables and echocardiographic parameters, but no moderate or strong correlations between body weight or sex and these parameters were noted. Of the 51 geriatric rhesus macaques evaluated, 36 (71%) fulfilled the criteria for diastolic dysfunction. Valve regurgitation, especially tricuspid regurgitation (43%), and aortic regurgitation (51%) also were common in geriatric rhesus macaques. Although these findings merit follow-up, they are unlikely to have clinical significance given their prevalence in these apparently healthy animals.**

**Abbreviations:** BSA, body surface area; BW, body weight; HR, heart rate; LVDd, left ventricular internal diameter in diastole; LVDs, left ventricular internal diameter in systole; RI, reference interval

Using animal models to study human diseases has contributed greatly to advancing research and our clinical understanding of human diseases. Although murine models are most commonly used, the intrinsic differences in rodent cardiovascular physiology and their resistance to arrhythmia prevent direct translation to human cardiovascular diseases. Rhesus macaques (*Macaca mulatta*) are thus frequently used as an animal model of human cardiovascular disease because they closely resemble humans in their anatomy and physiology.

Echocardiography is a commonly used, noninvasive antemortem diagnostic tool in veterinary and human cardiology, allowing assessment of chamber size, valvular anatomy, and myocardial function. A few previous studies report echocardiographic parameters of young and older adult rhesus monkeys in small populations.[20,37] The first report comprised the findings of echocardiographic images and parameters with their reference intervals on 28 healthy young rhesus monkeys.[20] This study, however, only included young monkeys (1 to 9 y old) and did not report parameters to determine diastolic dysfunction of the left ventricle due to the lack of appropriate technology. Another study reported the reference intervals of echocardiographic parameters including ones for determining diastolic dysfunction.[37] However, that study assessed only small numbers of young and older adult rhesus macaques (7 to 20 y old) and did not include geriatric monkeys older than 20 y. The availability of reference intervals for echocardiographic parameters in a large population of geriatric rhesus macaques is fundamental to assess their age-related cardiovascular changes when these animals are used for cardiovascular and noncardiovascular research. Some cardiovascular diseases are associated with significant health concerns in geriatric monkeys and may negatively affect the design of experiments and the interpretation of results. In addition, establishing the reference intervals of geriatric macaques will help to identify the cardiac-disease–free animals and to set their pedigrees as controls when inherited cardiovascular disease, such as hypertrophic cardiomyopathy, is investigated.

The purpose of the current study was to acquire and derive systolic and diastolic reference values of echocardiographic measurements from a large cohort of healthy geriatric rhesus macaques. To our knowledge, this cohort of geriatric rhesus macaques represents the largest sample size in the literature.

## Materials and Methods

**Subjects and housing.** All study procedures and methods were conducted at the California National Primate Research Center and were approved in advance by the IACUC of the University of California–Davis. All animals were cared for in accordance with the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals*.[19] The animal care and use program at the University of California–Davis is USDA-registered, maintains a Public Health Services Assurance, and is an AAALAC-accredited. Outdoor macaques were group-housed in 0.5-acre rectangular enclosures (field pens). Indoor macaques were pair-housed in stainless steel cages sized in accordance with primary cage-space regulations. Routine husbandry parameters included controlled temperature, humidity, and ventilation. Room lightening was controlled to provide alternating 12-h periods of light and darkness. All macaques were housed with species-appropriate environmental enrichment, fed commercial primate chow twice daily (LabDiet Monkey Diet 5047, Purina Mills International, St Louis, MO) supplemented with fruits

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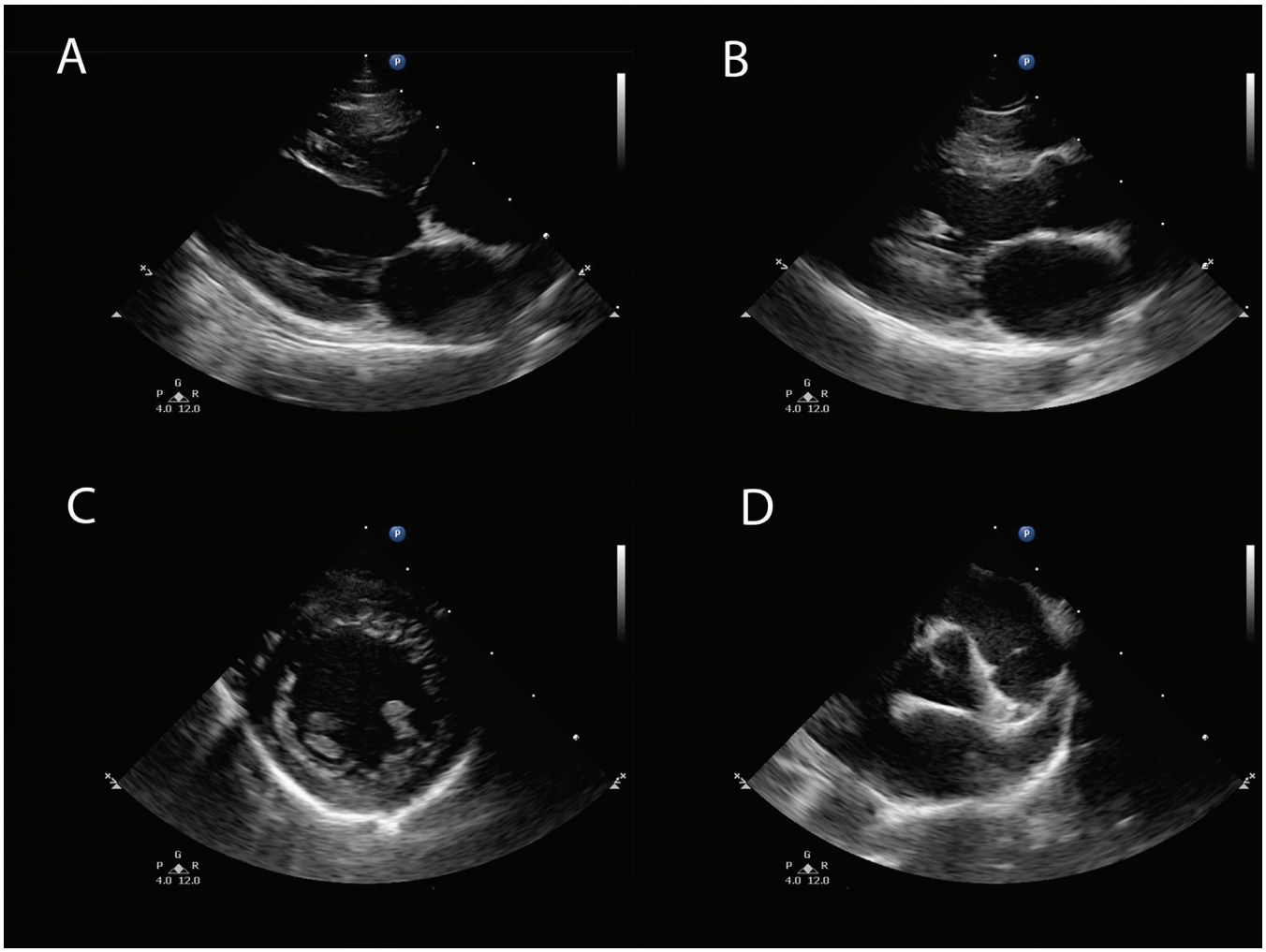



**Figure 1.** Right parasternal long and short-axis echocardiographic images. (A) Right parasternal long-axis view of 4 chambers. (B) Right parasternal long-axis view of 5 chambers. (C) Right parasternal short-axis view of the left ventricle. (D) Right parasternal short-axis basilar view of the aorta and left atrium.

and vegetables biweekly, offered water without restriction through automatic watering devices. All animals were sedated at least annually to undergo complete physical examination, weighing, tuberculosis testing, dental prophylaxis, and blood testing including CBC analysis and serum biochemistry. When any chronic health issue, including diabetes mellitus is diagnosed, these monkeys are transferred to another colony and were excluded from this study. Furthermore all monkeys were monitored periodically for bacterial and viral infections (herpes B virus, simian T-lymphotropic virus, SIV, and simian type D retrovirus).[2,9]

**Sedation.** Prior to echocardiographic examination, rhesus macaques were fasted overnight. The macaques were sedated by using ketamine hydrochloride (10 mg/kg IM; Ketaject, Phoenix Pharmaceutical, St Joseph, MO).

**Echocardiographic measurement.** The sedated macaques were consecutively positioned in right and left recumbency for echocardiographic examination (CX50 Ultrasound System, Philips, Best, Netherlands). All echocardiographic examinations were performed by 2 veterinary cardiologists (JS and CGH) using a 4- to 12-mHz sector-array transducer (S12-4) with color and spectral Doppler capabilities; results were reviewed by an ACVIM board-certified cardiologist (JS). Data analysis was completed by using standard offline analysis software (Syngo Dynamics, Siemens, Erlangen, Germany). All echocardiographic measurements were made in accordance with the guidelines of the American Society of Echocardiography by using the leading-edge to leading-edge method of measurement.

The following parameters were obtained from 2D views (Figure 1). The aortic root diameter and left atrial diameter were acquired from the right parasternal short-axis and long-axis 4-chamber views. From the right parasternal short-axis M-mode view at the chordae level, the interventricular septal thickness (IVS), left ventricular internal diameter during diastole (LVDd) and systole (LVDs), and left ventricular posterior wall thickness in diastole and systole as well as the E-point to septum separation were obtained (Figure 2). Peak velocities for pulmonary and aortic flow were measured from pulsed-wave and continuous-wave spectral Doppler, respectively, as well as from passive filling (E wave) and atrial contraction (A wave) velocities (Figure 3). Pulmonary flow was obtained from the right parasternal short-axis view of the right ventricular outflow tract at the level of the aortic valve, with the sample gate positioned in the pulmonary artery just distal to the pulmonic valve; aortic flow was obtained from the subcostal view with parallel alignment to the aorta, bisecting the valve leaflets.[3,33] Transmitral flow velocities were obtained from the left parasternal apical 4-chamber view, with the sample gate positioned at the tips of the mitral valve leaflets when they were wide open. No angle corrections were necessary, given that parallel alignment of

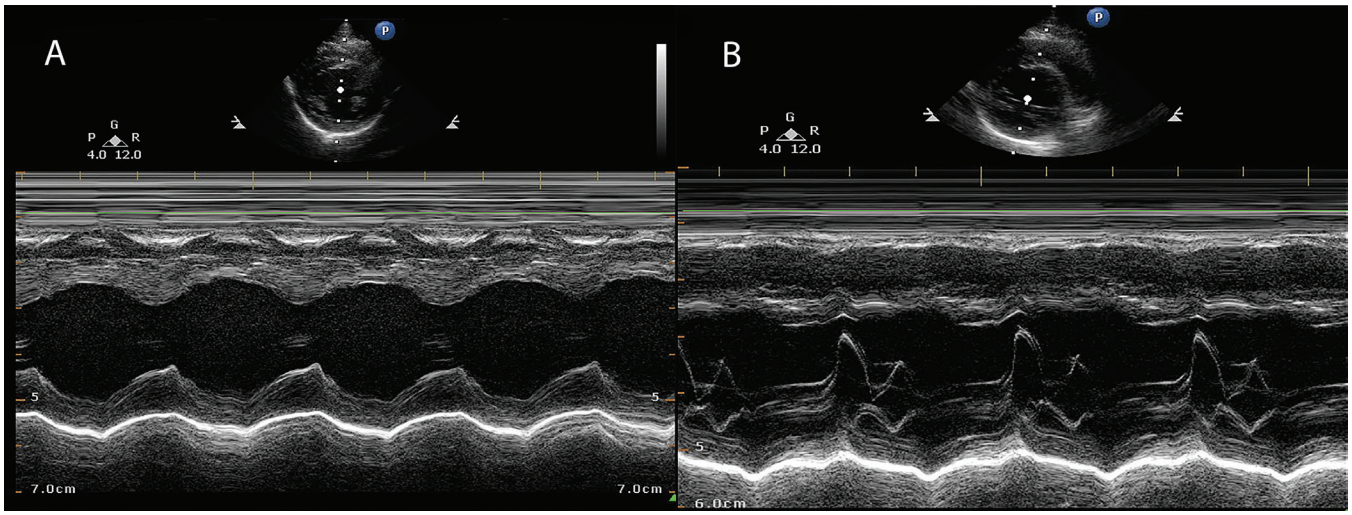



**Figure 2.** M-mode echocardiographic images at the (A) left ventricle and (B) mitral valve.

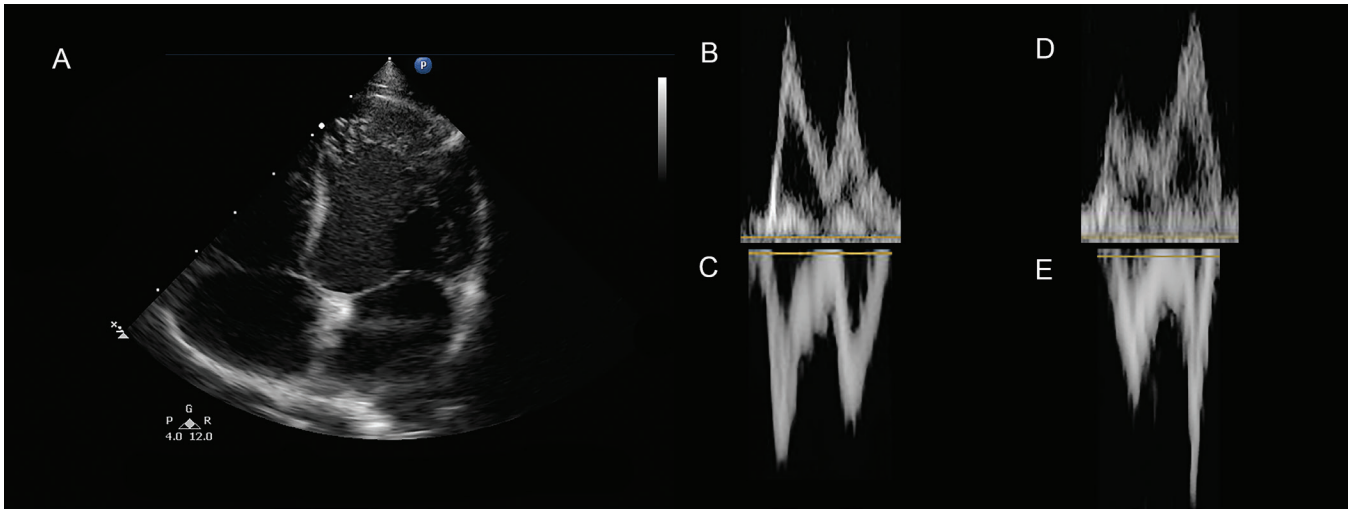



**Figure 3.** Left apical 4 chamber view and pulsed-wave spectral Doppler images with passive filling (E wave) and atrial contraction velocity (A wave) and 2D color tissue Doppler images with peak velocities measured in early (E′) and late (A′) diastole. (A) Left apical 4-chamber view. (B) Normal E:A ratio. (C) normal E′:A′ ratio. (D) Decreased E:A ratio with diastolic dysfunction. (E) Decreased E′:A′ ratio with diastolic dysfunction.

the Doppler gate was possible in all monkeys. Color-tissue 2D Doppler image examinations were performed to obtain the septal (medial) and free-wall (lateral) mitral annulus motions from the left apical 4-chamber view, and peak velocities were measured in early and late diastole (Figure 3).

Regurgitations through each of the 4 valves were subjectively quantified from color Doppler profiles. Aortic and pulmonic valve regurgitations were evaluated from the previously described views for spectral Doppler measurements. Aortic regurgitations were quantified as mild (ration of jet height to left ventricular outflow tract width, less than 24%), moderate (25% to 64%), and severe (greater than 65%). Pulmonic regurgitation was quantified as mild (thin narrow origin jet with normal right ventricular size), moderate (moderately wide origin jet with normal or mildly dilated right ventricular size), or severe (large wide origin jet with dilated right ventricular size).[5] Mitral and tricuspid valve regurgitations were evaluated from the previously described views for spectral Doppler measurements as well as from the right parasternal long-axis 4-chamber view. Mitral valve regurgitations were quantified as mild (jets occupying less than 30% of the left atrium area), moderate (31% to 70% of atrium), or severe (more than 70%). Tricuspid valve regurgitations were quantified as mild (right ventricle, right atrium, and vena cava all normal in size), moderate (normal or dilated right ventricle, right atrium, or vena cava), or severe (right ventricle, right atrium, and vena cava all dilated). The presence and severity of left ventricular outflow tract obstruction and systolic anterior motion of mitral valve was determined by using the color Doppler evaluation and were quantified subjectively as mild to severe by the cardiologist (JS). Fractional shortening, and left ventricular ejection fraction were calculated as

$$\text{fractional shortening (\%)} = [(\text{LVDd} - \text{LVDs}) / \text{LVDd}] \times 100\%, \text{ and}$$

$$\text{left ventricular ejection fraction (\%)} = [(\text{LVDd}^3 - \text{LVDs}^3) / \text{LVDd}^3] \times 100\%$$

End-systolic volume index was calculated according to the corrected Teichholz formula:

$$\text{ESVI (mL/m}^2) = (7 \times \text{LVDs}^3) / [(2.4 + \text{LVDs}) \times \text{BSA}],$$

with LVDs in centimeters and body surface area (BSA) in square meters.[38] Stroke volume (mL) was estimated as $\text{LVDd}^3 - \text{LVDs}^3$, and cardiac output (L/min) was calculated as stroke

**Table 1.** M-mode and 2D measurements in geriatric rhesus macaques.

| | | | | Reference interval | |
|---|---|---|---|---|---|
| Cardiac geometry[a] | Mean | 1 SD | Range | Lower limit | Upper limit |
| IVSd | 0.59 | 0.13 | 0.32–0.87 | 0.31 | 0.86 |
| LVDd | 2.07 | 0.29 | 1.52–2.77 | 1.46 | 2.64 |
| LVPWd | 0.66 | 0.06 | 0.55–0.77 | 0.53 | 0.78 |
| IVSs | 0.88 | 0.18 | 0.44–1.29 | 0.52 | 1.24 |
| LVDs | 1.16 | 0.31 | 0.64–1.9 | 0.49 | 1.77 |
| LVPWs | 1.03 | 0.15 | 0.79–1.34 | 0.71 | 1.34 |
| EPSS | 0.27 | 0.12 | 0.08–0.53 | 0.02 | 0.51 |
| Ao (sa) | 1.53 | 0.22 | 1.11–2.1 | 1.09 | 1.97 |
| LA (sa) | 1.82 | 0.24 | 1.44–2.41 | 1.32 | 2.29 |
| LA/Ao (sa) | 1.21 | 0.21 | 0.79–1.71 | 0.77 | 1.63 |
| Ao (la) | 1.17 | 0.19 | 0.85–1.62 | 0.78 | 1.56 |
| LA (la) | 2.11 | 0.25 | 1.58–2.67 | 1.61 | 2.61 |
| LA/Ao (la) | 1.87 | 0.35 | 1.12–2.75 | 1.15 | 2.57 |

Ao (la), aortic root diameter from long-axis view; Ao (sa), aortic root diameter from short-axis view; EPSS, E-point septal separation; IVSd, interventricular septal thickness in diastole; IVSs, interventricular septal thickness in systole; LVDs, left ventricular internal diameter in systole; LVPWd, left ventricular posterior wall thickness in diastole; LVPWs, left ventricular posterior wall thickness in systole; LA (la), left atrial diameter in diastole from long-axis view; LA (sa), left atrial diameter from short-axis view
[a]All measurements are in centimeters except for LA/Ao, which is a ratio.

**Table 2.** Left ventricular functional parameters in geriatric rhesus macaques.

| | | | | Reference interval | |
|---|---|---|---|---|---|
| | Mean | 1 SD | Range | Lower limit | Upper limit |
| Fractional shortening (%) | 43.62 | 10.65 | 20.8–69 | 20.7 | 64.43 |
| LVEF (%) | 76.57 | 10.24 | 54.1–95.6 | 55.75 | 97.52 |
| ESVI (mL/m$^2$) | 3.22 | 2.17 | 0.6–9.26 | –1.62 | 7.35 |
| Stroke volume (mL/beat) | 10.35 | 3.46 | 3.8–18.0 | 3.12 | 17.23 |
| Stroke volume index (mL/m$^2$) | 21.53 | 7.31 | 8.5–37.5 | 6.34 | 36.24 |
| Cardiac output (L/min) | 1.57 | 0.50 | 0.69–2.77 | 0.54 | 2.57 |

ESVI, end-systolic volume index; LVEF, left ventricular ejection fraction.

volume × heart rate (bpm) / 1000. BSA (m$^2$) was calculated as $(10.1 \times BW^{2/3}) / 10^4$, with BW expressed in grams.

**Statistical analysis.** All statistical analyses were performed using commercial software packages (MedCalc version 12.7.4, MedCalc Software, Ostend, Belgium, and Prism version 7, GraphPad Software, La Jolla, CA). Descriptive statistics (mean, SD, and range) were calculated for all measurements and are provided as mean ± 1 SD (range). For each measurement, outliers were determined and excluded by using the Tukey test, and the double-sided 95% reference intervals were constructed thereafter. The 90% confidence intervals for the reference limits were obtained by using the robust method, and these intervals were estimated with bootstrap methods. Normality testing for continuous data was performed by using D'Agostino–Pearson test.

For each parameter, a model was performed stepwise by using age (in years), BW (kg), BSA, sex, and heart rate (HR; bpm) as explanatory variables. Because of very strong correlation between BW and BSA ($R^2 = 0.996$), BSA was not considered further in the analysis. For each variable included in the model, the coefficient of the linear association with an echocardiographic parameter was obtained as well as its associated *P* value. The coefficients represent the mean change in parameters for an increase of one unit of the explanatory variable. The $R^2$ values represent the strength of the association, or the percentage of the variation in the parameters that is explained by the variables in the model. Significant correlation was reported as weak ($R^2 < 0.2$), moderate ($0.2 \leq R^2 < 0.4$), or strong ($0.4 \leq R^2$) ($P < 0.05$).[16]

Rhesus macaques in this study were then allocated by age into 3 groups (18 to 21 y, 22 to 25 y, and 26 to 29 y). The proportions of the macaques with diastolic dysfunction (mitral E:A ratio ≤ 0.9 or mitral E:A ratio ≥ 0.9 with a lateral or medial E′:A′ ratio ≤ 0.9) were compared between groups by using $\chi^2$ and Fishers exact tests. A *P* value less than 0.05 was considered statistically significant.

## Results

The study sample consisted of 51 geriatric rhesus macaques (age [mean ± 1 SD], 22.4 ± 3.0 y [range, 18.4 to 29.3 y]; BW, 11.3 ± 2.6 kg [5.2 to 17.0 kg]). Their mean BSA was 0.5 ± 0.08 m$^2$ (0.3 to 0.7 m$^2$). A total of 34 rhesus macaques were female; 17 were male. The mean HR during the studies was 153 ± 21 bpm (117 to 217 bpm).

M-mode and 2D measurements of all geriatric macaques are listed in Table 1. Left-ventricular functional parameters, BW, BSA, and HR are presented in Table 2, and Doppler-derived parameters are presented in Table 3. Of the 51 geriatric rhesus macaques, 26 (51.0%) had aortic regurgitation, 22 (43.1%) had tricuspid regurgitation, 13 (25.5%) had mitral regurgitation, and 5 (9.8%) had pulmonic regurgitation. In addition, 10 (19.6%) macaques had signs of left ventricular outflow tract obstruction, and 6 (11.8%) of them had signs of systolic anterior motion. All

**Table 3.** Doppler-derived echocardiographic parameters in geriatric rhesus macaques

| | | | | Reference interval | |
|---|---|---|---|---|---|
| | Mean | 1 SD | Range | Lower limit | Upper limit |
| $V_{Ao}$ (m/s) | 0.92 | 0.17 | 0.56–1.33 | 0.56 | 1.27 |
| E wave (m/s) | 0.59 | 0.11 | 0.38–0.88 | 0.35 | 0.82 |
| A wave (m/s) | 0.70 | 0.19 | 0.31–1.2 | 0.32 | 1.08 |
| E/A | 0.82 | 0.14 | 0.61–1.14 | 0.49 | 1.08 |
| $V_{pulm}$ (m/s) | 1.00 | 0.15 | 0.71–1.36 | 0.70 | 1.30 |
| PV AT (msec) | 72.02 | 12.73 | 52–106 | 42.88 | 96.09 |
| PV ET (msec) | 186.59 | 26.82 | 115–244 | 133.58 | 242.61 |
| PV AT/ET | 0.39 | 0.08 | 0.27–0.58 | 0.21 | 0.56 |
| $V_{tricusp}$ (m/s) | 2.09 | 0.32 | 1.47–2.74 | 1.35 | 2.77 |
| TR Pk Grad (mm Hg) | 15.68 | 7.53 | 1.6–30.1 | 0.14 | 32.49 |
| RVSP (mm Hg) | 20.68 | 7.53 | 6.6–35.1 | 5.14 | 37.49 |
| E′ medial (m/s) | 0.05 | 0.01 | 0.04–0.07 | 0.04 | 0.07 |
| A′ medial (m/s) | 0.08 | 0.01 | 0.07–0.1 | 0.07 | 0.1 |
| E/E′ medial | 10.74 | 2.15 | 6.21–14.93 | 6.33 | 15.27 |
| E′/A′ medial | 0.67 | 0.11 | 0.44–0.97 | 0.42 | 0.90 |
| E′ lateral (m/s) | 0.07 | 0.02 | 0.04–0.12 | 0.032 | 0.11 |
| A′ lateral (m/s) | 0.08 | 0.02 | 0.04–0.14 | 0.036 | 0.13 |
| E/E′ lateral | 8.68 | 2.43 | 4.12–16.32 | 2.89 | 13.58 |
| E′/A′ lateral | 0.89 | 0.28 | 0.52–1.52 | 0.28 | 1.45 |

A wave, passive filling velocity; A′ wave, late diastolic mitral annulus motion; E wave, atrial contraction velocity; E′ wave, early diastolic mitral annulus motion: PV AT, pulmonary valve acceleration time; PV ET, pulmonary valve ejection time; RVSP, right ventricular systolic pressure; TR pk grad, peak gradient of tricuspid regurgitation; $V_{Ao}$, peak velocity for aortic flow; $V_{pulm}$, peak velocity for pulmonary flow; $V_{tricusp}$, peak velocity for tricuspid flow.

of these abnormalities were graded as mild, except for one case of moderate mitral regurgitation.

The correlation between the echocardiographic parameters and the sex, BW, HR, and age of the geriatric macaques were analyzed by using linear models. Strong and significant correlations with HR were revealed for lateral late diastolic mitral annulus motion (A′ lateral; Table 4). Aortic root diameter from the long-axis view, left atrial diameter from the long-axis view, medial late diastolic mitral annulus motion, and pulmonary valve ejection time had moderate correlations with HR, whereas aortic root diameter from the long-axis view and tricuspid flow velocity showed moderate associations with age in this model. Fifteen parameters had weak correlations and significant linear association between the parameters and the variables; however, these associations explained only a small portion of the variation in the parameters, and a large amount of unexplained variation remained (Table 4).

Of the 51 geriatric rhesus macaques, 36 (70.6%) met the criteria for diagnosis of diastolic dysfunction used previously (Figure 3).[36] After we allocated the macaques into 3 groups by age (18 to 21 y, 22 to 25 y, and 26 to 29 y), the incidence of diastolic dysfunction was 20 of 24 (83.3%), 11 of 19 (57.9%), and 5 of 8 (62.5%), respectively. The prevalence of diastolic dysfunction did not differ significantly by age (Figure 4), suggesting the incidence of diastolic dysfunction does not necessarily increase with advancing age.

## Discussion

In the present study, we developed reference intervals for cardiac geometry and left-ventricular systolic and diastolic function in geriatric rhesus macaques (age, 18 to 29 y). Diastolic dysfunction of the left ventricle and valve regurgitation, especially aortic and tricuspid regurgitations, were highly prevalent in this population.

The term 'geriatric' is somewhat ambiguous, and the definition can be changed depending on the population characteristics. In the present study, we defined geriatric rhesus macaques in the research facility as older than 18 y, because their handling requirements and experimental utility due to age differ significantly from younger macaques'. The previous study that reported echocardiographic parameters defined 'older adult' macaques as 18 to 20 y.[37] Because most of the rhesus macaques in the present study population are older than 20 y, our current data reflect an older cohort of animals than in previous reports.

In the current study, the large sample size for establishing the echocardiographic parameters improved the description of the statistical distribution for each parameter and clarified the variability inherent in the data. Another important feature that supports the reliability of our reported echocardiographic parameters and their defined reference intervals is the study design, in which all echocardiographic measurements were confirmed by a ACVIM board-certified veterinary cardiologist (JS) using standard methodology.[22,26,31] Echocardiography is a noninvasive and highly informative antemortem diagnostic tool, but it is very operator-dependent.[10,29,39] Therefore, this research must be conducted by a single investigator–cardiologist who has substantial experience in and skills for echocardiographic examination. In addition, it is worthwhile to note that multiple echocardiographic parameters were weakly to moderately correlated with BW, BSA, age, and HR. These values should be interpreted cautiously when a macaque's size, age, or HR are markedly different from those in this population.

In elderly people, decreased diastolic function and various valvular changes are prevalent, and, depending on their severity, these conditions may or may not become significant health concerns associated with cardiovascular morbidity and mortality.[4,11,41] These abnormalities are often attributed to decreases in myocyte number, increases in myocyte size, and increases in

**Table 4.** Echocardiographic parameters showing significant correlations with age, body weight, or heart rate are listed as coefficients, with the *P* value in parentheses

| | Age | Body weight | Sex | Heart rate | $R^2$ |
|---|---|---|---|---|---|
| LA (sa) | | | | −0.0037 (0.015) | 0.122 |
| Ao (sa) | −0.000059 (0.042) | | | −0.0031 (0.035) | 0.157 |
| LA/Ao (sa) | | | 0.15 (0.017) | | 0.118 |
| LA (la) | | | | −0.0081 (<0.0001) | 0.340 |
| Ao (la) | 0.000074 (0.0058) | | | −0.0029 (0.03) | 0.249 |
| LVDd | | | | −0.0048 (0.032) | 0.155 |
| LVPWd | | | | −0.0013 (0.0055) | 0.153 |
| LVDs | | −0.046 (0.036) | | −0.0047 (0.048) | 0.152 |
| SV | | | | −0.057 (0.039) | 0.137 |
| SI | | −1.11 (0.036) | | | 0.097 |
| EPSS | | −0.027 (0.017) | | | 0.128 |
| $V_{Ao}$ | | | | −0.0041 (0.0039) | 0.164 |
| A wave | | | | −0.0053 (0.0037) | 0.192 |
| $V_{tricuspid}$ | 0.00028 (0.0445) | | | | 0.330 |
| PV AT | | | | −0.22 (0.044) | 0.084 |
| PV ET | | | | −0.65 (0.00014) | 0.267 |
| E′ medial | | | | 0.00019 (0.025) | 0.147 |
| A′ medial | | | | 0.00035 (0.0029) | 0.245 |
| E′ lateral | | | | 0.00038 (0.048) | 0.099 |
| A′ lateral | | | | 0.00093 (<0.0001) | 0.454 |
| E′/A′ lateral | | | | −0.0049 (0.043) | 0.103 |

A wave, passive filling velocity; A′ wave, late diastolic mitral annulus motion; Ao (la), aortic root diameter from long-axis view; Ao (sa), aortic root diameter from short-axis view; E′, early diastolic mitral annulus motion; EPSS, E-point septal separation; LA (la), left atrial diameter from long-axis view; LA (sa), left atrial diameter from short-axis view; LVDd, left ventricular internal diameter in diastole; LVDs, left ventricular internal diameter in systole; LVPWd, left ventricular posterior wall thickness in diastole; PV AT, pulmonary valve acceleration time; PV ET, pulmonary valve ejection time; SI, stroke volume index; SV, stroke volume; $V_{Ao}$, peak velocity for aortic flow; $V_{tricusp}$, peak velocity for tricuspid flow.
All parameters with weak ($R^2 < 0.2$), moderate ($0.2 \leq R^2 < 0.4$), or strong ($0.4 \leq R^2$) correlations are shown in the table.
[a]A′ lateral and heart rate were the only strongly correlated parameters.

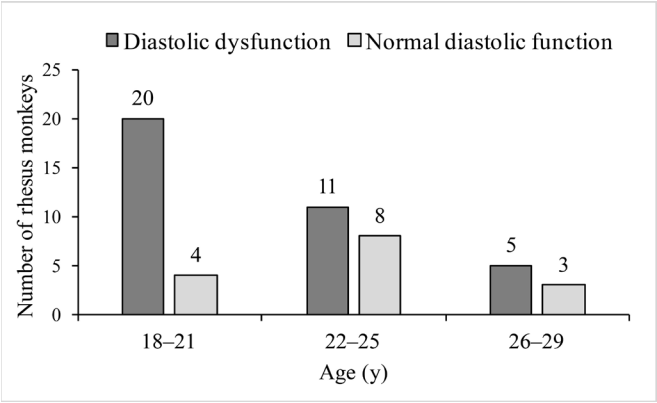



**Figure 4.** Numbers of rhesus macaques with diastolic dysfunction in different age groups. The incidence of diastolic dysfunction in the geriatric rhesus macaques in these age groups was 20 of 24 (83.3%) in 18–21-y macaques, 11 of 19 (57.9%) in 22–25-y macaques, and 5 of 8 (62.5%) the 26–29-y macaques. No significant differences were noted among these groups.

the amount of connective tissue matrix.[13,21] Myocyte number decreases because of cell necrosis and apoptosis. As myocytes are lost, they are replaced with fibroblasts, and the remaining myocytes hypertrophy. As the fibroblasts produce collagen, interstitial fibrosis occurs, and the heart becomes stiffer and less compliant. The stiff and less-compliant ventricle affects diastolic mechanics, leading to diastolic dysfunction.[8,12,18,23] In the present study, 36 of the 51 geriatric macaques (70.6%) met the criteria for diastolic dysfunction in the absence of left-ventricular hypertrophy. Although clinical significance was not assessed fully, these macaques were deemed clinically healthy, without any other functional abnormalities.

In addition, diastolic dysfunction can be an early sign or consequence of ventricular hypertrophy, but none of these geriatric rhesus macaques had abnormally thick interventricular or posterior ventricular walls despite their geriatric age. Some metabolic diseases in humans, such as diabetes mellitus, are known to be major risk factors for the development of heart failure due to left ventricular diastolic and subsequent systolic dysfunction.[7] Two recently published studies revealed that type 2 diabetes mellitus is associated with left ventricular diastolic dysfunction in rhesus macaques, similar to the key characteristics in humans.[14,29] In the present study, the annual blood tests, physical examinations, and apparently healthy status of these animals excluded rhesus macaques with diabetes mellitus. Ultimately no physiologic conditions in the rhesus macaques enrolled in our study were consistent with type 2 diabetes, making it an unlikely source of the diastolic dysfunction in the study population. Therefore, the highly prevalent diastolic dysfunction in the geriatric rhesus macaques in this population is likely to be an age-related change. This finding is very similar to the changes seen in elderly humans and has considerable value for future translational research studying the clinical and pathologic characteristics of diastolic dysfunction of the elderly.

Valve regurgitations were common in our population, especially aortic (51.0%) and tricuspid (43.1%) regurgitation. This finding is consistent with the high prevalence of aortic and tricuspid regurgitations in aging humans (typically 70 y

and older).[27,34,35] In addition, several studies revealed age as a significant risk factor in humans for developing valve regurgitation.[24,35] The various causes of these valvular abnormalities included not only primary valve disease but were also secondary effects due to other cardiac (for example, left-sided congestive heart failure for tricuspid regurgitation) and noncardiac conditions (for example, systemic and pulmonary hypertension for aortic and tricuspid regurgitation, respectively).[27,28] In addition, sedative drugs alone have been shown to result in trivial to mild valve insufficiency in dogs and thus might have influenced the findings in the present study.[19] Although we did not further investigate the causes of these valvular regurgitations and thus the significance of these abnormalities, this finding might offer important value for future translational research.

Several reports describe the spontaneous occurrence of vascular disease, valvular disease, and other congenital cardiac anomalies in rhesus macaques.[6,25,36,40] Recently, a unique animal model of left ventricular hypertrophy was observed in the rhesus macaques housed at the California National Primate Research Center.[32] Left ventricular hypertrophy is defined as the presence of thickened left ventricular septal and free walls and results in diastolic dysfunction. Over the past 10 y, this disease has been observed within the colony and slowly characterized from a pathology perspective. Recent pedigree investigation suggests that this condition is familial and likely heritable.[17] Currently, antemortem investigation of the colony has been performed to aid in identifying this condition in the living colony.[15] Moreover, genetic and molecular evaluations will be performed to evaluate subclinical disease within the colony. To accurately determine the structural abnormalities of this inherited disease, it is crucial to have standardized echocardiographic values from a large cohort of healthy geriatric rhesus macaques, given that the abnormalities and associated clinical signs of LVH can develop in later life stages. Because sudden cardiac death is a prominent feature of this described condition, we expect the incidence of this disease in geriatric rhesus macaques to be extremely low, making them a phenotypically perfect control group. The reference intervals that we established in the present study are specific for geriatric rhesus macaques and thus will help to standardize proposed control groups in future genetic studies of inherited LVH.

Several moderate to strong associations were found between echocardiographic parameters and variables (age, BW, sex, and HR). All of these correlations were noted with HR, and therefore these parameters need to be interpreted cautiously when HR is significantly low or high. Importantly, because no moderate to strong correlations were noted between the BW or sex and any parameters, the reference ranges established in the present study are applicable to the general geriatric population. However, despite the lack of patterns on visual inspection, we performed only linear associations in this study, and it therefore remains possible, albeit unlikely, that other types of associations may exist.

One limitation of the present study is the lack of thorough physical and biochemical evaluation concurrent with echocardiography. Although all animals were deemed apparently healthy and underwent routine health screenings including annual hematology and biochemical analysis, the possibility of subclinical systemic disease that affects echocardiographic findings remains. This effect is unlikely, however, and is minimized by the use of a sample size that is larger than that in any previously reported study. Another limitation of the present study is the lack of comparison of the echocardiographic parameters of the geriatric macaques among the different research facilities. These macaques were bred for research purposes and often are inbred in the facility where the present study was conducted, perhaps thus causing significant founder effects in the population. This concern would be particularly valid if any clearly defined cardiac disease were observed with high frequency. Given our findings and the lack of clinically relevant echocardiographic abnormalities, this potential disadvantage seems unlikely to be a detriment to the use of our proposed reference intervals.

In the present study, we report complete echocardiographic parameters for a population of geriatric rhesus macaques and established their reference intervals. Valve regurgitations, especially aortic and tricuspid, and diastolic dysfunction of the left ventricle were highly prevalent in the study population, even though the macaques were clinically healthy. Our findings closely mimic those found in the aging human population. For future studies of adult-onset disease, particularly cardiac abnormalities, these reference intervals are especially useful. Our proposed reference intervals make it possible to use geriatric rhesus macaques as well-phenotyped control animals in translational research.

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