Temporal Changes in Ventricular Function Assessed Echocardiographically in Conscious and Anesthetized Mice

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The mouse is an important model system for cardiovascular biology, with echocardiography a critical tool for noninvasive measurement of cardiac morphology and function. The feasibility and short-term temporal consistency of repeated echocardiographic measurements in conscious mice has not been previously evaluated. We performed serial 2-dimensional guided M-mode transthoracic echocardiographic measurements at 5- to 10-minute intervals over 60 minutes in conscious mice and in mice treated with 1 of 3 anesthetic regimens: ketamine and acepromazine (n = 14); pentobarbital (n = 14); and ketamine and xylazine (n = 13). Unanesthetized mice received intraperitoneal saline (n = 6) or no injection (n = 7). In sequentially repeated measurements over 1 hour in conscious mice, none of the measured or derived echocardiographic parameters differed from baseline, whereas all 3 anesthetic regimens produced significant, prolonged, and temporally variable decreases in heart rate and fractional shortening. The relationship between heart rate and fractional shortening was not altered by anesthetic choice. Serial echocardiographic assessments of cardiac function, dimension, and mass can be performed with high reproducibility in conscious mice. (J Am Soc Echocardiogr 2003;16:1150-7.)

Echocardiography is an essential and established tool in the study of normal and genetically altered mice. Technical limitations related to the small size and rapid heart rate (HR) in the mouse initially argued for the performance of echocardiography under anesthesia, even though echocardiographic measurements of chamber dimension and systolic function, and HR, are significantly altered by anesthesia. More recently, higher frame rates, and smaller probes operating at higher frequencies, have facilitated imaging of conscious mice in some, but not all, experimental protocols and conditions.

Physiologic studies can mandate frequently repeated serial echocardiographic determinations, but the consistency and practicality of such measurements in the same mouse under conscious conditions has not been evaluated. Furthermore, the time course of anesthetic effect on echocardiographic parameters has not been thoroughly described in relation to conscious measurements on the same mice. Knowledge of the magnitude and time-dependent variation echocardiographic measurements of cardiac function, with and without general anesthesia, is critical for proper interpretations and comparison of murine physiology studies, and for defining and minimizing sources of experimental error.

We hypothesized that serial echocardiographic measurements can be performed reliably and reproducibly in conscious mice, and tested this hypothesis by performing frequent timed serial echocardiographic studies over 1 hour in conscious mice. Comparisons were made with serial determinations of the same echocardiographic measurements over 1 hour in mice anesthetized using 1 of 3 routinely used regimens: ketamine and xylazine (KX), ketamine and acepromazine (KA), or pentobarbital (Pent). Echocardiographic measurements under anesthesia were compared with the conscious baseline measurements in the same mouse, and all conscious and anesthetic measurements were compared to determine whether they adhered to the same force-frequency relationship. These data show the feasibility and reliability of repeated echocardiographic measurements...
graphic measurements in conscious mice, reveal significant differences in the temporal cardiodepressive effects of commonly used anesthetic regimens, and establish the relationship between HR and fractional shortening (FS%) relationship as a viable tool to allow comparisons among studies with differing anesthetics.

**METHODS**

**Animals**

A total of 54 C57BL/6 mice (C57BL/6J, Jackson Labs, Bar Harbor, Me) (25 males, 29 females; ages 12-24.5 weeks, mean age 19.4 weeks; body weight [BW] 19.6-44.3 g, mean BW 27.3 g) were studied in this protocol. Mice were housed in an air-conditioned room with a 12:12-hour light-dark cycle and received standard mouse chow and water ad libitum. Study protocols were approved by the institutional animal care and use committee, and conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

**Experimental Protocol and Anesthetic Regimen**

All mice were studied first in the conscious state with echocardiography. In all, 41 mice were then randomized to receive 1 of 3 commonly used anesthetic regimens by intraperitoneal (IP) injection dosed according to BW: KA (ketamine [60 µg/g] and acepromazine [25 µg/g]), n = 14; Pent (50 µg/g), n = 14; or KX (ketamine [80 µg/g] and xylazine [12 µg/g]), n = 13. Anesthetic agents were prepared in 0.9% saline and IP injectate volume was 0.1 mL/10 g. In a second set of experiments, repeated measurements were made in the conscious state on an additional 13 mice: 6 received IP saline (SIP) (0.1 mg/10 g), and 7 received no injection (NI). Echocardiographic and HR measurements were obtained 5 minutes after anesthesia injection, at 5-minute intervals thereafter until 30 minutes, and then every 10 minutes until 60 minutes. Echocardiographic measurements of anesthetized mice were terminated when movement could be elicited in response to stimulation.

**Echocardiography**

Transthoracic echocardiography was performed using a system (Sonos 5500, Agilent, Andover, Mass) with a 15-MHz high-frequency linear transducer at a frame rate of 100 frames/s. All images were acquired at a depth setting of 20 mm. Before initiation of the study, the mice were trained on 2 separate occasions over 1 to 2 days. Training included holding the mice in the position required for echocardiographic imaging for at least 5 minutes, and touching the chest in simulation of probe contact. Chest contact was performed first with a plastic probe, and then, to acclimate the mice to the sensation of the gel, with a metallic probe at 34°C.

In the conscious mice, we performed echocardiography by picking up the mouse by the nape of the neck and holding it in 1 hand in the prone position, with the tail held between the last 2 fingers. The mouse chest area was not shaved; ultrasound-coupling gel heated to 34°C was applied to the precordium, with the ultrasound probe beneath the animal. The same positioning and approach were used in anesthetized mice, but a second operator was more often required to manipulate the probe and record images while the animal was fully supported. Optimal parasternal long- and short-axis views were obtained by adjusting gain settings for visualization of endocardial and epicardial walls. Two-dimensional targeted M-mode echocardiographic images were obtained at the level of the papillary muscles from the parasternal short-axis view and recorded at a speed of 150 cm/s (maximal temporal resolution) for measurement of HR. All other measurements were made on screen using the digitally recorded signals.

**Left Ventricular Wall Thickness and Chamber Dimensions**

Wall thickness and chamber dimension were determined from M-mode tracings. Left ventricular (LV) wall thickness was evaluated in the interventricular septum (IVS) and the LV posterior wall (LVPW). End-diastolic measurements (IVSd, LVPWd, and left ventricular internal dimension [LVIDd]) were obtained at the point of maximal LV diastolic dimension. LV end-systolic dimensions (IVSs, LVPWs, and LVIDs) were obtained at the time of most anterior systolic excursion of the LVPW associated with minimal chamber dimension. All LV dimensions are presented as the average of measurements using the leading-edge technique of 3 to 5 consecutive selected sinus beats by 2 experienced readers. Both observers were blinded to the anesthetic regimen.

The FS%, a measure of LV systolic performance, was calculated from M-mode-derived LV dimensions using the formula: \( \text{FS\%} = \frac{\text{LVIDt} - \text{LVIDs}}{\text{LVIDt}} \times 100\% \). HR was determined from the cardiac cycles recorded on the M-mode tracing, using at least 3 consecutive beats. An external time standard was used to adjust for variations in paper (printout) speed. An index linearly related to cardiac output (COx) was computed as: \( \text{COx} = \text{HR} \times (\text{LVIDt} - \text{LVIDs})^3 \).

Estimates of LV mass (LVM) on the basis of systolic and diastolic echocardiographic measurements were computed as: \( \text{LVMi} = 1.05 \left( \text{IVS} + \text{LVID} + \text{LVPW} \right)^3 - \text{LVID}^3 \), where \( i \) = systolic or diastolic.

**Statistical Analysis**

Data are presented as mean ± SE unless otherwise noted. Anderson-Darling and Kolmogorov-Smirnov specification tests were used to verify approximately normal statistical distributions. Statistical significance was assessed using analysis of variance with correction for multiple determinations.
**RESULTS**

**Data Quality and Reproducibility**

A complete set of echocardiographic variables (LVIDd, LVIDd, LVPWd, TVPWhd, IVSs, IVSd, FS%, and HR) could be determined on 135/138 (98%) measurements on conscious mice, and 202/206 (98%) measurements on anesthetized mice. Repeated echocardiographic measurements from single data acquisitions in both anesthetized and unanesthetized mice were highly reproducible (intraclass correlation coefficient > 95%).

**Effect of Anesthesia on HR and FS%**

The baseline HR, measured in the conscious state before the administration of anesthetic agent or saline, did not differ among the 5 groups (overall 681 ± 8.7 bpm encompassing the anesthetic groups KX, Pent, KA, and the nonanesthetic groups SIP and NI). Consistent with the random assignment, mean age and BW were not significantly different among the treatment groups. All 3 anesthetic regimens (KX, Pent, KA) resulted in significant depression of the HR from baseline (P < .05) (Figure 1, A). HR recovered toward but did not reach baseline in any of these 3 groups at 60 minutes. The HR nadir occurred at 10 minutes with KA, and at 15 minutes with Pent and KX. Differences among HRs in the anesthesia groups diverged significantly by 15 minutes (P < .05), and differences persisted to 60 minutes, with HR recovering most rapidly with Pent and least rapidly with KX.

FS%, measured in the conscious state before the administration of anesthetic agent or saline, also did not differ among groups. FS% was depressed by all 3 anesthetic regimens (P < .05) (Figure 2, A). Recovery of FS% to baseline values occurred most rapidly in the anesthesia group with Pent (no significant difference from baseline at 25 minutes or later), and was intermediate with KA (no significant difference from baseline at 40 minutes or later). At all measurements up to 60 minutes, FS% was less than at that baseline with KX. Thus, both HR and systolic function were significantly affected by 3 commonly used anesthetic regimens.

**Repeated Measurements of HR and FS% Under Conscious Conditions**

HR was unchanged from baseline at each serial measurement over 60 minutes in conscious mice, with or without an IP injection (SIP and NI) (Figure 1, B). Similarly, FS% was unaltered on repeated measurement in the SIP and NI groups (Figure 2, B).

**Other Echocardiographic Measurements**

With all 3 anesthetic regimens, LVIDd increased in parallel with the most pronounced depressions of HR and FS%. Results with KX anesthesia were illustrative of the results seen with each of the anesthetic agents: LVIDd increased from 0.30 ± 0.01 cm at baseline to 0.36 ± 0.01 cm at 5 minutes postanesthesia (P < .001), with significant increases sustained from 5 to 20 minutes (Figure 3, A). As expected, LVIDd increased significantly, in parallel with the decrease in FS%, with all anesthetic regimens (Figure 3, A). Significant decreases could also be detected in the systolic wall thickness measurements IVSs and LVPWd with anesthesia. Again, results with KX were typical: IVSs decreased from baseline to 5 minutes, 0.16 ± 0.01 cm to 0.11 ± 0.01 cm, P < .001, with significant decreases sustained from 5 to 30 minutes (Figure 3, B). No significant changes occurred over time in the measurements of diastolic LV wall thickness (IVSd or LVPWd) (Figure 3, B). All anesthetic regimens decreased both HR and FS% substantially, and to a greater extent than they increased diastolic filling as reflected in LVIDd. Consequently, COx was substantially decreased (to 33% of baseline with KX, 53% of baseline with Pent, and 57% of baseline with KA) (Figure 3, C). Significant differences in COx were present throughout the 60-minute period of measurement with KX (Figure 3, C), whereas significant differences from baseline were less persistent with Pent (5-25 minutes, not shown) and KA (5-30 minutes, not shown).

LVM does not change during the cardiac cycle, but the echocardiographic dimensions used to calculated systolic and diastolic mass represent the extreme values of these measurements. Thus, the comparison of calculated systolic and diastolic mass can provide information about the quality of the echocardiographic information. Calculated systolic and diastolic LVM were highly correlated (Figure 4) (coefficient 1.04 ± .05, r = .94, F = 389, P < 10^-8), and did not change over time in the anesthesia (KX, Pent, KA) and conscious (SIP, NI) groups (data not shown).

**Relationship Between HR and FS%**

Although anesthesia depressed HR, the HR:FS% relationship assessed by regression analysis was independent of the presence or choice of anesthesia (Figure 5). Thus, an overall relationship could be predicted. A variety of linear, piecewise linear, and complex relationships were explored, but a logarithmic relationship explained the greatest fraction of the variance (66%): FS% = 17.17 ln(HR) − 53, F = 652, r = .81 (P < 10^-8).
domized to an anesthetic regimen, and the additional 13 mice studied only under conscious conditions, form the basis for a set of normal echocardiographic values, presented in Table 1. In this population the influence of sex and the approximately normally distributed covariates of age and BW on echocardiographic measurements was evaluated by regression analysis. Multivariate analysis demonstrated that body mass predicted echocardiographic LVM. With body mass entered into the regression, the trends for sex and age as independent predictors did not achieve statistical significance (diastolic LVM vs BW, slope/coefficient 0.0026 ± 0.0009 g/g, P < .01; systolic LVM vs BW, slope/coefficient 0.0022749 ± 0.0008 g/g, P < .01). HR decreased with advancing age (HR vs age, slope/coefficient -4.54 ± 1.92 bpm/wk, P = .02). The regression analysis of echocardiographic dimensions with the same covariates demonstrated that the increase in heart mass was a consequence of increased wall thickness (increased IVSd and LVPWd with increased BW, P < .05), rather than an increase in

Figure 1 Heart rate (HR) response during repeated echocardiographic assessment with (A) and without (B) anesthesia. None (A) represents aggregate baseline data from B, encompassing both no injection (NI) and intraperitoneal saline (SIP) groups, for comparison. HR is bpm; time is minutes after onset of anesthesia. There were no significant differences from control in NI and SIP injection time courses. Error bars, SE; KA, ketamine and acepromazine; KX, ketamine and xylazine; Pent, pentobarbital; *all sample means different from preanesthesia control, P < .05; §significant difference among sample means, P < .05.

Figure 2 Fractional shortening (FS%) during repeated echocardiographic assessment with (A) and without (B) anesthesia. None (A) represents aggregate baseline data from B for comparison. Time is minutes after onset of anesthesia. There were no significant differences from control in no injection (NI) and intraperitoneal saline (SIP) injection time courses. Error bars, SE; *ketamine and xylazine (KX) different from preanesthesia control, P < .05; +pentobarbital (Pent) different from preanesthesia control, P < .05; §ketamine and acepromazine (KA) different from preanesthesia control, P < .05.
chamber dimension ($P = \text{not significant}$). Because these measurements encompass a set of mice with a large variation in BW, we also repeated the statistics on the middle 2 quartiles of mice by BW (23.1-28.1 g inclusive, $N = 30$). When restricted to this subset, no echocardiographic parameter mean differed by more than 2%, nor did any SD differ by more than 15%, arguing strongly for the representative and robust nature of these echocardiographic normal values.

**DISCUSSION**

Repeated and highly reproducible transthoracic echocardiographic measurements are feasible in conscious mice. In conscious mice, 97% of the variance in FS% on sets of sequential determinations occurred between mice, rather than within sequential measurements, reflecting the most common definition of the intraclass correlation coefficient. Therefore, the mean FS% was unchanged on sequential determinations in conscious mice (59.6% ± 1.8% vs 59.7% ± 1.7%, $n = 56$ pairs of sequential determinations, mean ± SD). In contrast, with each of the anesthetic regimens the mean FS%, after decreasing substantially from conscious values after the induction of anesthesia, then increased on each sequential determination (eg, with KX anesthesia, 38.2% ± 9.4% vs 41.5% ± 10.3%, $n = 69$ pairs of sequential determinations, $P = .05$ by analysis of variance). If disregarded, this effect could result in significant experimental bias. Thus, when the experimental protocol requires repeated measurements but does not otherwise impose a requirement for anesthesia, sequential measurements under conscious conditions are likely to be possible, can provide consistent values, and are consequently preferable. In the conscious mouse the echocardiogram also allows assessment of the ventricular HR, an important physiologic parameter. When anesthesia is required, appropriate choice of an anesthetic agent, a narrow time window for performance, and concurrent measurement of HR...
Comparison with Other Studies

This study supports a remarkably consistent set of data from other studies showing marked changes in echocardiographic variables in mice as a consequence of anesthesia.\(^5\),\(^7\),\(^14\)-\(^17\) These include the recent report by Roth et al\(^{16}\) describing the time course of changes in murine echocardiography with several anesthetic agents including KX. Taken together, these studies suggest that the cardiodepressive effects apply to an extensive set of anesthetic agents, although the magnitude of the effect may differ among agents. Our report extends this literature in several respects, including the large number of measurements in the conscious state, and the concurrent comparison of anesthetic measurements with conscious measurements in the same mice. These differences allow us to estimate the magnitude of anesthesia effects, and to conclude that effects of anesthesia on HR and FS\% can persist for at least 1 hour. Most importantly, we can reject the hypothesis that technical limitations or variations in sympathetic or parasympathetic tone preclude the experimental application of serial echocardiography in mice in the conscious state,\(^{16}\) and in doing so, significantly broaden the range of physiologic experiments capable of being addressed with this powerful tool.

Conscious Responses to Echocardiography

We considered the possibility that conscious echocardiography in mice is associated with increased sympathetic tone and increased contractile function.\(^{16}\) Such an effect is almost certainly present, because resting HRs as low as 501 bpm have been reported in C57BL/6 mice\(^{18}\) and our mean HR

![Figure 5](https://example.com/figure5.png)

**Figure 5** Relationship between heart rate (HR) and fractional shortening (FS\%), conscious conditions (no injection [NI], intraperitoneal saline [SIP]) and with indicated anesthetic agents (ketamine and xylazine [KX], ketamine and acepromazine [KA], pentobarbital [Pent]). Least squares log-linear relationships between HR (bpm) and FS\% with different anesthetic agents are indicated.

Table. Normal echocardiographic measurements in conscious C57BL/6 mice

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<td>Computed LV mass</td>
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<td>mL/m</td>
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CO\(x\), Cardiac output; d, diastolic; FS\%, fractional shortening; HR, heart rate; IVS, interventricular septum; LVID, LV internal dimension; LVM, LV mass; LVPW, LV posterior wall; s, systolic.

with the echocardiographic dimensions should improve repeatability.
during conscious echocardiography was 683 bpm. However, considerable variability in HRs among mouse strains and with modest differences in level of activity (eg, quietly resting vs feeding/grooming) has been noted,\textsuperscript{18,20} as has phenotypic variation within the C57BL/6 strain.\textsuperscript{16} In our facility awake resting adult C57BL/6 mice of the same background and age have average rates of >600 bpm with minimal activity by continuous telemetry, and HRs considerably higher than 700 bpm were seen with exogenous augmentation of sympathetic activity.\textsuperscript{21} The essential observation here is that the level of sympathetic drive, as reflected in HR, did not change during the 1-hour period of experimental observation despite frequently repeated echocardiographic measurements; thus, there was no trend in mean values for HR and FS% in conscious mice over 1 hour. Finally, the conscious measurements of HR and FR are at the asymptotic limit of the measurements performed under anesthesia. It is important to note that bradycardia with transducer placement was obviated in these studies by training and maintenance of the prone position during imaging, and this is likely to be a significant factor in the consistency of the repeated conscious measurements.

Normal Echocardiographic Values

Given the consistency of conscious echocardiographic measurements, it becomes practical to compile a table of normal values for adult C57BL/6j mice (Table). This inbred strain represents the predominant strain basis for mouse metabolic physiology, in part because of its susceptibility to diet-induced obesity and insulin resistance,\textsuperscript{22} and represents the background for a broad range of transgenic and germline-altered models (http://www.informatics.jax.org/external/testing/mouse/STRAINS.shtml). The high degree of reproducibility in our conscious echocardiographic measurements suggests that problems with consistent transducer placement previously posited in the conscious state can be satisfactorily addressed.\textsuperscript{10} The regression relationships developed can guide the application of these normal values to adult mice with differing BW. The trends relating age and sex to echocardiographic cardiac dimension did not reach statistical significance when BW was included as a covariate in the regression relationship. This indicates that body mass, which is itself a function of age and sex, served as the most proximate predictor of cardiac mass in this population. Further studies of mice encompassing a greater variation in age from weaning to senescence, or encompassing a broader range of weights or genotypes, may reveal an independent role for sex and age in addition to BW in predicting cardiac dimension.

When it is not feasible to perform echocardiographic measurements under conscious conditions, the relationship of HR and FS% (Figure 4) provides a standard against which deviations from expected function can be evaluated. A relationship between HR and FS% is expected because of the force-frequency effect,\textsuperscript{23} but it is striking that the relationship is so constant among the set of anesthetic agents used in this study. Because of the large number of observations reported in this study, it is possible to appreciate the dramatic differences associated with profoundly depressed HRs (eg, in Hart\textsuperscript{5} with HR < 300 and KX anesthesia) and the minimal changes in FS% observed with HR \textasciitilde 450 bpm. An alternative piecewise linear fit explained slightly less total variance (55%) than the continuous logarithmic formulation. In piecewise linear regressions the slope coefficient for the upper segment did not differ significantly from 0, whereas in the logarithmic regression, this portion of the curve was asymptotically flat. Thus, for both formulations, the minimal change in FS% in response to HRs exceeding 450 bpm was well modeled.

Study Limitations

The rapid HR in conscious mice complicates Doppler measurements,\textsuperscript{24-27} which were not performed as part of this study. In the absence of Doppler data, the echocardiographic index of COx cannot be directly converted to true flow. In this study, LVM was estimated from M-mode measurements. Good agreement was noted between end-systolic and end-diastolic estimates of LVM, and mass measurements were not affected by marked anesthetic effects on HR, LV dimension, and wall thickening. However, area-length measurements may provide a somewhat more accurate measure of LVM, because the standard formula appears to consistently overestimate mass in comparison with necropsy measurements.\textsuperscript{5,12,28}

We obtained excellent quality images without shaving in C57BL/6 mice using the techniques described, but this might be required in other strains or in the presence of specific genetic mutations affecting hair.

A consequence of the study population is that the normal values, and the regression relationship reported, may not extend to juvenile or senescent mice, to alternative dosing or classes of anesthetic or sedative agents, or to various genetic models of extreme weight distribution, although the echocardiographic approach should be applicable. With the increasing use of echocardiographic approaches in mice it will be necessary to perform interlaboratory standardization, and carefully consider and report the precise strain backgrounds of experimental murine models. For example, variation in anesthetic response and echocardiographic measurements of HR and FS% in animals obtained from different vendors (Jackson Labs and Charles River Laboratories, Wilmington, Mass) but within the same C57BL/6 inbred strain has been reported.\textsuperscript{16} It is notable that all HR:FS% pairs encompassing the different vendors, derived from Figure 4 in the report by Roth et al,\textsuperscript{16} are along the HR:FS% curve (Figure 5), even though the HRs are at different points along the ordinate, supporting the use...
of this relationship for comparison between studies when necessary.

In summary, we have shown that anesthetic agents cause a profound time-dependent alteration in murine cardiac function assessed echocardiographically. These anesthesia effects will necessarily confound interpretation of data or complicate physiologically relevant comparison of data within groups or between groups, unless the timing between induction and measurement can be carefully controlled. In conscious mice, in contrast, frequent repeated echocardiographic measurements can be performed with highly consistent results. This is particularly valuable if serial or comparative studies are necessary as in the evaluation of genetic or pharmacologic manipulations.

REFERENCES