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Echocardiographic and Electrocardiographic Characteristics of Male and Female Squirrel Monkeys (*Saimiri* spp.)

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Cardiomyopathy is a leading cause of mortality in aging squirrel monkeys (*Saimiri* spp.). However, data regarding echocardiographic measures obtained from clinically healthy nonsedated squirrel monkeys have not been published, and few electrocardiographic data are available. Here we obtained echocardiographs without sedation and electrocardiographs with minimal sedation from 63 clinically healthy squirrel monkeys that ranged from 3 to 20 y in age. 2D and M-mode echocardiography were performed on nonsedated monkeys to determine the left ventricular internal diameters at systole and diastole and the ejection fraction. Electrocardiography was performed under sedation with ketamine (15 mg/kg). Parameters evaluated included heart rate; P-wave duration; lengths of the PR, QRS, and QT intervals; R-wave amplitude, and P-wave amplitude. Initial physical examination, electrocardiography, and echocardiography indicated normal cardiac function for all monkeys. The objectives of this study were to provide reference values for nonsedated echocardiography and ketamine-sedated electrocardiography of clinically normal squirrel monkeys and to determine correlates of age and sex in these values.

Squirrel monkeys (*Saimiri* spp.) are the most commonly studied neotropical nonhuman primate in biomedical research in the United States.[2] Heart failure due to cardiomyopathy is a leading cause of mortality in squirrel monkeys, particularly in large colonies of aging animals. The initial case report of dilative cardiomyopathy in a squirrel monkey came from our colony,[26] and in subsequent years, cardiomyopathy and heart failure have been the primary health concern in the geriatric population at our institution. In reviewing necropsy records over a 4-y span (census of approximately 70), we noted that 9 adult squirrel monkeys were euthanized for medical reasons related to cardiac changes. Another institution reported cases of dilative and hypertrophic cardiomyopathy in adult female Bolivian squirrel monkeys (*S. bolivinesis*) and found that 23 of 88 adult animals had lesions consistent with heart failure or cardiomegaly on necropsy.[3] Affected squirrel monkeys often do not present with clinical signs until cardiomegaly is severe, and often euthanasia is performed due to the lack of treatment options in the late stage of disease.

Squirrel monkey cardiomyopathy has not been characterized as primarily hypertrophic or dilative, and the causative agent is currently unknown.[3] Potential causes of cardiomyopathy in these species include *Trypanosoma cruzi*,[6,16] taurine deficiency,[19,26] virus-mediated myocarditis,[1] and captivity,[8] but the evidence is unclear. Current diagnostic methods available for the detection of cardiomyopathy include echocardiography, electrocardiography, radiology, and physical examination. Echocardiography enables the evaluation of cardiac function through the estimation of ejection fraction, chamber size, and wall thickness and the evaluation of ventricular outflow track. This noninvasive diagnostic method can be performed on nonsedated squirrel monkeys accustomed to handling. Anesthetics can cause cardiovascular and respiratory system depression, resulting in jeopardized cardiac function, confounding hemodynamic data, or underestimation of left ventricular function; therefore, performing cardiac evaluations on nonsedated animals may be preferable.[28] Electrocardiography provides insight into conductive abnormalities within the normal or diseased heart. However, changes can be very subtle and difficult to detect, and good-quality tracings are therefore necessary.[9] Sedation is often used to minimize muscle movement and aid in electrode placement.[25]

Few data regarding normal echocardiographic and electrocardiographic reference ranges in squirrel monkeys are available.[2,3,27] Previously published echocardiographic data were obtained from animals sedated with ketamine and xylazine, and electrocardiographic data were obtained after sedation with either sodium thiopental or ketamine and xylazine.[2,3,27] Furthermore, these previous studies assessed only single-sex populations or animals within a narrow age range, and the subspecies of *Saimiri* was mixed or not reported.[2,3,27] In the current study, we characterized echocardiographic data obtained from nonsedated, clinically healthy male and female Guyanese squirrel monkeys of various ages. In addition, we performed electrocardiography on these same monkeys after their sedation (15 mg/kg ketamine hydrochloride). These data establish new reference ranges for echocardiographic and electrocardiographic diagnostics in squirrel monkeys and may facilitate improved assessment of cardiac function in both healthy and diseased squirrel monkeys.

Materials and Methods

Animals. The study population comprised 63 (19 male and 44 female) adult Guyanese squirrel monkeys (*Saimiri sciureus sciureus*) that ranged from 4 to 20 y in age (Figure 1) and that were housed in same-sex groups of 2 to 10 members. Approximately 95% of the animals were born and raised at Stanford University;

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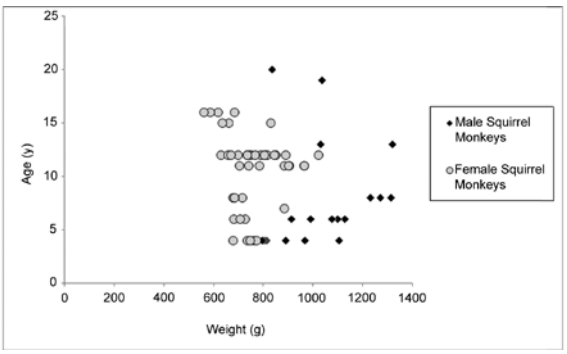

Figure 1. Scatter plot of age, weight, and sex distribution of study population.

the remaining 5% were domestically bred at other facilities. All monkeys had been screened previously for *T. cruzi* and were clinically normal. All animals were housed and handled in accordance with the *Guide for the Care and Use of Laboratory Animals* in an indoor facility accredited by AAALAC and were assigned to behavioral science research protocols.[11] All procedures for this study were approved by the IACUC of Stanford University.

The daily diet consisted of free-choice water and primate chow (5040 New World Primate Diet, PMI Nutrition International, St Louis, MO). Room conditions included a 12:12-h light:dark cycle, temperature of 24.6 to 29 °C, relative humidity of 30% to 70%, and 10 to 15 air changes hourly. A variety of manipulable objects, fruits, and vegetables were provided as a part of standard enrichment practices. All squirrel monkeys received annual exams that included an intradermal mammalian tuberculin test (Synbiotics, San Diego, CA).

Cardiac and clinical assessment. Echocardiography (Sonovet R3, Samsung Medison, Seoul, Korea) was performed on all monkeys by a board-certified physician cardiologist using a 6.5-MHz transducer adjusted for optimal image with the lowest possible power. Penetration depth was set at 5 to 50 mm. For echocardiographic examination, animals were handheld in left lateral recumbency on fleece pads. The transducer was placed in left parasternal short-axis view. A full cardiac assessment was made, and parameters measured included left ventricular internal diameter at diastole, left ventricular internal diameter at systole, and calculation of ejection fraction.

One week after echocardiographic assessment, all squirrel monkeys were sedated with ketamine hydrochloride (15 mg/kg IM; Fort Dodge Animal Health, Fort Dodge, IA) and received a complete physical examination as a part of their annual evaluation. This examination included assessing core body temperature, heart rate, respiratory rate, and body weight as well as completing tuberculosis testing and dental prophylaxis. In addition, electrocardiography (SurgiVet Advisor, Smiths Medical PM, Waukesha, WI) was performed at this time. Electrocardiograms were recorded by using a chart speed of 50 mm/s. For all recordings, gain was adjusted for 10 mm of deflection per millivolt. Standard blunt-toothed alligator clips were applied to the forearms and thighs. Electrical contact was enhanced by the use of electrode paste. The electrocardiograms were recorded from each monkey in the right lateral decubitus position (right side dependent, limbs partially flexed). Leads I and II were measured, and the following parameters were assessed: atrial and ventricular rates (derived from an average of 5 P-P and R-R intervals), P-wave duration, PR interval, QRS interval, QT interval, R-wave amplitude, and P-wave amplitude.

Statistical analysis. Statistical testing was conducted by using SPSS (version 21; SPSS IBM Software, Armonk, NY). All descriptive measurements from echocardiographic and electrocardiographic data are expressed as group mean ± 1 SD. Nonparametric Mann–Whitney procedures were conducted to determine whether echocardiographic or electrocardiographic findings differed between sexes. Nonparametric Kendal τ correlations between age and major study variables were calculated. Statistical significance was defined as a P value less than 0.05.

Results

A representative echocardiogram from a 7-y-old adult female squirrel monkey is shown in Figure 2. Echocardiographic parameters did not differ between male and female squirrel monkeys, therefore these values are reported for the overall study population.The mean left ventricular internal diameter at diastole was 0.87 ± 0.15 cm; the mean left ventricular internal diameter at systole was 0.53 ± 0.14 cm; and the mean ejection fraction was 74.5% ± 8.9%. However, electrocardiograms revealed that male monkeys had longer PR intervals (P = 0.02) and higher mean R-wave amplitudes (P = 0.004) than did female monkeys (Table 1). A representative electrocardiograph from a 4-y-old adult female squirrel monkey is shown in Figure 3. The findings in Table 2 indicate that age was negatively correlated with P-wave amplitude (τ = –0.27, P = 0.02) and QRS interval scores (τ = –0.24, P = 0.04).

Discussion

This study provides the first echocardiographic data collected from nonsedated clinically healthy male and female squirrel monkeys and provides new reference ranges for the clinical assessment of cardiac function. One previous study[3] reported echocardiography values obtained from healthy female squirrel monkeys anesthetized with ketamine and xylazine. Values obtained in our study concur with those obtained previously,[2] except for cardiac ejection fraction. Specifically, we found a mean ejection fraction of 74.5% in nonsedated squirrel monkeys, whereas a previous study[3] reported a mean ejection fraction of 59% in monkeys sedated with ketamine and xylazine—a difference of approximately 15% This difference can be explained by the known effect of xylazine on heart rate, which can decrease cardiac output by as much as 30%.[21] In fact, several studies have demonstrated xylazine's effect on measures of cardiac performance, including ejection fraction, left ventricular wall amplitude, aortic amplitude, and mitral valve E point septal separation.[4,12]

Electrocardiographic recordings from 176 squirrel monkeys sedated with sodium thiopental (112 were 2 to 3 y of age, and 54 were of unknown age) were collected previously by using a 9-lead electrocardiogram.[27] In comparison, our electrocardiographic data were obtained by using a standard anesthetic monitoring device and ketamine sedation—a more typical clinical setup than that used in the previous study[26] and thus perhaps more applicable to current practice. Cardiac output is increased with ketamine anesthesia and remains unchanged by thiopental anesthesia.[10,22] Although ketamine can cause increases in cardiac output and heart rate,[20] previous studies have shown that ketamine hydrochloride does not cause significant changes in the ECG of primates.[7] Our findings confirm this lack of effect, given that the values for the ECG indices that we obtained were very similar to those published previously.[27] Other colleagues[3] also reported ECG indices obtained from female squirrel monkeys after their sedation with ketamine and xylazine. ECG changes due to xylazine administration include bradycardia

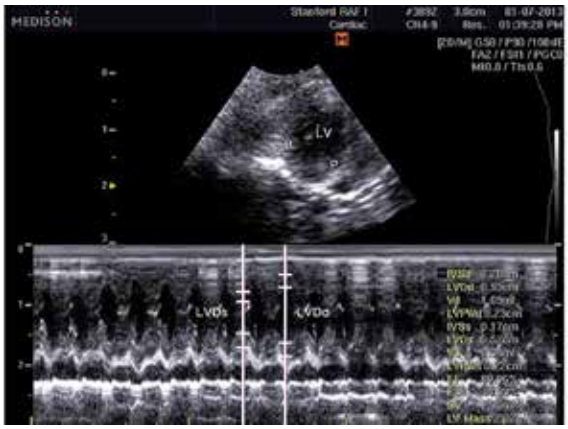

Figure 2. Representative 2D-guided M-mode echocardiograph of the left ventricle (LV) and papillary muscles (P) in short-axis view. Left ventricular internal diameter at diastole (LVDd) and left ventricular internal diameter at systole (LVDs) are labeled in the M-mode tracing.

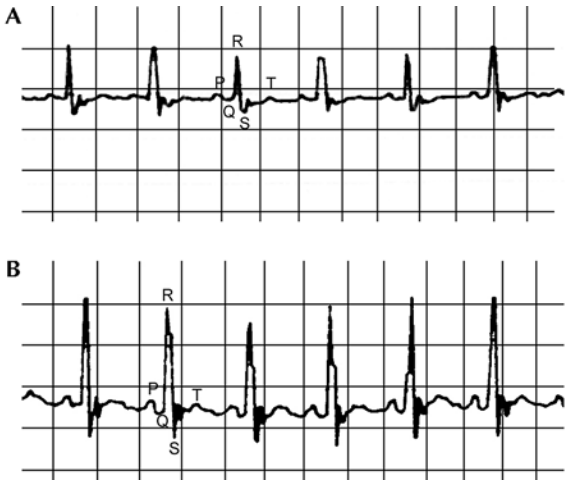

Figure 3. Representative electrocardiograph with labeled waves of (A) lead I and (B) lead II recorded at 50 mm/s and 1 mV = 10 mm.

Table 1. Electrocardiographic parameters (mean ± 1 SD) for colony animals

	Female	Male
Atrial rate (bpm)	282 ± 43.0	277.6 ± 41.8
Ventricular rate (bpm)	283 ± 43.2	278.8 ± 42.1
P-wave duration (s)	0.01 ± 0.0	0.01 ± 0.0
PR interval (s)	0.02 ± 0.0[a]	0.03 ± 0.0[a]
QRS interval (s)	0.02 ± 0.0	0.02 ± 0.0
QT interval (s)	0.06 ± 0.0	0.06 ± 0.0
P-wave amplitude (mm)	1.56 ± 0.56	1.76 ± 0.56
R-wave amplitude (mm)	9.07 ± 3.38[b]	11.4 ± 2.4[b]

1 mm = 0.1 mV

[a]PR interval was significantly (P <0.05) different between male and female squirrel monkeys.

[b]R-wave amplitude was significantly (P <0.05) different between male and female squirrel monkeys.

Table 2. Kendall τ correlation results for age and study variables

	τ	P
Ejection fraction	-0.12	0.21
P-wave duration	-0.10	0.33
PR interval	-0.02	0.84
QRS interval	-0.24	0.04
QT interval	-0.06	0.56
P-wave amplitude	-0.19	0.02
R-wave amplitude	-0.27	0.05
Atrial rate	-0.16	0.12

and increased PR and QT intervals;[13,14] therefore our indices likely vary from those published earlier[3] largely because of the difference in anesthetic technique.

The electrocardiographic results we obtained indicate significant (P < 0.05) differences between male and female squirrel monkeys in the PR interval and R-wave amplitude. Previous studies have shown significant sex-associated differences in wave duration and amplitude in humans that can be attributed to the relatively larger body and heart sizes of men as compared with women.[15,18,23,24] In women, the smaller absolute size of the heart as well as its smaller size relative to body weight both contribute to differences in wave amplitude on ECG.[23] Our squirrel monkey colony demonstrated similar sex-associated differences in body size (Figure 1), but published data confirming correlated differences in heart size are unavailable. We surmise that male squirrel monkeys have a larger heart mass, leading to increased R-wave amplitude when compared with that of female squirrel monkeys. Similarly, the longer PR interval of male monkeys might reflect increased atrial mass, thus prolonging the time until depolarization is complete within the atrium. Alternatively, the sex-associated difference in PR interval in squirrel monkeys may be attributable to the slower heart rate of male monkeys (277.6 ± 41.8 bpm) when compared with female monkeys (282 ± 43.0 bpm), given that heart rate was negatively correlates (P < 0.05) with PR interval.

We did not find a correlation between echocardiographic measurements and age, perhaps because all colony animals appeared to be clinically healthy at the time of this study. However, electrocardiographic measurements revealed statistically significant negative correlations between age and P-wave amplitude as well as age and QRS interval scores. If the study population had included cases of cardiomyopathy, which would be more likely to occur in older animals, expected ECG changes would include increased P-wave amplitude, R-wave amplitude, and QRS duration due to the associated changes in heart size.[5,17] We therefore conclude that the observed decrease in P-wave amplitude can be attributed to the smaller body size seen in older, clinically healthy animals (Figure 1).

The data we present provide valuable diagnostic reference values for clinicians assessing squirrel monkeys for evidence of heart disease. Our echocardiographic examinations indicate that ejection fraction remains consistent across age and sex and is a suitable method to measure cardiac function in squirrel monkeys. Ejection fraction provides a noninvasive, quantitative measure of heart function that can be performed on nonsedated squirrel monkeys for early detection of cardiac disease. In addition, electrocardiographic parameters were consistent across age, but male monkeys had significantly longer PR intervals and significantly higher mean R-wave amplitude than did female monkeys. These differences therefore should be considered when interpreting conduction abnormalities that may be sequelae of cardiomyopathy.

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