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ORIGINAL PAPER

# High-throughput phenotypic assessment of cardiac physiology in four commonly used inbred mouse strains

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**Abstract** Mice with genetic alterations are used in heart research as model systems of human diseases. In the last decade there was a marked increase in the recognition of genetic diversity within inbred mouse strains. Increasing numbers of inbred mouse strains and substrains and analytical variation of cardiac phenotyping methods require reproducible, high-throughput methods to standardize murine cardiovascular physiology. We describe methods for non-invasive, reliable, easy and fast to perform echocardiography and electrocardiography on awake mice. This method can be used for primary screening of the murine cardiovascular system in large-scale analysis. We provide insights into the physiological divergence of C57BL/6N, C57BL/6J, C3HeB/FeJ and 129P2/OlaHsd mouse hearts and define the expected normal values. Our report highlights that compared to the other three strains tested C57BL/6N hearts reveal features of heart failure such as hypertrophy and reduced contractile function. We found several features of the mouse ECG to be under genetic control and obtained several strain-specific differences in cardiac structure and function.

**Keywords** Echocardiography · Electrocardiography · Heart · Screening · C57BL/6

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## Introduction

The profound influence of genetic background is accepted as being part and parcel of the gene × gene interactions that underlie complex phenotypes (Kiselycznyk and Holmes 2011). Hence, careful characterization and comparison of strain phenotypes are necessary when transgenic or knockout mice are to be analyzed. Over the last years not only the number of inbred strains used in medical research, but also the number of in vitro and in vivo studies reporting strain-dependent differences in cardiovascular function increased progressively (Barnabei et al. 2010; Stull et al. 2006; Appleton et al. 2004). Even less data exist on substrains within single strains and their variations. The C57BL/6 mouse is one of the most commonly used strain in experimental research (Barnabei et al. 2010; Simon et al. 2013), but little is known about the cardiovascular variability within its common substrains C57BL/6N and C57BL/6J.

To identify the differences in cardiovascular physiology that arise from the genetic diversity, it is necessary to develop precise, reproducible, and non-invasive methods. Various techniques have been used for the characterization of cardiac phenotypes, such as histological analysis, electrocardiography, blood pressure analysis, cardiac imaging and determination of circulating biomarker concentrations. Different test systems, the fast improvement of instrumentation resulting in more accurate assessment (Collins et al. 2003) and the use of anesthetic agents (Collins et al. 2003; Berthonneche et al. 2009; Schoensiegel et al. 2011; Appleton et al. 2004; Lairez et al. 2013; Roth et al. 2002) led to various sets of data making it difficult to find reliable reference values to define cardiac phenotypes of background strains.

Over the last 10 years echocardiography has been increasingly applied to identify cardiac phenotypes and pathophysiological responses to surgical and pharmacological interventions (Collins et al. 2003). This technique uses ultrasound for visualization and provides information on the heart anatomy, blood flow pattern and function of heart muscle, vessels and valves. As a high-quality, non-invasive, reproducible method echocardiography became the most important technique to analyze the physiology of the murine heart and is now available in most research laboratories (Mitchell et al. 1998; Fayssoil and Tournoux 2013).

Yet, largely unknown are the complex mechanisms of murine cardiac electrophysiology. By electrocardiography (ECG), the electrical activity of the heart's conduction system and myocardial cell membrane currents are measured and recorded. Even though ECG differences between mice are reported for more than 40 years (Goldbarg et al. 1968), the variety of methods used, the inconsistent adjustments for analysis parameters and the multiple conditions for ECG recording make it difficult to compare the results. In 2001 Hampton (Chu et al. 2001) developed an ECG system to non-invasively obtain and analyze ECGs in large cohorts of conscious mice and made it available to the biotechnology community.

During the last 2 years we have performed over 8,000 echocardiograms and 4,000 electrocardiograms on awake mice using state-of-the-art ultrasound and ECG technologies. In our opinion, the combination of both diagnostic tools allows a first characterization of cardiovascular function. These studies are important since these genetic characteristics of traits interact with multiple pathological processes and disease states, such as heart failure and cardiomyopathy. We are member of the International Mouse Phenotyping Consortium (IMPC) that plans over the next 5 years to generate and carry out broad-based phenotyping of 5,000 mouse mutant lines as the first step towards a comprehensive encyclopedia of mammalian gene function (Brown and Moore 2012). To that end, we set up a practical guide for echocardiography and ECG in conscious mice that allows us the screening of the cardiovascular system of one mouse in 10 min. We report the phenotyping of C57BL/6N, C57BL/6J, C3HeB/FeJ and 129P2/OlaHsd mouse hearts, indicating that various strain-specific cardiac differences exist, and that compared to the other three strains tested, C57BL/6N hearts reveal a heart failure phenotype. Collectively, this study reports high-throughput screening of murine cardiovascular physiology with state-of-the-art technology, highlights strain-dependent differences on cardiovascular physiology between four commonly used inbred mice and substrains provide reference values.

## Methods

### Inbred mice

Four inbred mouse strains were used in this study: C57BL/6N, C57BL/6J, C3HeB/FeJ and 129P2/OlaHsd. Mice were bred in the animal facility of the Helmholtz Zentrum München. Experiments were done according to the German laws for animal protection and by permission of the Regierung von Oberbayern. Echocardiograms and electrocardiograms were recorded on 16-week-old mice. They were allowed to adjust to the experimental area at least 30 min before measurements and all examinations were performed in a conditioned quiet room to reduce external stimuli that could interfere with mouse physiology. Examinations were performed on conscious animals to prevent anesthesia-related impairment of cardiac function (Roth et al. 2002). All echocardiograms and electrocardiograms

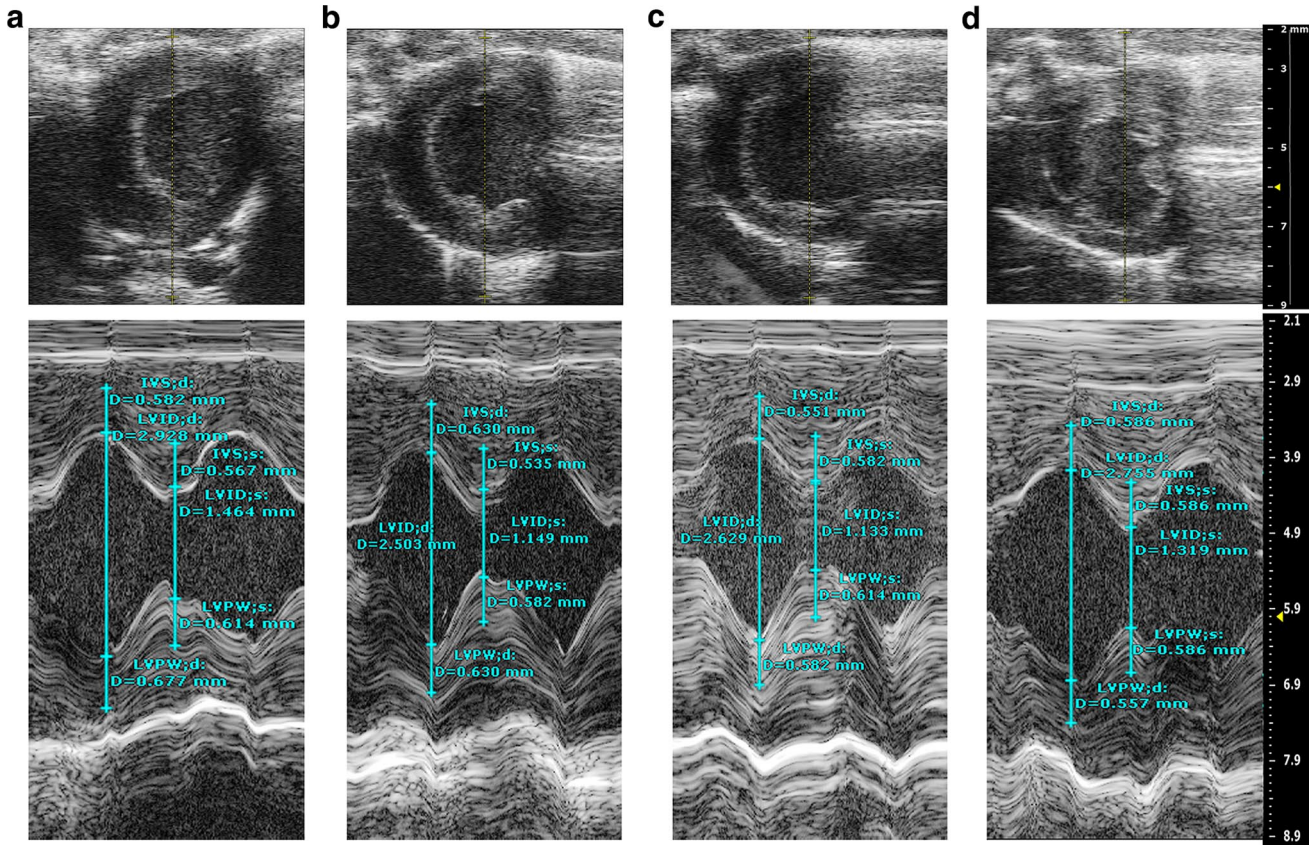


**Fig. 1** M-mode recordings through a short-axis view at the papillary muscle level from representative C57BL/6N, C57BL/6J, C3HeB/FeJ and 129P2/OlaHsd males. M-mode images of the left ventricle display dimensions of the ventricular walls, ventricular cavity, and cardiac function measurements. Y-axis represents the distance (in mm) from the transducer; time (in ms) is on the X-axis. Images show the interventricular septum (IVS), left ventricular posterior wall (LVPW), and left ventricular internal diameter (LVID) throughout diastole (d) and systole (s). **a** C57BL/6N, **b** C57BL/6J, **c** C3HeB/FeJ and **d** 129P2/OlaHsd

were recorded and analyzed by the same person, blinded to the genotypes of mice.

## Echocardiographic phenotyping

Cardiac function was evaluated with transthoracic echocardiography in 50 male and female mice of each inbred strain using a Vevo2100 Imaging System (VisualSonics Inc., Toronto Canada) with a 30 MHz probe. The day before the first examination the mouse chests were depilated using a topical depilatory agent. Bodyweights were taken shortly before transthoracic echocardiography. In order to eliminate circadian influences ultrasound was performed between 8 am and 11 am.

For echocardiographic examinations the mice were firmly held by the nape of the neck (in the supine position) in the palm of one hand with the tail held tightly between the last two fingers. Pre-warmed ultrasound gel was placed on the chest at the image location. For two-dimensional (2D) imaging ('B-mode') view along the parasternal long axis the transducer was placed vertically to the animal body on the left side of its sternum with the notch of transducer pointing to the animal head. Optimal parasternal views were obtained by adjusting gain settings for visualization of endocardial and epicardial walls (approximately 45 dB). A proper image in this orientation included the left atrium and ventricle, a slight portion of the right ventricular wall and the output of the aorta, with the heart forming a gourd-like structure. The beginning ascending aorta and the apex of the heart lay on the same horizontal line. The movement of the myocardium, valves, and vessel walls were analyzed macroscopically. For accurate linear measurements of left ventricular internal dimensions and wall thicknesses an M-mode image of the heart in parasternal short-axis view was acquired (Fig. 1). The transducer was rotated approximately 90° counterclockwise starting from the parasternal long-axis view. The M-mode cursor was placed perpendicularly to the interventricular septum and posterior wall of the left ventricle at the level of the papillary muscles. For calculation of the respiration rate the transducer was turned to a vertical position, moved to the diaphragm and at least three respiratory intervals were monitored. At the end of

an imaging session, ultrasound gel was removed from the mouse with water dampened gauze.

Qualitative and quantitative measurements were made offline using analytical software (VisualSonics Inc.). Left ventricular dimension in systole (LVIDs) and diastole (LVIDd), systolic and diastolic interventricular septum thickness (IVSs, IVSd) and systolic and diastolic posterior wall thickness (LVPWs, LVPWd) were measured in three consecutive beats according to the American Society of Echocardiography leading edge method (Sahn et al. 1978) as a measure of the actual visualized thickness of the ventricular septum and other chamber dimensions as defined by the actual tissue–blood interface (Fig. 1). The papillary muscles should be excluded from the cavity in the tracing. Fractional shortening (FS) was calculated as FS % = [(LVIDd − LVIDs)/LVIDd] × 100. Ejection fraction (EF) was calculated as EF % = 100 × [(LVvolD − LVvol S)/LVvolD] with LVvol = [(7.0/(2.4 + LVID) × $LVID^3$]. The corrected left ventricular mass (LV MassCor) was calculated as LV MassCor = 0.8(1.053 × [(LVIDd + LVPWd + IVSd)$^3$− $LVIDd^3$)]. The stroke volume (SV) is the volume of blood pumped from one ventricle of the heart with each beat. The stroke volume of the left ventricle was obtained by subtracting end-systolic volume (LVvolS) from end-diastolic volume (LVvolD). Heart rate was determined from the cardiac cycles recorded on the M-mode tracing, using at least three consecutive systolic intervals. In addition, respiratory rate was calculated by measuring three consecutive respiratory intervals.

### Electrocardiographic phenotyping

ECGs were recorded on 20 males and females using the ECGenie system (Mouse Specifics, Inc., Boston, MA) (Fig.S1). Since even modest handling of mice may induce alterations in heart rate (Desai et al. 1997), each mouse was permitted to acclimatize on the ECG recording platform 10 min prior to measurement. Furthermore, cage mates were placed on the adjacent platform unit to provide company. In order to eliminate circadian influences ECGs were recorded between 1 pm and 3 pm. A disposable lead plate (Mouse specifics Inc.) was embedded in the floor of the platform and spaced to provide contact between the electrodes and animals' paws providing an ECG signal equivalent to Einthoven lead II. Only runs where at least 15 ECG beats could be included in the analysis were chosen. Data were analyzed using standard protocols for ECG signal analysis by eMouse™ (Mouse Specifics, Inc.). The software uses a peak detection algorithm to find the peak of the R-waves and to calculate heart rate (HR). The software plots its interpretation of P, Q, R, S, and T for each beat so that HR, QRS duration, PQ interval, PR interval, QT interval and ST interval are measured and reported automatically (Fig. 2). In addition, each trace was examined for clear P, Q, R, S, T peaks before accepting the automatic calculations. Sensitivity was corrected manually in case R peaks were not chosen correctly. Noise and motion artifacts are rejected automatically by the software. As in mice the T-wave often merges with the final part of the QRS complex (Wehrens et al. 2000) the software automatically defines the end of the T-wave of each signal as the point where the signal intersects the isoelectric line. Heart rate variability (HRV) was calculated as the mean of the differences between sequential heart rates for the complete set of ECG signals. The QT intervals were rate corrected (QTc) by application of the equation recommended by Mitchell et al. (1998).

### Statistical analysis

Differences between strains were assessed using the Mann–Whitney rank sum test. Data were analyzed on SigmaPlot 12.0 analysis software (Systat Software, Inc.). The minimal significant probability value was set at 0.05. Note that *P* values are not corrected for multiple testing.

## Results

Cardiac function was assessed using the combined protocol of echocardiography and electrocardiography in high throughput. Multiple statistically significant differences were found between inbred strains (Figs. 2, 4; Tables 1, 2).

### Echocardiography shows genetic variability

Echocardiography of C57BL/6N hearts revealed significantly increased left ventricular mass (median for males 33 vs. 28 mg; for females 27 vs. 23 mg), left ventricular dimensions (median systole for males 1.6 vs. 0.96 mm; for females 1.4 vs. 0.81 mm; diastole for males 2.8 vs. 2.6 mm; for females 2.5 vs. 2.3 mm) and interventricular septum width (median systole for males 0.60 vs. 0.51 mm; for females 0.58 vs. 0.50 mm; diastole for males 0.57 vs. 0.51 mm; for females 0.51 vs. 0.51 mm) compared to mice of the C57BL/6J strain, although the bodyweight was the same (median for males 29 vs. 29 g; for females 23 vs. 22 g). The cardiac performance was reduced in C57BL/6N mice as indicated by significantly decreased fractional shortening (median for males 43 %; for females 44 %) and ejection fraction (median for males 75 %; for females 77 %) compared to mice of all other tested strains (Fig. 2). In contrast to the marked differences in C57BL/6N hearts, differences in heart performance between C57BL/6J, C3HeB/FeJ and 129P2/OlaHsd mice were much less pronounced. Hearts of the C57BL/6J strain performed better

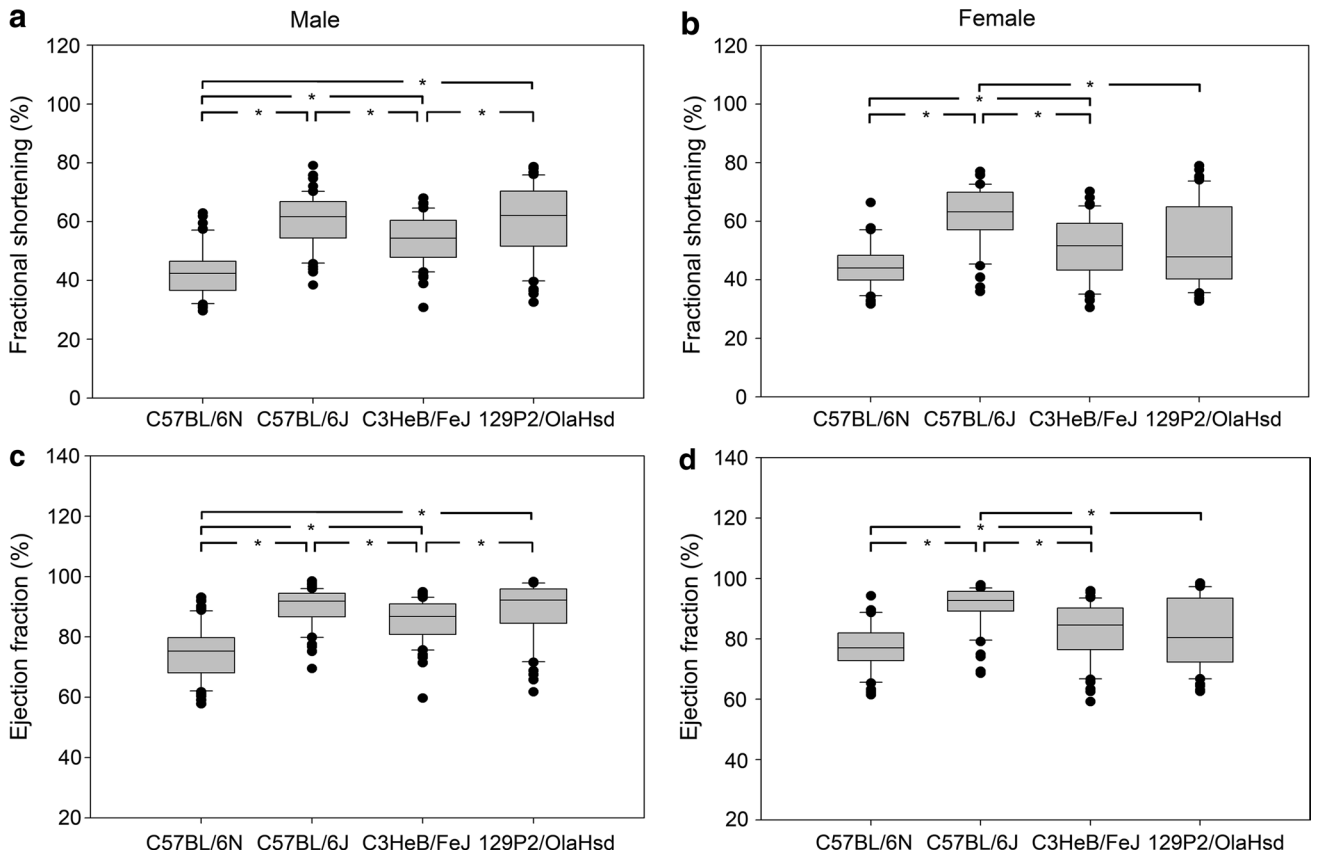


**Fig. 2** Strain differences in fractional shortening and ejection fraction. Functional cardiac alterations between C57BL/6N, C57BL/6J, C3HeB/FeJ and 129P2/OlaHsd mice. Fractional shortening males (**a**) and females (**b**) ejection fraction males (**c**) and females (**d**). *Single values* are presented in *boxplots* with 10th and 90th percentile whiskers. Each data point outside the 10th and 90th percentiles is plotted. *P* values are calculated by Mann–Whitney rank sum test; * $P \leq 0.05$; $n = 50$

than C3HeB/FeJ hearts (FS median for males 62 vs. 54 %; for females 63 vs. 52 %; EF median for males 92 vs. 87 %; for females 93 vs. 85 %). Significant differences in heart performance between C57BL/6J and 129P2/OlaHsd mice could only be obtained in female mice (Fig. 2). The calculated stroke volume did not differ much in male C57BL/6N (23 µl), C57BL/6J (22 µl), C3HeB/FeJ (24 µl), and 129P2/OlaHsd (23 µl) mice. However, statistically significant differences were found in between females, with the lowest stroke volume observed in C57BL/6 mice (C57BL/6N (19 µl) and C57BL/6J (16 µl) vs. C3HeB/FeJ (24 µl) and 129P2/OlaHsd (24 µl). The left ventricular mass was significantly altered by comparing C57BL/6N and C57BL/6J mice. In C57BL/6N mice the left ventricular mass was highly elevated compared to C57BL/6J mice (median for male 33 vs. 28 mg; for females 27 vs. 23 mg). Also differences in left ventricular mass were found for C3HeB/FeJ and 129P2/OlaHsd mice, alterations were reflected by differences in body weight and might be secondary effects (Table 1). Both C3HeB/FeJ and 129P2/OlaHsd mice are much heavier than C57BL/6J mice (median for males C3HeB/FeJ 33 g, 129P2/OlaHsd 33 g, C57BL/6J 29 g; for females C3HeB/FeJ 31 g, 129P2/OlaHsd 27 g, C57BL/6J 22 g) and show increased left ventricular dimension, mass and wall thickness compared to mice of the C57BL/6J strain (Table 1).

## Strain differences in ECG characteristics

We recorded differences in HR and ECG time intervals of 20 males and females each of C57BL/6N, C57BL/6J, C3HeB/FeJ, and 129P2/OlaHsd mice, four commonly used inbred strains. Figure 4 and Table 2 show all analyzed ECG parameters.

The heart rate was reduced in C57BL/6J (median for males 741 bpm; for females 734 bpm) and 129P2/OlaHsd (median for males 738 bpm; for females 710 bpm) mice compared to C57BL/6N (median for males 765 bpm; for females 785) and C3HeB/FeJ (median for males 781 bpm; for females 787 bpm) mice (Fig. 4). C57BL/6N mice showed reduced HRV compared to all other tested mice (median for males 5.0 bpm; for females 8.6 bpm; (Table 2).

**Table 1** Echocardiographic variables in C57BL/6N, C57BL/6J, C3HeB/FeJ and 129P2/OlaHsd conscious mice

| Parameter | C57BL/6N | | C57BL/6J | | C3HeB/FeJ | | 129P2/OlaHsd | |
|---|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female | Male | Female |
| LVIDd (mm) | 2.8** (2.5, 3.1) | 2.5**, **** (2.4, 2.8) | 2.6* (2.4, 2.8) | 2.3*, ***, **** (2.0, 2.6) | 2.8 (2.2, 3.0) | 2.7** (2.4, 3.0) | 2.6 (2.4, 3.3) | 2.9*, ** (2.5, 3.2) |
| LVIDs (mm) | 1.6**, ***, **** (1.4, 1.9) | 1.4** (1.2, 1.7) | 1.0*, *** (0.8, 1.2) | 0.8*, ***, **** (0.7, 1.0) | 1.1*, ** (0.9, 0.5) | 1.4** (1.0, 1.6) | 1.0* (0.7, 1.4) | 1.5** (0.9, 1.9) |
| LVPWd (mm) | 0.6***, **** (0.5, 0.7) | 0.6**, ***, **** (0.5, 0.6) | 0.6***, **** (0.6, 0.7) | 0.6*, **** (0.5, 0.7) | 0.7*, ** (0.6, 0.7) | 0.6*, **** (0.6, 0.6) | 0.6*, ** (0.6, 0.7) | 0.7*, **, *** (0.6, 0.7) |
| LVPWs (mm) | 0.6***, **** (0.5, 0.7) | 0.6***, **** (0.5, 0.7) | 0.6***, **** (0.6, 0.7) | 0.6***, **** (0.5, 0.6) | 0.7*, ** (0.6, 0.7) | 0.7*, **, **** (0.6, 0.7) | 0.6*, ** (0.6, 0.7) | 0.7*, **, *** (0.6, 0.7) |
| IVSd (mm) | 0.6** (0.5, 0.6) | 0.5**** (0.5, 0.6) | 0.5*, ***, **** (0.5, 0.6) | 0.5***, **** (0.4, 0.5) | 0.6** (0.5, 0.6) | 0.5** (0.5, 0.6) | 0.5** (0.5, 0.6) | 0.6*, ** (0.5, 0.6) |
| IVSs (mm) | 0.6** (0.6, 0.7) | 0.6** (0.5, 0.6) | 0.5***, **** (0.5, 0.6) | 0.5*, ***, **** (0.6,0.6) | 0.6** (0.6, 0.6) | 0.6** (0.6, 0.6) | 0.6** (0.5, 0.7) | 0.6** (0.5, 0.7) |
| Stroke volume (µl) | 23 (16, 27) | 19***, **** (15, 20) | 22 (18, 26) | 16***, **** (12, 21) | 24 (14,30) | 24 *, ** (18, 28) | 23 (20, 29) | 24*, ***, (19, 32) |
| LVmassCorr (mg) | 33** (27, 45) | 27**, ***, **** (23, 32) | 28*, ***, **** (23, 33) | 23*, ***, **** (18, 26) | 33** (24, 43) | 33*, **, **** (27, 37) | 32** (26, 45) | 41*, **, *** (29, 49) |
| Heart rate (bpm) | 710***, **** (646, 750) | 711***, **** (667, 751) | 709***, **** (675, 738) | 699***, **** (659, 720) | 662*, **, (593, 715) | 633 *, **, **** (494, 692) | 646*, **, (543, 709) | 584*, **, *** (451, 684) |
| Respiration rate (1/min) | 258**, ****, (224, 312) | 241** (200, 291) | 310*, ***, **** (273, 343) | 279*, **** (241, 330) | 268**, **** (243, 316) | 258**** (222, 299) | 225*, **, *** (198, 264) | 216**, *** (184, 289) |
| Bodyweight (g) | 29 (27, 31) | 23***, **** (21, 24) | 29 (28, 31) | 22***, **** (21, 24) | 33 (30, 35) | 31* **, **** (28, 35) | 33 (31, 35) | 27*, **, *** (24, 30.) |

Echocardiographic characteristics across the analyzed inbred mouse strains

Medians, first and third quartile and *P* values calculated by a Mann–Whitney Rank sum test

* $P < 0.05$ age and sex-matched differences versus C57BL/6N mice found

** $P < 0.05$ age and sex-matched differences versus C57BL/6J mice found

*** $P < 0.05$ age and sex-matched differences versus C3HeB/FeJ mice found

**** $P < 0.05$ age and sex-matched differences versus 129P2/OlaHsd mice found; $n = 50$

However, HRV did not vary much among mice of the four inbred strains analyzed (Table 2). The RR interval duration, defined as the inverse of heart rate, and the mean duration between depolarization–repolarization cycles does reflect the findings observed by analyzing the heart rate (Fig. 3). Male C3HeB/FeJ mice had the highest HR and thus the shortest RR interval duration (77 ms). By analyzing females we found the shortest RR interval durations for C57BL/6N (77 ms) and C3HeB/FeJ (77 ms) mice.

In C3HeB/FeJ hearts we found the shortest conduction between atria and ventricles measured by analyzing the duration between peak of P-wave and the beginning of the QRS complex (PQ) [median for males 15 ms; for females 17 ms; (Fig. 4)]. Only slight differences in duration of the PQ interval could be found between mice of all other strains (Fig. 4). The PR interval (the time from the onset of atrial depolarization to the onset of ventricular depolarization) was in C3HeB/FeJ hearts the shortest [median for males 19 ms; for females 22 ms; (Fig. 4)]. In female C57BL/6J hearts the conduction through the AV node took the longest [28 ms; (Fig. 4)]. The time necessary for the impulse to be distributed over the entire ventricular myocardium was found to be the shortest in C3HeB/FeJ hearts (median for males 9 ms; for females 9 ms) and the longest in 129P2/OlaHsd hearts [median for males 10 ms; for females 10 ms; (Fig. 4)]. ECG interval durations for ST and QT intervals were the shortest in C57BL/6N mice (median for males 29 and 37 ms; for females 27 and 36 ms, respectively) compared to the other strains tested (Table 2), even though not always statistically significant. Mice with the lowest heart rate showed the longest QT interval duration (Table 2) and after correction of the QT interval [the time of ventricular depolarization and repolarization] for heart rate main alterations were found between females. For C57BL/6N females we obtained the shortest QTc interval duration (median 21 ms). By recording ECGs of female

**Table 2** Electrocardiographic variables in C57BL/6N C57BL/6J C3HeB/FeJ and 129P2/OlaHsd conscious mice

| Parameter | C57BL/6N | | C57BL/6J | | C3Heb/FeJ | | 129P2/OlaHsd | |
|---|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female | Male | Female |
| HRV (bpm) | 5.0**, **** (2.8, 10) | 8.6**** (6.0, 20) | 9.4* (8.1, 19) | 19 (8.8, 30) | 7.1 (5.2, 14) | 7.8**** (5.1, 16) | 13* (6.8, 28) | 22*, *** (11, 78) |
| RR (ms) | 79** (77, 80) | 77**, **** (75, 82) | 81*, *** (79, 83) | 83*, *** (80, 85) | 77**, **** (75, 79) | 77**, **** (76, 81) | 82*** (79, 84) | 85*, *** (81, 87) |
| QT (ms) | 37 (36, 41) | 36**, **** (35, 38) | 39**** (37, 40) | 40*, *** (39, 42) | 38 (35, 40) | 38**, **** (34, 40) | 41** (38, 43) | 40*, *** (38, 44) |
| ST (ms) | 29 (27,33) | 27**, **** (26, 29) | 30 (29, 31) | 31* (29, 32) | 29 (27, 32) | 29 (26, 32) | 32 (28, 34) | 31* (28, 34) |
| QTc (ms) | 43 (41, 47) | 42**, **** (40, 43) | 43 (42, 45) | 44* (42, 46) | 43**** (40, 45) | 43 (39, 44) | 45*** (43, 48) | 44* (42, 46) |

Heart rate variability (HRV) and ECG time intervals across the analyzed inbred mouse strains

Medians, first and third quartile and *P* values calculated by a Mann–Whitney rank sum test

* $P < 0.05$ age and sex-matched differences versus C57BL/6N mice

** $P < 0.05$ age and sex-matched differences versus C57BL/6J mice

*** $P < 0.05$ age and sex-matched differences versus C3HeB/FeJ mice

**** $P < 0.05$ age and sex-matched differences versus 129P2/OlaHsd mice; $n = 20$

C57BL/6N, C3HeB/FeJ, and 129P2/OlaHsd mice median QTc interval durations of 44, 43, and 44 ms were obtained, respectively.

## Discussion

In the present study, we evaluated the cardiac physiology of four inbred mouse strains by awake echocardiography and electrocardiography in high-throughput testing. Our findings of significant differences between C57BL/6N, C57BL/6J, C3HeB/FeJ and 129P2/OlaHsd inbred mouse hearts show the impact of genetic divergence between strains and substrains. The principle finding of this study is that C57BL/6N hearts reveal a heart failure phenotype as indicated by a higher muscle mass and a poorer left ventricular function as compared to the other three strains tested.

On the one hand, strain-dependent differences in physiological function can be challenging when comparing findings of studies in which different inbred strains have been used as shown by Lygate et al. (2012) and others (Arber et al. 1997; Dansky et al. 1999); but on the other hand, their complex variation can help us to understand disease processes. For example, inbred strains have been used to identify loci responsible for resistance to infectious disease, cancer, behavior, and metabolism (Hoit et al. 2002). Single-nucleotide base substitutions (SNPs) at certain gene or genomic position represent the major part of inter-individual variability. Common genetic variants have been shown to increase the risk of cardiomyopathy (Ivandic et al. 2012), cardiac arrhythmias, and SNPs can alter cardiac electrical manifestations in certain populations (Kaab and Schulze-Bahr 2005). Mekada et al. demonstrated genetic differences between the C57BL/6J substrains and C57BL/6N substrains at 12 SNP loci, indicating genetic diversity in 0.8 % of SNP loci (Mekada et al. 2009). One of the differences found is associated with the gene locus *Lims1*. The protein encoded by this gene is PINCH1 which is ubiquitously expressed and provides physical linkage between integrin receptors and the actin cytoskeleton and serves as a signaling mediator for downstream effectors (Meder et al. 2011; Liang et al. 2009). The specific deletion of *Lims1* results in various cardiovascular phenotypes like abnormal arteries, defective cardiac outflow tract septation (Arnold et al. 2013), dilated cardiomyopathy (Liang et al. 2009). There is strong evidence that PINCH1 plays a role in processes leading to hypertrophy (Chen et al. 2005). Our finding that C57BL/6N hearts are hypertrophic may be partially caused by the genetic divergence of *Lims1* found for the C57BL/6N substrains. This would need to be addressed in further studies. Furthermore, we explored the hypertrophic phenotype of C57BL/6N hearts, but did not define why this phenotype has developed. As such echocardiographic analysis of the contractile parameter are incomplete without knowledge of the afterload against which the heart must work. One could define the hypertrophic phenotype by secondary screening, i.e., using blood pressure measurements and evaluating circulating disease marker.

Future work has to aim at associating the rich phenotypic data of this study and other reports with genetic markers. Excellent studies following this issue were done by Simon et al. (2013) and Auerbach et al. (2010). In

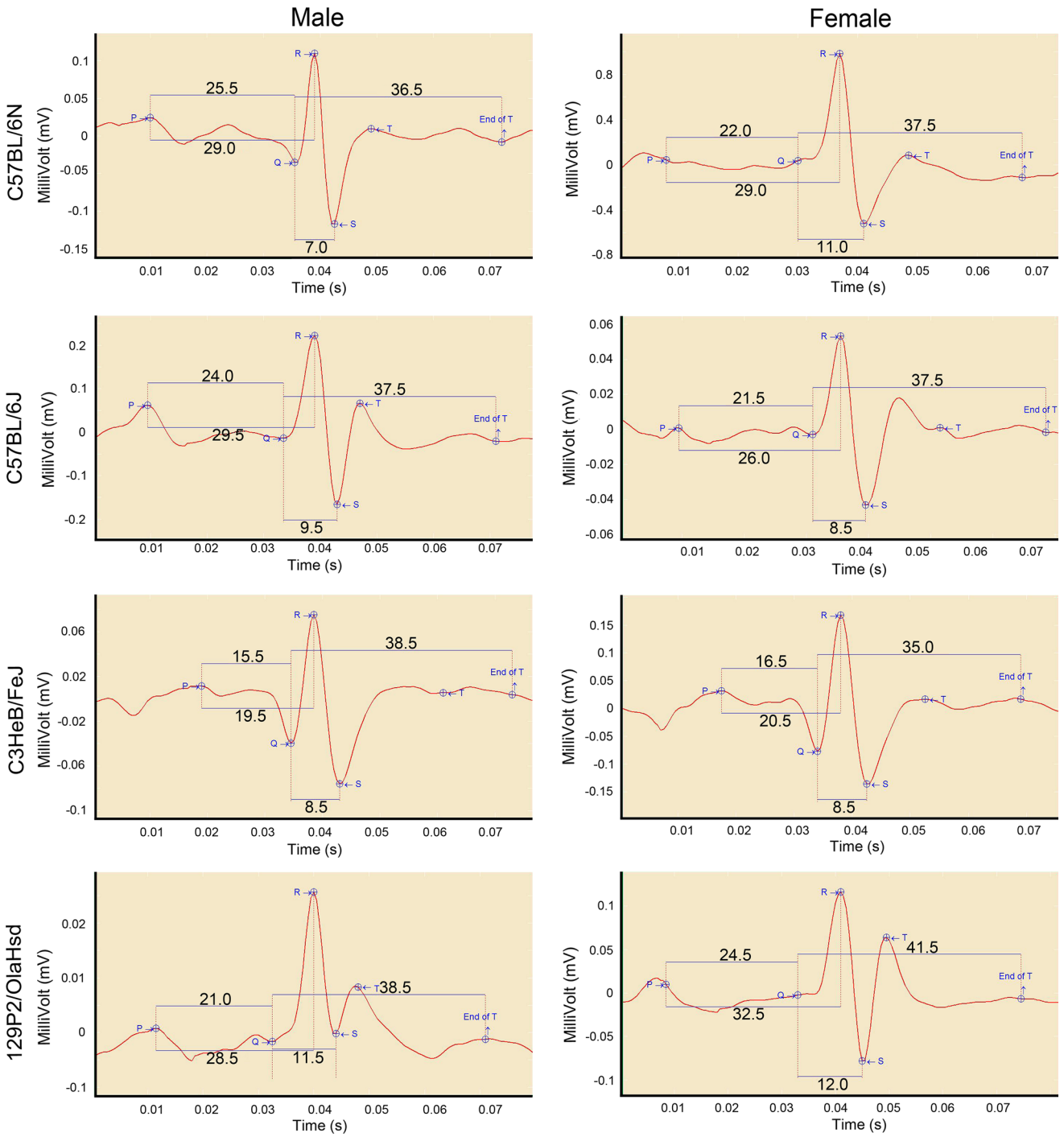


**Fig. 3** Representative electrocardiograms from conscious male and female C57BL/6N, C57BL/6J, C3HeB/FeJ and 129P2/OlaHsd mice with indication of ECG parameters and interval durations, as presented by Mouse Specific Software. Interval durations are given in milliseconds

the latter genetic composition and basic physiology of C57BL/6J and C3H/HeJ hearts were analyzed and genes identified that were populated in pathways associated with metabolic disease or cardiomyopathy, respectively. In line with this is the study of Lerman et al. (2002) in which hearts of C3H/HeJ and C57BL/6J mice were compared and greater diastolic and systolic ventricular wall thickness and reduced fractional shortening for C3H/HeJ hearts were found. We obtained comparable data by analyzing C57BL/6J and C3HeB/FeJ mice, even though they performed echocardiography under light sedation and analyzed only males. Considering the last aspect we found in

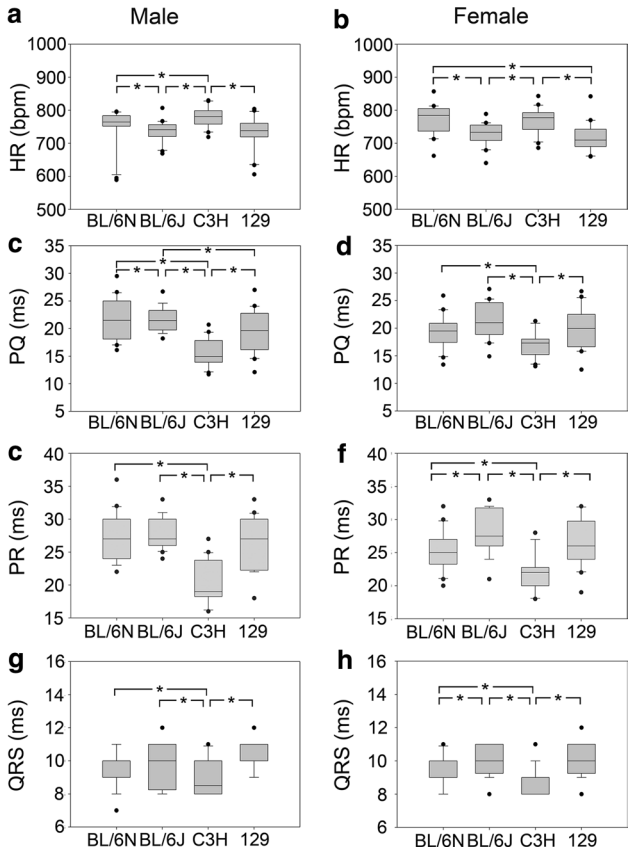


**Fig. 4** Strain differences in heart rate [in beats/min (bpm)] between male (**a**) and female (**b**), in PQ intervals between male (**c**) and female (**d**), in PR intervals between male (**e**) and female (**f**), and in QRS interval duration between male (**g**) and female (**h**) C57BL/6N (BL/6N), C57BL/6J (BL/6J), C3HeB/FeJ (C3H) and 129P2/OlaHsd (129) mice. *Single values* are presented in *boxplots* with 10th and 90th percentile whiskers. Each data point outside the 10th and 90th percentiles is plotted. *P* values are calculated by Mann–Whitney rank sum test; $^*P \leq 0.05$; $n = 20$

addition that no gender differences exist. By contrast, Barnabei et al. (2010) used for their analysis an isolated heart model and reported a greater ejection fraction for C3H/HeJ mice than for C57BL/6J mice. This might be explained by the existing discrepancies in cardiac function between ex vivo and in vivo analysis. It is to mention that, we chose the C3HeB/FeJ substrain as this was used in large-scale systematic phenotypic analysis of *N*-ethyl-*N*-nitrosourea (ENU)-induced mouse mutants (Hrabe de Angelis et al. 2000; Soewarto et al. 2000), which resulted in generation of hundreds of new mouse lines on this background available for the research community.

Studies have shown that different strains of mice represent characteristic variations in cardiac physiology (Hoit et al. 2002; Shah et al. 2010; Berthonneche et al. 2009). Our finding of a relative hypertrophy and reduced contractile performance of the hearts of C57BL/6N vs. C57BL/6J mice is in agreement with a study by Roth et al. (2002), even though they sedated the mice for analysis which might have influenced cardiac physiology. Our characterization of cardiac performance revealed that in relation to the other three strains the tested hearts of C57BL/6N mice performed the worst. Reported FS % has not been consistent in the published data. The cutoff in fractional shortening for conscious or light sedated mice displaying cardiac phenotypes is reported in the range of 40 % (Messaoudi et al. 2009)–60 % (Schoensiegel et al. 2011). Given that EF and FS are often used as indicators for contractile dysfunction in animal models of disease, our findings further highlight the need to better define "normality" and to consider genetic variability in animal studies.

Gender differences could only be obtained among female and male C57BL/6 in bodyweight, wall width and chamber dimensions. This agrees with a study by Hoit et al. (2002), although their measures of left ventricular dimension in sedated mice are more than one-third higher than ours. Anesthetic agents can significantly affect cardiovascular parameters and may have influenced echocardiographic measurements in that study. Inter-observer variability among studies could also have an impact, as one of the most prominent causes of inter-observer variability in echocardiographic evaluations is defining border limits. Ultrasound signals are reinforced where surface change density, allowing definition of limits between surface layers. The inclusion or exclusion of these echoes from interfaces of the left ventricular cavity or myocardial wall can cause significant discrepancies in the overall measurements (Foppa et al. 2005).

The genetic background influences the cardiac conduction system, and duration of action potentials of myocardial cells as indicated by QT intervals as shown by significant differences in HRs and ECG interval durations among inbred strains (Figs. 3, 4; Table 2). Our study showed that C3HeB/FeJ mice have higher HRs and reduced PR and QRS interval durations compared to C57BL/6J mice, which agrees with other reports (Auerbach et al. 2010; Xing et al. 2009), even though they analyzed the C3H/HeJ substrain. The lowest heart rates and highest HRV were recorded in 129P2/OlaHsd mice with a median of 738 bpm in males and 710 bpm in females, and 13 and 22 bpm, respectively. However, distribution of HR values and HRV was found for all strains studied which suggests that their regulation is a complex trait and influenced by multiple genes. In line with this is a study from Howden et al. (2008) which reports about some quantitative trait loci for basal HR and HRV phenotypes in quiescent mice.

The QRS complex duration represents ventricle excitation time and marks the time required to depolarize the entire contractile myocardium. In 16-week-old mice, the main difference in QRS duration was found for C3H/FeJ

mice, which had the shortest QRS durations. That QRS durations vary among inbred strains is known (Xing et al. 2009; Appleton et al. 2004). It has been suggested that the distribution pattern of the Purkinje fiber network in the myocardium and ventricular wall thickness play an important role in ventricle excitation time and thus interstrain differences in the QRS complex duration may reflect differences in ventricular wall thickness (Jay et al. 2004; Oosthoek et al. 1993; Xing et al. 2009). Indeed, we found that C3H/FeJ males had the thickest ventricular walls among the tested inbred strains. However, among females only 129P2/OlaHsd mice showed an equal distribution throughout the echocardiographic analysis of wall width, with a relative thickening compared to the other female inbred mice. Unfortunately, no data for distribution of Purkinje fibers in these mice were available for us to explore the remaining assumption.

Overall, no strong influence of sex on HRs and ECG time intervals were found. This agrees with the study by Mitchell et al. (1998), but not with Chu et al. (2001) who reported intersex differences in 2-month-old mice of strains different from the ones we analyzed. Studies show that sex differences can be observed in some but not all strains (Xing et al. 2009; Appleton et al. 2004). In addition, age-related ECG changes could modulate the result. In order to limit the number of variables, we used fully grown mice at the age of 16 weeks in this study. Nevertheless, we cannot exclude that the results might be different when mice with other characteristics are used.

There exists a scarcity of T-wave and the resulting T interval duration measures of mice in the literature. The repolarization in the mouse heart is characterized by a large rapidly activating and inactivating transient outward potassium current, which in effect causes repolarization to start before depolarization ends (Appleton et al. 2004). As a result a clear and separate T-wave cannot always be reliably distinguished in the mouse ECG. QT and ST intervals were measured in all mice and both correspond with changes in QRS duration in several instances in our study, even after correction for heart rate. We found that ECG interval durations for ST, QT and QTc intervals were the shortest in C57BL/6 mice compared to the other strains tested.

Both genetic and environmental factors influence HR which may consequently affect measurements (Seed et al. 1987). To obtain comparable and reliable data, we controlled possible extraneous variables by the following strategies: (1) the environment (light, temperature, noise, etc.) was carefully controlled; (2) to eliminate circadian influences, ECHOs and ECGs were measured at fixed times; and (3) analyses were performed in conscious mice acclimated to the instrument. As the number of experimental mice continues to increase, there is the need to identify differences in cardiovascular physiology and conduction system that arise from the genetic diversity within mouse strains with little effort, low costs and reproducible for the research community. Combination of echocardiography and electrocardiography in awake mice made it possible to identify strain-specific differences. Using our system, phenotypic variances were generally smaller within than between strains, indicating elevated trait heritabilities.

Multiple methods for cardiac imaging have been developed over the years for the visualization and assessment of cardiac function. Among these cardiac micro-CT, PET scan, and cardiac MRI might be limited by expense, frequent need for contrast material and availability (Collins et al. 2003). Although, high potential for assessing murine cardiac physiology is offered by three-dimensional (Pistner et al. 2010) Doppler echocardiography and transesophageal imaging (Steudel et al. 1998), these techniques are still technically too challenging for broad application under high throughput. By echocardiography, both systolic and diastolic cardiac function can be measured with high precision and reproducibility to allow for the monitoring of cardiac pathophysiology, and the analysis of intervention. Thus, echocardiography remains the most frequently used modality for the routine evaluation of cardiac function in mice (Collins et al. 2003). A matter of debate in the research community is if or if not to sedate the mice during echocardiography. Most available narcotics have an impact on the cardiac function of the mouse (Stypmann et al. 2009) particular on HR, FS %, and end-diastolic diameter (Lairez et al. 2013; Roth et al. 2002), and might mask left ventricular dysfunction and cardiomyopathy (Lairez et al. 2013). Furthermore, strain-dependent cardiodepressive effects have been shown (Barnabei et al. 2010; Berthonneche et al. 2009). Few groups have performed echocardiography in awake mice (Schoensiegel et al. 2011; Messaoudi et al. 2009) but this technique might enhance sympathetic tone and heart rate (Gao et al. 2011), and heart rate in mice correlate well with cardiac contractility (Palakodeti et al. 1997). The physiological heart rate for a conscious mouse is near 550–620 bpm, while maximal rates are near 720–800 bpm (Stull et al. 2006). We obtained median heart rates between 682 (620; 732) bpm for males and 679 (563; 716) bpm for females, still in the physiological range. As shown by us and others (Schoensiegel et al. 2011; Semeniuk et al. 2002; Esposito et al. 2000) performing echocardiography in the conscious state allows phenotypic characterization with sufficiently high sensitivity and specificity. In addition, we have shown that the method is reliable, reproducible and with low intra-observer variability (Schoensiegel et al. 2011). Despite the above-mentioned possible adverse effects of narcotics there are further disadvantages which led us to convert our protocol from performing echocardiography in nonconscious to conscious mice. In our hands echocardiography of a conscious mouse takes <2 min, of a

nonconscious mouse 10 min. The fast data acquisition not only saves time, but also reduces the stress level of mice and increases the probability of finishing the examination without too many movements of the mouse. Further, for echocardiography and the accurate monitoring of body temperature and sedation status two observer are needed. However, one examiner can perform echocardiography in a conscious mouse. Further, once people are used in handling mice there is not much training needed to switch from performing echocardiography on a fixed unconscious mouse to a conscious one held in the own hand. Therefore, for high-throughput phenotyping we recommend to use the fast and reliable method with conscious mice, particularly when done frequently (in our case daily). In case a more precise and then time-intensive analysis, e.g., valve function analysis is needed, the method needs further adjustment.

Because mice differ in their cardiovascular physiology already in between substrains, it is not possible to define normal lab values for the species mouse as commonly used in human diagnostics. We present a fast, easy and reproducible protocol for non-invasive echocardiography and electrocardiography in conscious mice. Using this system, we demonstrate significant strain-dependent differences in echocardiograms and electrocardiograms in C57BL/6N, C57BL/6J, C3HeB/FeJ and 129P2/OlaHsd mice. The presented normal values of 16-week-old commonly used inbred mice and the detailed methodological report may increase the quantity of data collected from mouse models and improve the quality of data.

## Conclusion

Combination of awake echocardiography and electrocardiography allows high-quality phenotyping of cardiac physiology in a high-throughput setting. Using this system, we demonstrated significant differences between inbred mice even among substrains. Here, we identified C57BL/6N mice to have a hypertrophic heart and a reduced heart performance compared to C57BL/6J, C3HeB/FeJ and 129P2/OlaHsd mice.

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**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical standards** The experiments comply with the current laws of the government of Germany.

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