

# Echocardiographic Reference Intervals with Allometric Scaling of 823 Clinically Healthy Rhesus Macaques (*Macaca Mulatta*)

**Yu Ueda**

North Carolina State University

**Laetitia MM Duler**

University of California Davis

**Kami J Elliot**

California National Primate Research Center

**Paul-Michael D Sosa**

California National Primate Research Center

**Jeffrey A Roberts**

California National Primate Research Center

**Joshua A. Stern** (✉ [jstem@ucdavis.edu](mailto:jstem@ucdavis.edu))

University of California Davis <https://orcid.org/0000-0001-5611-5745>

---

## Research article

**Keywords:** heart, cardiac, cardiovascular, ultrasound, non-human primate, cardiomyopathy, hypertrophic cardiomyopathy, left ventricular hypertrophy

**Posted Date:** March 18th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-17866/v1>

**License:** © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

**Version of Record:** A version of this preprint was published on September 22nd, 2020. See the published version at <https://doi.org/10.1186/s12917-020-02578-y>.

# Abstract

**Background:** Echocardiography is commonly used for assessing cardiac structure and function in various species including non-human primates. A few previous studies reported normal echocardiographic reference intervals of clinically healthy rhesus macaques under sedation. However, these studies were under-powered, and the techniques were not standardized. In addition, body weight, age, and sex matched reference intervals should be established as echocardiographic measurements are commonly influenced by these variables. The purpose of this study was to establish reference intervals for complete echocardiographic parameters based on a large cohort of clinically healthy rhesus macaques with wide ranges of weight and age distributions using allometric scaling.

**Results:** A total of 823 rhesus macaques (ages 6 months to 31 years old; body weights 1.4 to 22.6 kg) were enrolled. Of these rhesus macaques, 421 were males and 402 were females. They were assessed with a complete echocardiographic examination including structural and functional evaluation under sedation with ketamine hydrochloride. The reference intervals of the key echocardiographic parameters were indexed to weight, age, and sex by calculating the coefficients of the allometric equation  $Y = aMb$ . On correlation matrix, body weight, age, sex, and heart rate were significantly correlated with various echocardiographic parameters and some of the parameters were strongly correlated with body weight and age. Multiple regression analysis was also performed to predict various echocardiographic parameters from heart rate, body weight, age and sex. Heart rate and body weight statistically significantly predicted various echocardiographic parameters. Valve regurgitation including tricuspid, aortic, pulmonic, and mitral regurgitations without other cardiac structural and functional abnormalities are common in clinically healthy rhesus macaques under ketamine sedation.

**Conclusions:** In this study, the reference intervals of echocardiographic parameters were established by performing complete echocardiographic examinations on a large number of clinical healthy rhesus macaques. In addition, allometric scaling was performed based on their weight, and further indexed to age and sex. These allometrically scaled reference intervals can be used to accurately evaluate echocardiographic data in rhesus macaques and diagnose structural and functional evidence of cardiac disease.

## Background

Echocardiography is a noninvasive imaging technique commonly used for assessing cardiac structure and function. It includes the measurement of cardiac chamber size and ventricular wall thickness, and an assessment of valve structure and function, systolic and diastolic function, and blood flow patterns in the heart. In clinical settings, findings from complete echocardiographic assessment are used as important information to determine medical and surgical treatment of cardiac diseases. In research settings, echocardiographic evaluation of subjects is inevitable to fully assess the cardiac condition for cardiovascular and non-cardiovascular research.

A few previous studies reported normal echocardiographic reference ranges of clinically healthy rhesus macaques under sedation[1, 2]. In addition, the author of the present study reported echocardiographic reference intervals in geriatric rhesus macaques older than 18 years[3]. These studies reported that the reference intervals of various echocardiographic parameters in geriatric rhesus macaques are different from those in younger adult rhesus macaques. This study also reported that diastolic dysfunction without other obvious cardiovascular abnormalities in geriatric rhesus macaques is common. However, these previous studies did not have sufficient power due to their small sample sizes. In addition, these reference values were not indexed to body weight (BW). Weight matched reference intervals should be established due to the knowledge that various echocardiographic parameters are influenced by BW [4–6]. In addition, the reference intervals should be indexed to age and sex since some of variables are known to be influenced by these variables[7–9]. Previously, naturally occurring hypertrophic cardiomyopathy (HCM) and other cardiomyopathies have been reported in rhesus macaques and they could represent an important non-human primate model of human familial HCM[10–15]. Applying BW independent reference intervals to rhesus macaques may result in misdiagnosis of HCM as well as other cardiac diseases. The establishment of echocardiographic reference intervals indexed to these variables in a large population of rhesus macaques of various age and sex groups is fundamental to assessing their cardiovascular conditions and abnormalities in clinical and experimental settings. Knowing the normal reference values is of utmost importance to aid in the identification of the presence and severity of cardiovascular abnormalities.

The purpose of the present study was to establish reference intervals of key echocardiographic parameters based on a large cohort of clinically healthy rhesus macaques with wide ranges of weight and age distributions and establish these reference intervals with allometric scaling to BW. They are also indexed to other patient characteristics including the sex and age. To our knowledge, this cohort of rhesus macaques represents the largest sample size for establishing echocardiographic parameters in the literature in any other veterinary patients.

## Results

A total of 823 rhesus macaques were enrolled in the present study. The average age (+/- standard deviation [SD]) and BW (+/-SD) were 7.8 (+/- 5.4) years and 8.49 (+/-3.52) kg, respectively. The mean heart rate (HR) during the echocardiographic studies was 134 (+/-24.6) bpm. Of 823 rhesus macaques, 402 rhesus macaques were female and 421 were male. The mean age (+/- SD) in males and females were 7.9 (+/- 5.4) years and 7.8 (+/- 5.4) years, respectively. The mean BW in males and females were 8.46 (+/- 3.54) kg and 8.46 (+/- 3.53) kg, respectively. The mean HR during the studies was 133 (+/- 24.6) bpm in males and 134 (+/- 24.6 bpm) in females.

None of the rhesus macaques had evidence of significant cardiac structural abnormalities or moderate to severe valve regurgitation. None of the rhesus macaques had systolic dysfunction determined based on left ventricular fractional shortening (LVFS) and left ventricular ejection fraction (LVEF). However, some of the older animals in the study group had evidence of diastolic dysfunction without other structural and/or

functional abnormalities, which could be attributed to the age-related findings based on previous studies in rhesus macaques and other species [3]. Of the 823 rhesus macaques, 303 had tricuspid (36.8%), 119 had aortic (14.5%), 107 (13.0%) had pulmonic, and 92 had mitral (11.2 %) valve regurgitation. All of these regurgitations were graded as trace or mild and they were not associated with chamber enlargement and/or cardiac dysfunction.

Intra-observer measurement variability for all echocardiographic variables measured in 10 rhesus macaques was between 0 and 6.5 %, and interobserver measurement variability was between 0 and 10.7 %.

Mean, SD, range, and reference interval with 90% confidence intervals of upper and lower limits for each 2D and M-mode echocardiographic measurement are listed in Table 1. Continuous and pulsed-wave Doppler-derived parameters are listed in Table 2. On correlation matrix, WT, age, sex, HR, and blood pressure (BP) were significantly correlated with various echocardiographic parameters (Table 3) with some parameters demonstrating strong correlation to BW and/or age.

Multiple regression analysis was performed to predict various echocardiographic parameters from HR, weight, age and sex. HR and weight statistically significantly predicted various echocardiographic parameters (Table 4). Regression analysis using logarithmically transformed echocardiographic parameters including the proportionality constant (a) and allometric scaling exponents (b) are reported in Table 5. The analysis was repeated after the animals were divided based on their sex (Supplement Table 1 and 2). The rhesus macaques were also sub-grouped into 0–5, 6–10, 11–15, and  $\geq 16$  years old groups, and allometric scaling was performed within each age group (Supplement Table 3 - 6).

## Discussion

In the present study, reference intervals for cardiac structure as well as left and right ventricular systolic and diastolic function of rhesus macaques were established. This study is highlighted by a large sample size with a broad range of age and weight as well as evenly distributed sexes. This is important because the statistical method for generating reference intervals and its accuracy with lower confidence intervals of reference limits are dependent on the total sample size and its distribution[16]. Another positive feature of the present study is that all echocardiographic images were obtained or assessed by an American College of Veterinary Internal Medicine board-certified veterinary cardiologist (JS) using standardized methodology. This is also important because echocardiography is highly operator dependent and echocardiographic images obtained with poor techniques might result in misdiagnosing cardiac conditions[17]. Although a few previous studies reported the reference intervals for various echocardiographic parameters in rhesus macaques, the sample numbers were relatively small and techniques to obtain echocardiographic images were not standardized[1–3]. Furthermore, allometric scaling of echocardiographic evaluation based on BW was not performed in any of the previous studies. The present study provides more precise reference intervals based on BW as well as age and sex. These

updated reference intervals will be used to assess cardiovascular condition as well as aid in diagnosing cardiac diseases such as HCM in rhesus macaques.

The results of the present study in 823 clinically healthy rhesus macaques demonstrated that BW, age, HR and sex have a significant effect on various two-dimensional (2D) and M-mode echocardiographic parameters. Among all these variables, as reported in the previous studies, BW has the most significant impact on the echocardiographic measurement of left atrial and aortic diameter as well as left ventricular wall thickness during systole and diastole and performing allometric scaling eliminated the effect of BW on these measurements[18–22]. Therefore, using BW-based 95% prediction intervals based on allometric scaling is recommended when evaluating individual rhesus macaques. Since the age of rhesus macaques also has a significant impact on various echocardiographic parameters, using the 95% prediction intervals for the parameters in each age group (0–5, 6–10, 11–15,  $\geq 16$  years old) should be considered, in particular when the left ventricular wall thickness is close to the upper end of the weight-based 95% prediction intervals. Although significant correlations were identified between various echocardiographic parameters and HR as well as sex, the correlations were all weak and less likely to have significant on echocardiographic assessment. However, due to the fact that the sex often has a significant impact on the variabilities of cardiac structure and function as well as progression of various cardiac diseases such as HCM in other species, BW-based 95% prediction intervals by allometric scaling were provided for each sex group for various echocardiographic variables (Table 3,4 and Supplement Table 1,2) [7–9].

Mild valve regurgitation especially at the tricuspid and aortic valves were common in this population of rhesus macaques, particularly in older patients. This is consistent with the findings of a previous study[3]. Mild forms of valve regurgitation could be associated with sedation but also could be due to nonclinical mild valvular degeneration or other clinically insignificant congenital or acquired valve diseases[23, 24]. Valve regurgitation could also be associated with pulmonary or systemic hypertension, however these findings were ruled out in our study based upon systolic blood pressure measurements performed in a portion (n = 182) of animals and lack of quantitative or qualitative structural heart changes expected with these conditions [24, 25]. This represents one minor limitation of this study since systolic BP measurements were not performed in all animals as it was added to the routine echocardiographic protocol at a later date during data collection. Thus, although unlikely, mild systemic hypertension could not be ruled out as a sole or partial cause of trace or mild aortic or mitral valve regurgitation in these animals[26]. Ultimately, the authors content that the absence of other cardiac structural and functional abnormalities as well as the absence of clinical signs in association with systemic hypertension rules out significant systemic hypertension as a cause of valve regurgitation in these cases[27]. No further investigation into the incidence of trace or mild valve regurgitation was performed in this study but could offer important value for future translational research.

The use of experimental animal models is imperative to advance our understandings of the pathogenesis and pathophysiology of human diseases[28]. It is also essential to study efficacy and safety of pharmaceutical compounds. Murine models of various diseases are the most commonly used as

experimental animal models for studying human diseases including cardiovascular diseases. However, these animal models possess various hurdles which make the direct translational approach difficult[29]. These challenges include their small body size and their different cardiovascular physiology and kinetics. Large animal models including dogs, cats and pigs are also used for studying various diseases, but they still possess inherent physiological, biochemical and genetic differences from human beings[28, 30, 31]. Non-human primate models of cardiovascular diseases have the greatest advantages for translational research because of their physiological, biological, metabolic and genetic similarities to humans and thus they have played a key role to advance our understanding of diseases and clinical medicine[28, 32, 33]. Among them, rhesus macaques (*macaca mulatta*) have been extensively used as a non-human primate model of human diseases. The reference intervals established by this study will therefore be important to utilize when researchers conduct future translational research in rhesus macaques for cardiovascular and non-cardiovascular diseases.

Accurate reference intervals are essential to diagnose various cardiac diseases impacting the health of rhesus macaques in research and clinical settings. For example, HCM has been reported at the California National Primate Center (CNPRC) and this naturally occurring cardiomyopathy is related to sudden cardiac death in this facility[10–12]. Diagnosis of HCM was historically made based on gross necropsy examination documenting severely thickened left ventricular walls with or without prominent papillary muscles[12]. However, HCM could also be diagnosed by antemortem echocardiographic examination assisted by complimentary electrocardiographic, radiographic and cardiac biomarker analyses[10, 34]. Therefore, precise reference intervals are essential to accurately diagnose HCM using echocardiographic examination. In the present study, the reference intervals of left ventricular wall thickness obtained by the conventional method were different with clinical significance to the ones reported in the previous studies[1, 2, 10]. In addition, allometric scaling analysis revealed that the reference interval is highly dependent on the weight and age, while less dependent on sex. Diagnosis of HCM and other cardiac diseases in rhesus macaques therefore should be done based on the allometric scaling prediction intervals reported in this study. This technique will provide the best opportunity to accurately diagnose this condition and perhaps aid in understanding this non-human primate model of HCM.

There are a few limitations to report in this study. One limitation is a lack of thorough physical and biochemical examination concurrently performed with echocardiographic examination. Although semiannual to annual examination was performed on these animals and they were deemed to be healthy without any health concerns at the time before sedatives are administered, the possibility of having subclinical systemic disease could not be completely ruled out. This is however unlikely to have a serious impact on the reference intervals and is minimized by the use of a large sample size. In addition, systolic BP measurement was not performed in all rhesus macaques enrolled in the study due to later implementation of routine BP protocol during echocardiographic examination. This is also less likely to impact the findings of this study with a lack of other clinical and echocardiographic signs consistent with systemic hypertension. The impact of possible systemic hypertension is also minimized by a large sample size. Another limitation is a lack of comparison of the echocardiographic parameters in rhesus macaques among different research facilities. Since animals are often inbred in a facility,

echocardiographic findings could be different in animals in other facilities. However, due to the fact that rhesus macaques are often exchanged among facilities to avoid development of serious health problems due to inbreeding, it is unlikely to find significant differences in the reference intervals of echocardiographic parameters in clinically healthy rhesus macaques from different research facilities. Nevertheless, further study should be performed with multi-center settings to prove that the reference intervals established in the present study can be applied accurately at other facilities and used to diagnose cardiac diseases such as HCM. Lastly, in all rhesus macaques, echocardiographic examination was performed under sedation with ketamine, and the effect of sedatives on echocardiographic parameters should not be ignored. Thus, reference intervals of these parameters under different conditions such as in awake animals or those sedated with a different protocol could differ from those reported in the present study.

## Conclusions

This study is the first to report allometrically scaled echocardiographic reference intervals in a large population. It is also the first to accurately describe the reference intervals with regard to the impact of age and sex in the population. These reference intervals will assist clinicians and researchers to accurately determine the cardiac status of rhesus macaques under ketamine sedation. In addition, mild valve regurgitation is not uncommon in clinically healthy rhesus macaques presumably due to sedative effect or mild valve degeneration. Ultimately, the proposed reference intervals make it possible to accurately identify cardiovascularly healthy rhesus macaques for use as control animals in translational research.

## Methods

### Subjects and housing:

This study was conducted at the CNPRC which is the USDA-registered and AAALAC-accredited facility. The CNPRC maintains an approval from the Institutional Animal Care and Use Committee of the the University of California-Davis and Public Health Services Assurance. All rhesus macaques enrolled in the study were housed in the CNPRC. Echocardiographic examination and blood pressure measurement under sedation were implemented as a part of routine examinations in the CNPRC, and these examinations were performed to all healthy rhesus macaques before assigned to other experiments, allocations, and transportation to other facilities. All rhesus macaques were returned to their cages after the examination once they were fully recovered from sedation. None of animals were euthanized for completion of this study.

All rhesus macaques at this facility are taken care in accordance with the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act [35]. Outdoor rhesus macaques are all housed as groups in 0.5-acre rectangular enclosures. Most of indoor rhesus macaques are housed pairs in stainless steel cages sized correspondingly to primary cage-space regulations. Some of indoor rhesus macaques are

housed individually in the same indoor condition. All rhesus macaques including both indoor and outdoor animals are managed with species-appropriate environmental enrichment. They are fed primate chow twice daily (LabDiet Monkey Diet 5047, Purina Mills International, St Louis, MO) with biweekly vegetables and fruits supplement. Water is provided using automatic watering device to animals without any restriction to access. Room lighting in the indoor room for the indoor rhesus macaques is automatically controlled with alternating 12h:12 h light and darkness. Complete physical examination, tuberculosis testing, dental prophylaxis, and blood tests including complete blood count analysis and serum biochemistry are performed accordingly. When any health issues are noted, these rhesus macaques were transferred to a separated colony and excluded from the present study. All rhesus macaques were also monitored for possible viral infections (herpes B virus, simian type D retrovirus, simian immunodeficiency virus, and simian T-lymphotropic virus).

## **Sedation:**

Rhesus macaques were fasted overnight and sedated with ketamine hydrochloride (10 mg/kg IM; Ketaject, Phoenix Pharmaceutical, St. Joseph, MO) within five to ten minutes prior to echocardiographic examination and BP measurement. If necessary, an additional dose of ketamine (5–10 mg/kg IM) was given during echocardiographic assessment to maintain appropriate sedation.

## **Echocardiographic measurement:**

Echocardiographic examination of rhesus macaques was performed as previously reported as a part of routine screening and for other ongoing projects [3]. Echocardiographic examinations were performed using one of two echocardiographic ultrasound devices (Affiniti 50, Phillips, Best, Netherland, and CX50 Ultrasound System, Phillips, Best, Netherlands) with rhesus macaques positioned in right and left lateral recumbency subsequently. All echocardiographic examinations were performed by a veterinary cardiologist (JS) or a cardiology research fellow (YU) under the direction of a veterinary cardiologist using a 4- to 12-mHz sector-array transducer (S12–4) or a 1- to 5-mHz sector-array transducer (S5–1) depending on the size of the rhesus macaque. 2D and M-mode echocardiography with color and spectral Doppler was performed and saved simultaneously. Three consecutive measurements were obtained for each echocardiographic parameter. Using offline analysis software (Syngo Dynamics, Siemens, Erlangen, Germany), all results were analyzed by an author (YU) and reviewed by an ACVIM board-certified cardiologist (JS). All measurements were done in accordance with the guidelines for performing a comprehensive transthoracic echocardiographic examination in adults provided by the American Society of Echocardiography. In this study, the leading-edge to leading-edge method of measurement was used for all echocardiographic measurements [36]. After measurement and assessment, rhesus macaques without any significant cardiac abnormalities and changes were selected as control animals from the echocardiographic database developed by the authors (YU, JS).

In this study, the aortic root diameter (Ao) and left atrial diameter (LA) were acquired from right parasternal long-axis 4-chamber views (Ao [1a] and LA [1a]) from 2D views (Figure 1[a] and [b]) and the right parasternal short-axis view at the level of the aortic valve (Ao [sa] and LA [sa]) (Figure 1[c]). The interventricular septal thickness during diastole (IVSd) and left ventricular posterior wall thickness in diastole (LVPWd) were obtained from the two-dimensional (2D) right parasternal long-axis and short-axis 4-chamber views, and the maximal thickness of IVSd and LVPWd were reported as IVSd (2D) and LVPWd (2D) (Figure 1[a] and [d]). The interventricular septal thickness during diastole (IVSd) and systole (IVSs), left ventricular posterior wall thickness in diastole (LVPWd) and systole (LVPWs), and left ventricular internal diameter during diastole (LVDd) and systole (LVDs) as well as mitral valve E-point to septum separation (EPSS) were measured from the right parasternal short-axis M-mode views at the chordae level (Figure 1[d], 2[a], and [b]). Peak pulmonary flow velocity (PV Vmax) with its acceleration time (PVAT), and ejection time (PVET) were obtained from the right parasternal short-axis view of the right ventricular outflow tract at the level of the aortic valve. The sample gate using the pulsed-wave spectral Doppler technique was positioned in the pulmonary artery just distal to the pulmonic valve (Figure 2[c] and [d]). The left parasternal apical four-chamber view was used to measure the passive early filling (E wave) and atrial contraction later filling (A wave) velocities (Figure 1[e]). The sample gate using the continuous spectral Doppler technique was positioned at the tips of the mitral valve leaflets when they were wide open (Figure 2[e]). Color-tissue Doppler imaging (TDI) was performed to obtain septal (medial) and free-wall (lateral) mitral annulus motions from the left apical 4-chamber view, and peak velocities were measured in early (E' [medial] and E' [lateral]) and late diastole (A' [lateral] and A' [lateral]) (Figure 2[f]). Aortic flow profile with maximal aortic flow velocity (Ao Vmax) was obtained from the subcostal or left parasternal apical aortic outflow view with parallel alignment to the aorta using the continuous spectral Doppler technique (Figure 2[g] and [h]) [37, 38]. Acceptable parallel alignment of the Doppler gate was possible in all rhesus macaques, no angle corrections were performed. Using the same image, isovolumic relaxation time (LVIVRT) and mitral E deceleration time (MVDT) were also measured. On the left apical four-chamber view optimized for the right cardiac chambers, tricuspid annular plane systolic excursion (TAPSE) was obtained based on the M-mode by qualifying the maximal longitudinal displacement of the lateral tricuspid valve annulus toward the right ventricular apex during systole. During the measurements, the cursor was placed as parallel as possible to the majority of the right ventricular free wall (Figure 2[i]). Pulsed-wave TDI velocities of longitudinal myocardial motion at the lateral tricuspid annulus were also obtained to measure peak systolic annular velocity (RV S' Vmax) (Figure 2[j]).

Using the color Doppler technique, the presence and severity of valve regurgitations were determined on all four cardiac valves as previously performed [3]. Mitral and tricuspid valve regurgitations were evaluated from right parasternal long-axis four-chamber view or right parasternal short-axis view of the heart base with aorta or left apical four-chamber view. Mitral valve regurgitations were quantified as severe (jets occupying more than 70% of the left atrium area), moderate (30 to 69% of atrium), or mild (less than 29%). Tricuspid valve regurgitations were quantified as severe (right ventricular, right atrium, and vena cava all dilated), moderate (normal or dilated right ventricle, right atrium, or vena cava), or mild (all normal size). Aortic regurgitations were quantified as severe (ratio of jet height to left ventricular

outflow tract width, greater than 65%), moderate (25% to 64%), and mild (less than 24%) based on the right parasternal left ventricular outflow view or left parasternal left ventricular outflow flow view. Pulmonic regurgitation was quantified as severe (wide origin jet with severely dilated right ventricle), moderate (wide origin jet with normal or mildly dilated right ventricular size) from right parasternal short axis pulmonary outflow view, or mild (thin narrow origin jet with normal right ventricular size). The presence of left ventricular outflow tract obstruction (LVOTO) was determined by color Doppler evaluation from right parasternal long axis or left parasternal left ventricular outflow view. Rhesus macaques with moderate or severe valve regurgitation and those with LVOTO were excluded from the present study. Rhesus macaques with no or mild valve regurgitation were enrolled in the present study as long as no other structural and/or functional abnormalities were noted on the echocardiographic examination.

Left ventricular fractional shortening (LVFS) and ejection fraction (LVEF) were calculated as previously described and the animals with LVFS less than 25% and/or LVEF less than 50% were excluded from this study [3].

Intra-observer measurement variability was determined by having one of the authors (YU) measure blinded echocardiographic parameters twice on different days from saved echocardiographic images from ten randomly selected rhesus macaques with good image quality. Inter-observer measurement variability was calculated by having two of the authors (YU, LD) measure all echocardiographic parameters, while they are blinded to each other's measurements.

## **Blood pressure measurement:**

Under sedation, indirect BP measurement was performed in 181 rhesus macaques using an oscillometric systemic BP measurement device (Cardell 9401, Midmark Corp, Versailles, OH, USA) at the same time of echocardiographic examination. Briefly, animals had a systolic, mean and direct BP measured on the left forelimb while the animal was in right lateral recumbency[39]. BP was measured two to three times ensuring that the displayed heart rate on the BP measurement device was confirmed to match the heart rate obtained on echocardiogram and all obtained values were averaged.

## **Statistical analysis:**

This study is a prospective observational study to establish references intervals for echocardiographic parameters on healthy rhesus macaques. 823 rhesus macaques were enrolled in the study when echocardiographic examination was performed as a part of routine examination before assigning to experiments, allocation, and transportation. All data obtained were acquired by the authors (YU, JS) between January 2015 and November 2019 at the CNPRC.

The mean percent coefficient of variation (CV) was calculated for intraobserver and interobserver measurement variabilities using an equation:  $CV = (\text{SD of the measurement} / \text{average of measurement}) \times$

Descriptive statistics (mean, SD, and range) was calculated and provided as mean +/- SD for normally distributed parameters and median and interquartile range for non-normally distributed parameters. Outliers were determined using the Tukey test and removed before establishing double-sided 95% reference intervals. The 90% confidence intervals for the reference limits were obtained without the robust method. Normality testing for continuous data was performed using D'Agostino-Pearson test [16].

Pairwise Pearson correlation analysis was performed to assess the correlations between patient characteristics (BW, age, sex, HR, and systolic and mean BP) and all echocardiographic variables. The degree of correlation was determined with  $r$  as weak correlation with  $r < 0.3$ , moderate correlation with  $0.3 < r < 0.5$ , strong correlation with  $r > 0.5$ .

For each echocardiographic parameter, multiple regression analysis was performed between all patient characteristics and the echocardiographic parameters. A model was performed stepwise by using age (in days), BW (kg), sex, and heart rate (HR; bpm) as explanatory variables. 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 while other variables are kept constant.

Linear echocardiographic variables were normalized to BW (kg) using the constants obtained from allometric scaling or power equation,  $Y = ax^b$ . In this equation,  $a$  represents the proportionality constant,  $b$  represents the scaling exponent,  $Y$  represents the linear echocardiographic parameter, and  $x$  represents the BW. Linear regression analysis was then performed on  $\log_{10}(\text{BW})$  versus each  $\log_{10}(\text{echocardiographic parameter})$ . This produces the  $\log_{10}$  form of the allometric scaling equation,  $\log(Y) = \log(a) + b \times \log(x)$ . In this equation,  $b$  represents the slope and  $a$  represents antilog Y-intercept. Prediction intervals were determined from the constant ( $a_c$ ) using the formula:  $a_c = \log_{10}^{-1}[\log(a) +/- t \times S_{x,y}]$ . In this equation,  $a$  is the proportionality constant obtained from the linear regression equation,  $t$  is the desired Student's t-statistic for  $n-2$  degrees of freedom, and  $S_{x,y}$  is the standard error of the Y estimate obtained from linear regression.

A P-value of  $< 0.05$  was assigned as significant for all analyses including multivariable analysis. Statistical analysis was performed using commercially available software packages (MedCalc version 12.7.4, MedCalc Software, Ostend, Belgium).

## List Of Abbreviations

A wave, atrial contraction late 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; Ao Vmax, peak velocity for aortic flow; BP, blood pressure; BW, body weight; CNPRC, California National Primate Research Center; CV, coefficient of variation; DF, degree of freedom; E wave, passive filling early filling velocity; E'

wave, early diastolic mitral annulus motion; EPSS, E-point septal separation; HCM, hypertrophic cardiomyopathy; HR, heart rate; IVSd, interventricular septal wall thickness during diastole; IVSs, interventricular septal wall thickness during systole; LA(la), left atrial diameter in diastole 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, LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; LVIVRT, left ventricular isovolumic relaxation time; LVOTO, left ventricular outflow tract obstruction; LVPWd, left ventricular posterior wall thickness during diastole; LVPWs, left ventricular posterior wall thickness during systole; MVDT, left ventricular deceleration time; PVAT, pulmonary valve acceleration time; PVET, pulmonary valve ejection time; PV Vmax, peak velocity for pulmonary flow; RV S' Vmax, tricuspid peak systolic annular velocity; SAM, systolic anterior motion; SD, standard deviation; TAPSE, tricuspid annular plane systolic excursion; TDI, Color tissue Doppler image; 2D, two-dimensional

## Declarations

*Ethics approval and consent to participate:* This study was conducted at the CNPRC with the IACUC approval of the University of California-Davis. The CNPRC is USDA-registered and AAALAC-accredited facility and maintains a Public Health Services Assurance

*Consent for publication:* Not applicable

*Availability of data and materials:* The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

*Competing interests:* The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

*Funding:* This study was supported by the California National Primate Research Center Base Grant Award Number CNPRC-P51 OD011107.

*Author contributions:* JS and JR conceived the research and selected the study subjects. YU and JS collected and analyzed the data. YU wrote the manuscript. All the authors contributed to the revision of the manuscript and approved the final version of the manuscript for submission.

*Acknowledgements:* We acknowledge the expertise of Ross Allen who aided in the completion of this study.

## References

1. Korcarz CE, Padrid PA, Shroff SG, Weinert L, Lang RM: *Doppler echocardiographic reference values for healthy rhesus monkeys under ketamine hydrochloride sedation. J Med Primatol* 1997, 26(6):287–298.

2. Tang HL, Wang LL, Cheng G, Wang L, Li S: *Evaluation of the cardiovascular function of older adult Rhesus monkeys by ultrasonography. J Med Primatol* 2008, 37(2):101–108.
3. Ueda Y, Gunther-Harrington CT, Cruzen CL, Roberts JA, Stern JA: *Echocardiographic Parameters of Clinically Normal Geriatric Rhesus Macaques (Macacamulatta). J Am Assoc Lab Anim Sci* 2017, 56(4):361–368.
4. Cornell CC, Kittleson MD, Della Torre P, Haggstrom J, Lombard CW, Pedersen HD, Vollmar A, Wey A: *Allometric scaling of M-mode cardiac measurements in normal adult dogs. J Vet Intern Med* 2004, 18(3):311–321.
5. Sisson D, Schaeffer D: *Changes in linear dimensions of the heart, relative to body weight, as measured by M-mode echocardiography in growing dogs. Am J Vet Res* 1991, 52(10):1591–1596.
6. Kim SH: *Normalization of Cardiac Measurements: Isometric vs. Allometric Method. J Cardiovasc Imaging* 2020, 28(1):18–20.
7. Daimon M, Watanabe H, Abe Y, Hirata K, Hozumi T, Ishii K, Ito H, Iwakura K, Izumi C, Matsuzaki M *et al*: *Gender differences in age-related changes in left and right ventricular geometries and functions. Echocardiography of a healthy subject group. Circ J* 2011, 75(12):2840–2846.
8. Hayashi S, Yamada H, Nishio S, Hotchi J, Bando M, Takagawa Y, Saijo Y, Hirata Y, Sata M: *Age- and gender-specific changes of tricuspid annular motion velocities in normal hearts. J Cardiol* 2015, 65(5):397–402.
9. D'Andrea A, Vriza O, Carbone A, Ferrara F, Di Maio M, Cocchia R, Tagliamonte G, Acri E, Driussi C, Pezzullo E *et al*: *The impact of age and gender on right ventricular diastolic function among healthy adults. J Cardiol* 2017, 70(4):387–395.
10. Haertel AJ, Stern JA, Reader JR, Spinner A, Roberts JA, Christie KL: *Antemortem Screening for Left Ventricular Hypertrophy in Rhesus Macaques (Macaca mulatta). Comp Med* 2016, 66(4):333–342.
11. Kanthaswamy S, Reader R, Tarara R, Oslund K, Allen M, Ng J, Grinberg C, Hyde D, Glenn DG, Lerche N: *Large scale pedigree analysis leads to evidence for founder effects of Hypertrophic Cardiomyopathy in Rhesus Macaques (Macaca mulatta). J Med Primatol* 2014, 43(4):288–291.
12. Reader JR, Canfield DR, Lane JF, Kanthaswamy S, Ardeshir A, Allen AM, Tarara RP: *Left Ventricular Hypertrophy in Rhesus Macaques (Macaca mulatta) at the California National Primate Research Center (1992–2014). Comp Med* 2016, 66(2):162–169.
13. Shannon RP, Simon MA, Mathier MA, Geng YJ, Mankad S, Lackner AA: *Dilated cardiomyopathy associated with simian AIDS in nonhuman primates. Circulation* 2000, 101(2):185–193.

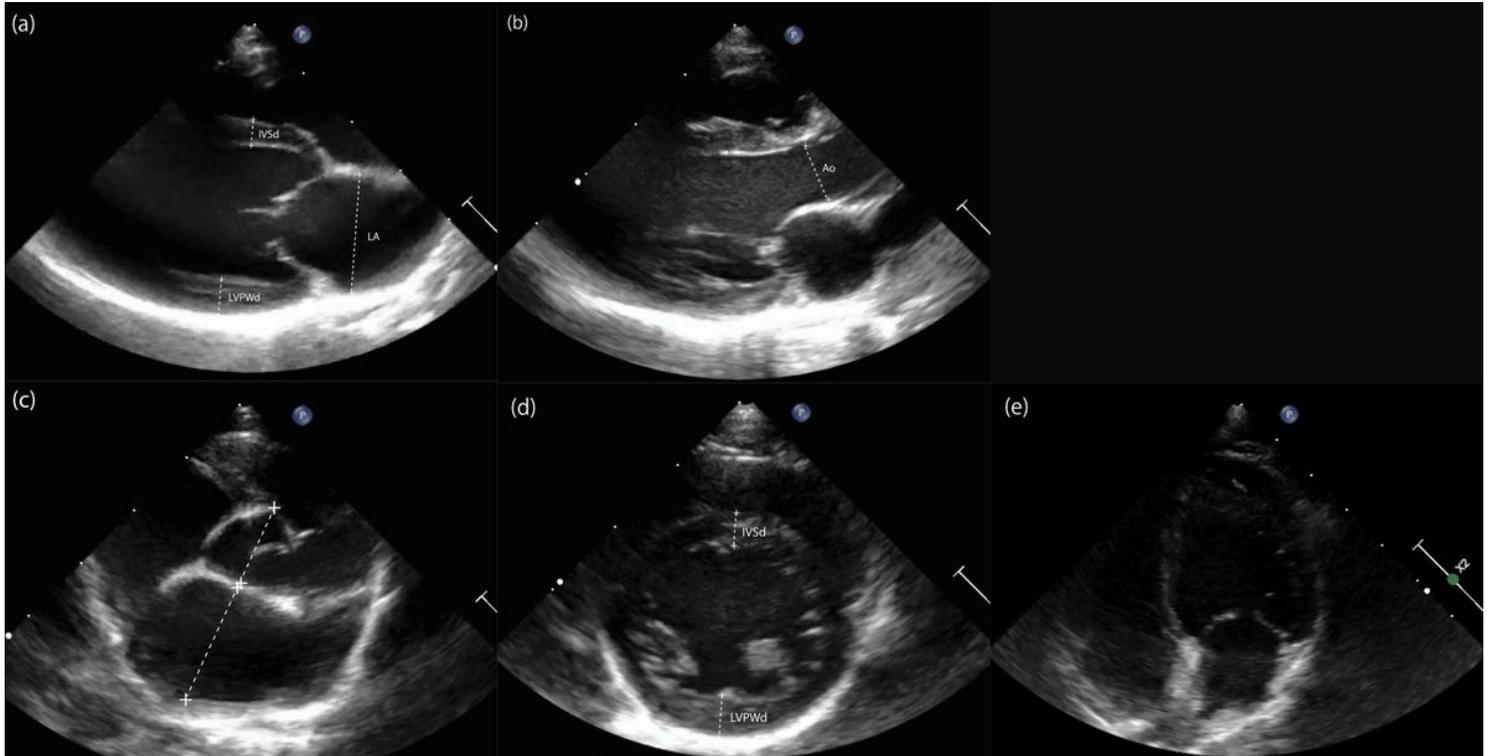
14. Shannon RP: *SIV cardiomyopathy in non-human primates. Trends Cardiovasc Med* 2001, 11(6):242–246.
15. Vogel P, Fritz D: *Cardiomyopathy associated with angiomatous pheochromocytoma in a rhesus macaque (Macaca mulatta). Vet Pathol* 2003, 40(4):468–473.
16. Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton RM, Barnhart KF, Blanco-Chavez J, American Society for Veterinary Clinical P: *ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Vet Clin Pathol* 2012, 41(4):441–453.
17. Popescu BA, Andrade MJ, Badano LP, Fox KF, Flachskampf FA, Lancellotti P, Varga A, Sicari R, Evangelista A, Nihoyannopoulos P *et al: European Association of Echocardiography recommendations for training, competence, and quality improvement in echocardiography. Eur J Echocardiogr* 2009, 10(8):893–905.
18. Rovira S, Munoz A, Rodilla V: *Allometric scaling of echocardiographic measurements in healthy Spanish foals with different body weight. Res Vet Sci* 2009, 86(2):325–331.
19. Rovira S, Munoz A: *Two-dimensional- and M-mode echocardiographic measurements and indices of cardiac function in Spanish colts and fillies of different age. J Vet Med Sci* 2009, 71(7):957–964.
20. Kayar A, Gonul R, Or ME, Uysal A: *M-mode echocardiographic parameters and indices in the normal German shepherd dog. Vet Radiol Ultrasound* 2006, 47(5):482–486.
21. Karsten S, Stephanie S, Vedat Y: *Reference intervals and allometric scaling of two-dimensional echocardiographic measurements in 150 healthy cats. J Vet Med Sci* 2017, 79(11):1764–1771.
22. Haggstrom J, Andersson AO, Falk T, Nilfors L, U Ol, Kresken JG, Hoglund K, Rishniw M, Tidholm A, Ljungvall I: *Effect of Body Weight on Echocardiographic Measurements in 19,866 Pure-Bred Cats with or without Heart Disease. J Vet Intern Med* 2016, 30(5):1601–1611.
23. Kellihan HB, Stepien RL, Hassen KM, Smith LJ: *Sedative and echocardiographic effects of dexmedetomidine combined with butorphanol in healthy dogs. J Vet Cardiol* 2015, 17(4):282–292.
24. Nkomo VT, Gardin JM, Skelton TN, Gottdiener JS, Scott CG, Enriquez-Sarano M: *Burden of valvular heart diseases: a population-based study. Lancet* 2006, 368(9540):1005–1011.
25. Passik CS, Ackermann DM, Pluth JR, Edwards WD: *Temporal changes in the causes of aortic stenosis: a surgical pathologic study of 646 cases. Mayo Clin Proc* 1987, 62(2):119–123.
26. Marwick TH, Gillebert TC, Aurigemma G, Chirinos J, Derumeaux G, Galderisi M, Gottdiener J, Haluska B, Ofili E, Segers P *et al: Recommendations on the Use of Echocardiography in Adult Hypertension: A Report from the European Association of Cardiovascular Imaging (EACVI) and the American Society of Echocardiography (ASE). J Am Soc Echocardiogr* 2015, 28(7):727–754.

27. Ravakhah K, Motallebi M: *Silent aortic regurgitation in systemic hypertension. J Heart Valve Dis* 2013, 22(1):64–70.
28. Phillips KA, Bales KL, Capitanio JP, Conley A, Czoty PW, Hart BA, Hopkins WD, Hu SL, Miller LA, Nader MA *et al*: *Why primate models matter. Am J Primatol* 2014, 76(9):801–827.
29. Milani-Nejad N, Janssen PM: *Small and large animal models in cardiac contraction research: advantages and disadvantages. Pharmacol Ther* 2014, 141(3):235–249.
30. Camacho P, Fan H, Liu Z, He JQ: *Small mammalian animal models of heart disease. Am J Cardiovasc Dis* 2016, 6(3):70–80.
31. Camacho P, Fan H, Liu Z, He JQ: *Large Mammalian Animal Models of Heart Disease. J Cardiovasc Dev Dis* 2016, 3(4).
32. Cox LA, Olivier M, Spradling-Reeves K, Karere GM, Comuzzie AG, VandeBerg JL: *Nonhuman Primates and Translational Research-Cardiovascular Disease. ILAR J* 2017, 58(2):235–250.
33. Ueda Y, Stern JA: *A One Health Approach to Hypertrophic Cardiomyopathy. Yale J Biol Med* 2017, 90(3):433–448.
34. Ueda Y, Slabaugh TL, Walker AL, Ontiveros ES, Sosa PM, Reader R, Roberts JA, Stern JA: *Heart Rate and Heart Rate Variability of Rhesus Macaques (Macaca mulatta) Affected by Left Ventricular Hypertrophy. Front Vet Sci* 2019, 6:1.
35. *Animal Welfare Act as Amended. 7 USC* 2013, S2131–2159.
36. Mitchell C, Rahko PS, Blauwet LA, Canaday B, Finstuen JA, Foster MC, Horton K, Ogunyankin KO, Palma RA, Velazquez EJ: *Guidelines for Performing a Comprehensive Transthoracic Echocardiographic Examination in Adults: Recommendations from the American Society of Echocardiography. J Am Soc Echocardiogr* 2019, 32(1):1–64.
37. Abbott JA, MacLean HN: *Two-dimensional echocardiographic assessment of the feline left atrium. J Vet Intern Med* 2006, 20(1):111–119.
38. Rishniw M, Erb HN: *Evaluation of four 2-dimensional echocardiographic methods of assessing left atrial size in dogs. J Vet Intern Med* 2000, 14(4):429–435.
39. France LK, Vermillion MS, Garrett CM: *Comparison of Direct and Indirect Methods of Measuring Arterial Blood Pressure in Healthy Male Rhesus Macaques (Macaca mulatta). J Am Assoc Lab Anim Sci* 2018, 57(1):64–69.

## Tables

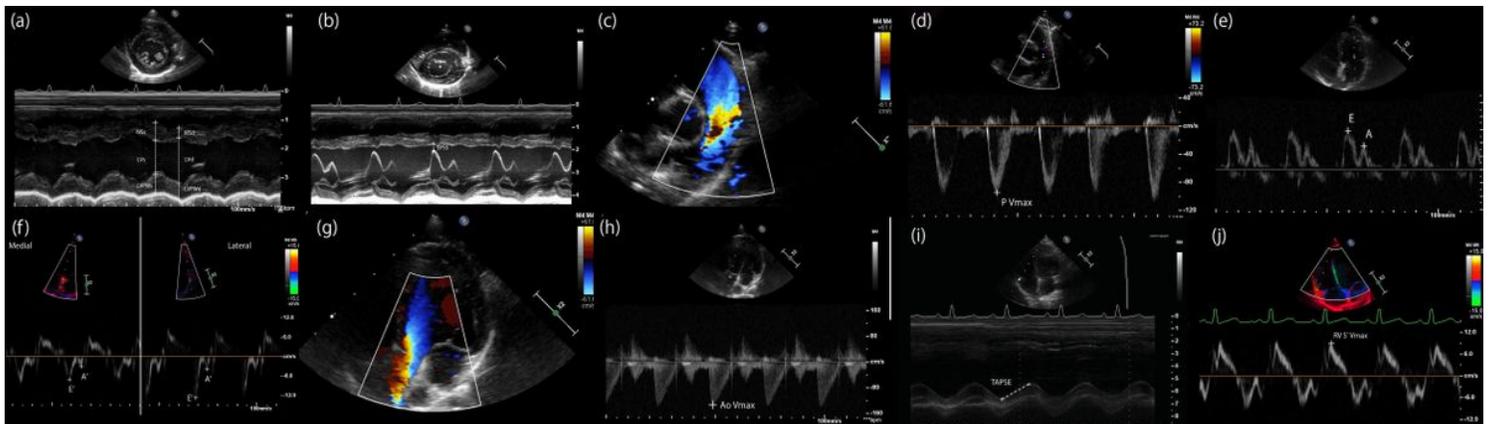
Due to technical limitations, all tables are only available for download from the Supplementary Files section.

## Figures



**Figure 1**

Examples of two-dimensional echocardiographic images. (a) Right parasternal long-axis four-chamber view, (b) right parasternal long-axis left ventricular outflow view, (c) right parasternal short-axis view of the heart base with aorta, (d) right parasternal short-axis left ventricle with papillary muscles view, (e) left parasternal apical four-chamber view



**Figure 2**

Examples of M-mode and Doppler echocardiographic image. (a) M-mode image of right parasternal short-axis left ventricle with papillary muscle view, (b) M-mode image of right parasternal short-axis mitral valve view, (c) color Doppler image with pulmonary flow in right parasternal short-axis view from heart base with pulmonary artery, (d) pulsed-wave spectral Doppler image with pulmonary flow in right parasternal short-axis view from heart base with pulmonary artery, (e) pulsed-wave spectral Doppler image of mitral flow from left parasternal apical four-chamber view, (f) color-tissue Doppler image with medial and lateral mitral annulus motions from left apical 4-chamber view, (g) color Doppler image of aortic outflow from left apical five-chamber view, (h) continuous-wave spectral Doppler image of aortic outflow from left parasternal five-chamber view (i) M-mode image of tricuspid annular plane systolic excursion from left apical 4-chamber view optimized for the right cardiac chambers, (j) color-tissue Doppler image with longitudinal myocardial motion at the lateral tricuspid annulus from left apical four-chamber view optimized for the right cardiac chambers

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [NC3RsARRIVEGuidelinesChecklist2014Ueda.docx](#)
- [AdditionalFileRhesusEchiRIUeda02.22.2020.docx](#)
- [Tables.docx](#)