Developmental changes in left and right ventricular diastolic filling patterns in mice

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ABSTRACT

Developmental changes in left and right ventricular diastolic filling patterns were determined non-invasively in isoflurane-anesthetized outbred ICR mice. Blood velocities in the mitral and tricuspid orifices were recorded in 16 embryos at day 14.5 (E14.5) and 17.5 (E17.5) of gestation using an ultrasound biomicroscope, and also serially in three groups of postnatal mice aged from 1 to 7 days (N=23), 1 to 4 weeks (N=18), and 4 to 12 weeks (N=27), using 20 MHz pulsed Doppler. Postnatal body weight increased rapidly to 8 weeks. Heart rate increased rapidly from ~180 beats/min at E14.5 to ~380 beats/min at 1 week after birth, then more gradually to plateau at ~450 beats/min after 4 weeks. Ventricular filling was quantified using peak E/A (ratio of peak velocity of early ventricular filling due to active relaxation (E wave) to that of the late ventricular filling caused by atrial contraction (A wave)), and peak E/total TVI (the ratio of peak E velocity to total time-velocity integral of E and A waves). Both ventricles had similar diastolic filling patterns in embryos (peak E/A ratio of 0.28 ± 0.02 for mitral flow and 0.27 ± 0.02 for tricuspid flow at E14.5). After birth, mitral peak E/A increased to >1 between the 3rd and 5th day and continued to increase to 2.25 ± 0.25 at ~3 weeks then remained stable. Tricuspid peak E/A ratio increased much less but stabilized at the same age (increased to 0.79 ± 0.03 at 3 weeks). Peak E/total TVI showed similar left-right differences, and changes with development. Age-related changes were largely due to increases in peak E velocity. Results suggest diastolic function matures ~3 weeks postnatally presumably in association with maturation of ventricular recoil and relaxation mechanisms.

Key words: Mitral orifice; tricuspid orifice; pulsed Doppler; cardiac hemodynamics; ultrasound biomicroscope; isoflurane.
INTRODUCTION

Ventricular diastolic function, particularly that of the left ventricle, has been extensively studied in humans using pulsed Doppler echocardiography. This method is used to noninvasively monitor the pattern of the ventricular filling waves produced by active ventricular relaxation during early diastole (E wave) and by atrial contraction during late diastole (A wave). The peak velocity ratio of the E and A waves (peak E/A ratio) is most often used to quantify ventricular diastolic function. The evaluation of ventricular diastolic filling pattern is important because diastolic dysfunction contributes substantially to the production of symptoms in various cardiac disorders including congestive heart failure, coronary artery disease, hypertension, dilated and hypertrophic cardiomyopathy and amyloid heart disease, and it often precedes the onset of systolic dysfunction (23;26;27). In human fetal cardiomyopathy, ventricular diastolic dysfunction, relative to systolic dysfunction, is associated with a significantly higher risk of perinatal mortality (32).

Genetically-engineered mice have been used to model human cardiac diseases. As in humans, diastolic dysfunction occurs in association with cardiac hypertrophy in various transgenic or mutant mouse models (3;4). By using Doppler methods, abnormal diastolic function has also been observed in phospholamban-deficient mice (16) and hyperthyroid mice (38). In senescent mice the mitral peak E/A ratio is significantly decreased (38) suggesting that, as in humans, diastolic dysfunction develops during aging. However, most studies reporting ventricular diastolic filling patterns in mice are limited to the adult left ventricle (16;33;40) or to the embryonic heart without differentiating the left and right ventricles (12;24;41). Little information on diastolic ventricular function is available either for the mouse neonate and juvenile, or for the right ventricle throughout development. This information is important for the critical evaluation of mice as potential models of human cardiac disease.

In the current study, left and right ventricular diastolic filling waveforms were examined during late gestational development in mice using newly-developed ultrasound biomicroscope (9;10;43;51). The study began on day 14.5 of gestation when the interventricular septum fully separates the ventricular inflow tracts of the embryonic heart (19;36), and measurements were made by placing the Doppler sample volume discretely within the left or right ventricular inflow tracts with the guidance of a high resolution ultrasound image. We also examined the left and right ventricular diastolic filling waveforms throughout postnatal development from birth to the young
adult. Measurements were made using a 20 MHz transcutaneous pulsed Doppler system in the absence of a two-dimensional ultrasound image, a method previously validated in studies of left ventricular filling in adult mice (15;38;39).

**MATERIALS AND METHODS**

The study protocol was approved by the Mount Sinai Hospital Animal Care Committee, and the study was conducted in accordance with the guidelines of the Canadian Council on Animal Care.

**Prenatal study.** Pregnant mice (ICR, wild type, Harlan Sprague Dawley, Indianapolis, IN) were studied on day 14.5 (E14.5) and day 17.5 (E17.5) of gestation (where 18.5 day is full term), and a total of 16 embryos (1 to 3 in each of 8 pregnant mice) were observed at each time point. Day 0.5 of gestation was defined as noon on the day a vaginal plug was found after overnight mating. Mice were anesthetized by face mask with ~1.5% isoflurane (the minimum required to suppress spontaneous body movements). Body temperature was monitored via rectal thermometer and maintained at 36-38°C using a heating pad and lamp, and heart rate was also monitored via transcutaneous electrodes (Indus Instruments, Houston, TX). All hair was removed from the abdomen by shaving followed by a chemical hair remover (Nair, Carter-Horner Inc., Mississauga, Ontario). To provide a coupling medium for the transducer, a warmed thick ultrasound gel was placed around the margin, and a thinner gel put in the central area. The recording was started after waiting approximately 1-2 minutes for the mouse to stabilize.

An ultrasound biomicroscope (VS40, VisualSonics Inc., Toronto) with the transducer frequency set at 40 MHz (for lateral and axial resolutions of 68 μm and 38 μm respectively) was used to image embryonic cardiac structures. The pulsed Doppler operating frequency was set at 20 MHz to measure blood flow velocities. The Doppler sample volume was 104 μm (lateral) by 257 μm (axial). The pulse repetition frequency was set at 25 kHz to achieve the maximum measurable velocity of ~50 cm/s (10;51). With these settings, we sampled flow spectra separately from mitral and tricuspid orifices of the embryonic heart on E14.5 and E17.5 (figure 1).

One to three embryos with an orientation which permitted a transverse section of the embryonic chest and a good overall view of the atrio-ventricular inflow channels were selected in each pregnant mouse (figure 1). The left and right ventricles were identified. Visible flow streams
generated by echogenic embryonic blood (37) facilitated accurate placement of the Doppler sample volume within the mitral and tricuspid orifices, and the intercept angle was measured and used when calculating blood flow velocity. Doppler flow spectra were recorded for at least 5 consecutive cardiac cycles and transferred to a Doppler signal processing workstation (DSPW, Indus Instruments, Houston, Texas) for post-analysis. The whole procedure (from onset of anesthesia to the end of data collection) took about 20 to 30 minutes. The mouse was returned to the cage after waking up in about 1 to 3 minutes.

**Postnatal study.** Three groups of wild-type ICR mice were studied serially in overlapping age ranges. We used multiple groups to limit repeated experiments on individuals thereby reducing possible developmental effects. In the neonatal group, 23 neonates from 2 litters were studied every other day from 1 to 7 days after birth. In the pre-weaning group, 18 mice from 2 litters were studied weekly from 1 to 4 weeks after birth. The gender was not identified in neonatal and pre-weaning groups. In the post-weaning group, 27 mice (12 males and 15 females from 2 litters) were followed every four weeks from 4 to 12 weeks of age.

Postnatal mice were anesthetized as described above for pregnant mice. Heart rate and body temperature were monitored as above in the postweaning group. Heat and anesthetic settings were the same in the neonatal and preweaning groups but heart rate and body temperature were not monitored. Any hair on the precordial region was cleanly shaved and the region was covered with pre-warmed ultrasound gel.

A 20 MHz transcutaneous pulsed Doppler instrument (Valpey-Fisher, Hopkinton, MA) was used to sample the flow spectrum from mitral and tricuspid orifices. The size of the transducer was ~2 mm in diameter, and the sample volume ~ 0.5 x 0.5 x 0.5 mm³. The ultrasound biomicroscope was not used for velocity measurements because postnatal blood velocities exceeded the biomicroscope’s upper limit. However, the biomicroscope was used to measure the range depth required to reach the atrioventricular orifices from a point on the chest near the apex of the heart (~3.5 mm for neonates and 5-7 mm for adults). The optimal transducer orientation during flow sampling was ~45 degrees to the body surface for postnatal mice at all ages.

The Doppler transducer was placed on the skin near the apical region of the heart then pointed rostrally to the animal’s posterior and right side to sample the mitral flow waveform first. Typically, two consecutive peaks (e.g. E and A waves) were observed in each cardiac cycle. For tricuspid flow, the transducer was slowly angled further toward the animal’s right until the mitral
flow disappeared, then further to the right until another waveform with two consecutive peaks appeared (figure 2). The tricuspid flow spectrum had a lower amplitude than the mitral flow spectrum, and exhibited a greater and opposite variation with respiration. During inspiration, tricuspid flow velocity increased (for both E and A waves, but to a variable extent for each) whereas mitral flow velocity slightly decreased (both E and A wave) (figure 2, 3B, 3D) as observed in humans (26). Systolic outflow and diastolic inflow waves were often detected at the same Doppler sample volume location in the left ventricle, but not in the right ventricle (figure 1-3). We adjusted the direction and sampling depth of the transducer to obtain the flow spectra with the highest possible amplitude. As suggested by human studies (26), the smallest flow area is at the level of the mitral and tricuspid valve tips and therefore the velocity is the highest there. Velocity at this site is believed to best reflect the pressure gradient between the atrium and ventricle. No angle correction was made because it was assumed that the intercept angle between the ultrasound beam and flow direction was close to zero so correction was not required. Doppler flow spectra for at least ten consecutive cardiac cycles were recorded, and then transferred to the Doppler signal processing workstation and saved for post-analysis. The procedure from the onset of anesthesia to the end of data collection took about 5 to 10 minutes for neonatal and preweaning groups and less than 15 minutes for the postweaning group. Then the mouse was returned to its cage after waking up in about 1 to 2 minutes.

The specific shape of the left and right ventricular diastolic filling waveforms were confirmed in two mice at 12 weeks of age by two-dimensional image-guided Doppler sampling using an Acuson Sequoia C256 with the transducer frequency at 13 MHz (figure 3), and also in six neonates at 1-9 days after birth, by image-guided Doppler sampling using new ultrasound biomicroscope prototype software which permitted velocity measurement up to ~80 cm/sec (data not shown).

To evaluate the effect of anesthesia on the ventricular diastolic filling pattern, the mitral and tricuspid flows were recorded in 10 mice at one week of age without anesthesia (at this age mice were small enough to be held still), and compared with results from the neonatal group at the 7th day after birth obtained under anesthesia. In addition, in 10 mice at two weeks postnatal age, after the mitral and tricuspid flow spectra were recorded under anesthesia as described above, the isoflurane vaporiser was turned off and the mouse moved away from the mask. Continuous alternate recordings of mitral and tricuspid flow spectra were made until the mouse awoke (in 1-2
min) and recording became impossible due to the movement of the mouse. The data obtained at arousal was compared with those during anesthesia for each mouse.

**Data analysis.** Doppler waveforms were quantitatively analyzed using a Doppler signal processing workstation and related software (Indus Instruments). The following parameters were measured and calculated: (1) R-R interval and heart rate; (2) the peak velocity of the E wave (peak E); (3) the peak velocity of the A wave (peak A); (4) the ratio of peak E to peak A velocities (peak E/A ratio); (5) the time–velocity integral (or area) under E and A waves (total TVI); (6) the ratio of peak E velocity to the total TVI (peak E/total TVI), which is considered a load independent index of ventricular diastolic function according to humans studies (17;25;29). The E and A waves were usually at least partially merged probably due to the high heart rate in mice, so it was impossible to define the real ending point of the E wave. For this reason, the time durations and areas (or TVIs) of the individual E wave and A wave and the related ratios were not determined. The left ventricular isovolumic relaxation time (IVRT) was measured when the diastolic inflow and systolic outflow waveforms were available in the same Doppler recording (figure 1-3). Left ventricular IVRT is the time interval between the end of the left ventricular outflow and the start of mitral inflow (24). To eliminate the effect of heart rate on this temporal parameter, the left ventricular IVRT was normalized to the R-R interval and expressed as a percentage of the cardiac cycle (%IVRT). Each parameter in the mouse embryo was averaged over five cardiac cycles. Each parameter in postnatal mice was averaged over ten consecutive cardiac cycles to minimize the effect of variations caused by respiration.

**Variability of tricuspid blood velocity measurements:** In contrast with the available data for mitral flow (15;38;39), little data has been reported concerning the measurement of tricuspid flow in mice. Therefore, we evaluated inter-observer variability in tricuspid flow measurement by comparing the results obtained by two independent operators within one session from 10 adult mice. For inter-session variability, the tricuspid flow spectra from the same 10 mice were recorded twice by the same observer with a time interval of one week. We chose the heart rate, peak E/A ratio, total TVI and peak E/total TVI for the evaluation of variability, because those parameters are most commonly used to quantify the ventricular diastolic filling pattern. Inter-observer variability within one session, and inter-session variability with the same observer were expressed as the percent discrepancy between two measurements (i.e. the absolute value of the
difference between the two measurements divided by the mean of the two, expressed as a percentage).

**Developmental changes in ventricular morphology**: The morphology of the left and right ventricles changes during development and would be anticipated to affect ventricular filling patterns. The gross morphology of the right and left ventricles was observed in mouse embryos at E14.5 and E17.5, neonates at one day after birth, and the adult at 8 weeks postnatal age. At day E14.5 and E17.5, one pregnant mouse was euthanized while anesthetized with isoflurane and two embryos were obtained. Two neonates and two adult mice were similarly euthanized and the hearts removed. Embryos and isolated postnatal hearts were fixed in 10% formalin for 24 hours. Embryonic hearts were then removed under a microscope. Embryonic, neonatal and adult hearts were embedded in paraffin, 10 μm serial sections obtained, and then slides stained with hematoxylin and eosin. The slides from the middle portion between the cardiac base and apex were compared between age groups.

**Statistical Analysis**: All results are presented as mean ± SEM. Unpaired t-tests were used in the comparison of: 1) mouse embryos at 14.5 vs. 17.5 days; 2) anaesthetized vs. unanaesthetized groups of one-week-old mice; and 3) male vs. female mice at both 8 and 12 weeks. A paired t-test was used to compare a group of two-week-old anaesthetized mice before and after waking up. To observe the developmental change with advancing age, the measurements from every first session in each of embryonic, neonatal, pre-weaning and post-weaning groups were compared with each other. In all comparisons between the four age groups of mice, differences in the standard deviations between groups made it necessary to use mixed linear models (45), with heterogeneous variances. For parameters with both mitral and tricuspid measurements, the correlation between the two orifices was modeled in the covariance matrix as well. Pair-wise significance tests were carried out for each parameter, with p-values adjusted for multiple comparisons using the simulated distribution of the maximum absolute value of a multivariate t random vector (7).
RESULTS

In the prenatal study, each pregnant mouse had ~10 embryos and it was easy to find 1 to 3 embryos with optimal orientation for Doppler recording. Recordings from all studied embryos were included in the analysis. In the postnatal study, some mice were excluded from the analysis at several time points. Reasons included the complete fusion of E and A waves caused by high heart rate (in some adults of the post-weaning group), death (neonatal group at the 7th day) or the poor quality of the Doppler waveform. The numbers of included measurements at different time points are presented in table 1.

There was a rapid postnatal increase in body weight from birth to 8 weeks followed by a more gradual increase to 12 weeks of age (figure 4A). Heart rate significantly and rapidly increased during embryonic and neonatal development (E14.5 to 7 days postnatally), then increased more gradually with advancing age into adulthood (table 2 and figure 4B).

As figure 4C shows, in embryo and early neonate, total TVIs in the mitral and tricuspid orifices were similar. Afterwards, the total TVI of mitral flow increased with age to ~3 weeks after birth, but that of tricuspid flow did not increase throughout postnatal development (table 2, figure 4C).

Ventricular diastolic filling patterns. Table 2 summarizes the developmental changes in left and right ventricular diastolic filling patterns by comparing the first time point in each of the four age groups. The peak E velocity, peak E/A ratio, and peak E/total TVI in the mitral and tricuspid orifices all significantly increased with age from E14.5 to 4 weeks after birth. These variables of mitral and tricuspid orifices did not significantly differ before birth, whereas after birth the values from the mitral orifice were significantly greater than those from the tricuspid orifice. In contrast, peak A velocity did not change significantly with age in either orifice.

In mouse embryos, the lateral dimension (~100 μm) of the Doppler sample volume was much less than the width of the mitral and tricuspid orifices (300-350 μm at E14.5, 450-500 μm at E17.5) so separate inflow waveform could be readily obtained. Embryonic left and right ventricles had similar diastolic filling patterns (figure 1), with peak E/A ratios less than 0.5 (figure 6A). From E14.5 to E17.5, there was a significant increase in heart rate (p<0.0001) (figure 4B), and approximately parallel increases for both ventricles in peak E velocity (p<0.0001), peak A velocity
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(mitral: p=0.004, tricuspid: p=0.0002), peak E/A ratio (p<0.0001), and peak E/total TVI (p<0.0001) were observed (figure 5 and 6).

In neonatal mice, the ventricular diastolic flow pattern in the mitral orifice changed markedly in the first week after birth (figure 2). At the time of birth, the peak E/A ratio was less than 1 for both ventricles (figure 6A). However, between the third and fifth day after birth, the mitral peak E/A ratio started to reverse from less than 1 to greater than 1, and then continued to increase, mainly due to an increase in peak E velocity (figure 5A). After about 3 weeks, the peak E/A ratio became relatively stable and remained at ~2 into adulthood (figure 6A). In the tricuspid orifice, peak E velocity showed a slight increase with age, but was always lower than peak A velocity (figure 5B). The peak E/A ratio therefore remained less than 1 throughout the studied age range (figure 6A). In general, both mitral and tricuspid peak A velocities stayed relatively stable throughout the observed postnatal age range, although small fluctuations were observed (figure 5). The peak E/total TVI significantly increased for both ventricles postnatally, but to a much lesser extent in the right ventricle than the left (figure 6A, B and table 2).

Left ventricular IVRT gradually and significantly decreased from 47.4 ± 2.4 ms at E14.5 to 13.1 ± 0.03 ms at 4 weeks after birth (table 2) and then showed little further change with age (data not shown). Left ventricular IVRT expressed as a percentage of cardiac cycle length (%IVRT) did not change significantly from 14.3 ± 0.6% at E14.5 to 1 week after birth, but then decreased significantly between 1 and 4 weeks after birth (to 9.8 ± 0.2%), and remained consistent between 4 and 12 weeks postnatal age (table 2, figure 6C). Doppler spectra collected for peak E and A velocity measurements were not always suitable for IVRT measurements, so the number of included measurements for IVRT at each time point were slightly less than for velocity measurements (table 1).

In the post-weaning group, the parameters from male and female subsets of mice were compared at 8 and 12 weeks, and significant differences found only in the body weight and total TVI of mitral flow (table 3).

Ventricular gross morphology at different ages. Figure 7 shows histological sections of the heart in the short axis plane near the middle of the ventricles from embryos at E14.5 and E17.5, a neonate at one day, and an adult at 8 weeks after birth. In adults, the left ventricular
chamber is round and its wall is morphologically dominant. In contrast, in the embryo at E14.5, both ventricular chambers were similar in shape and size, and their walls were of similar thickness. Left ventricle dominance appeared to emerge by 1 day after birth (figure 7).

**Effect of anesthesia on the ventricular diastolic filling patterns.** In one-week-old mice, ventricular diastolic filling patterns studied without anesthesia were similar to those studied under anesthesia (table 4). There was no significant difference between the two groups in the peak E/A ratio, which is the most commonly used variable reflecting the diastolic filling pattern. Somewhat higher heart rate, peak E and A velocities, and total TVI were observed in mice without anesthesia which may be due to the stress of restraint (table 4). In two-week-old mice, no significant differences were observed in parameters measured from mitral and tricuspid flow waveforms during anesthesia and at arousal, except for a slight increase of peak E/total TVI in mitral flow (table 5).

**Reproducibility of measurements from tricuspid flow waveforms.** The inter-observer variability within sessions was <7% and the inter-session variability was <13% for measurements of heart rate, peak E/A ratio, TVI, and peak E/total TVI from the tricuspid flow waveform (table 6).

**DISCUSSION**

The current study is the first to report the developmental changes of both left and right ventricular diastolic filling patterns in mice from embryonic to adult periods. Peak E/A ratio and peak E/total TVI progressively increased, and left ventricular %IVRT progressively decreased during late gestation to reach mature levels at ~3 weeks postnatally indicating that rapid functional maturation of the heart occurred over this developmental interval. Whereas left and right ventricular diastolic filling patterns were similar in the embryo, they diverged during the first ~3 weeks after birth. During this interval, there was a large increase in peak E velocity whereas peak A wave velocity showed little change. The increase in peak E was more marked in the mitral orifice than the tricuspid orifice. Peak E/A and peak E/total TVI showed similar left-right differences, and changes with development. Results indicate that diastolic function matures ~3 weeks postnatally presumably in association with maturation of ventricular recoil and relaxation mechanisms.
Developmental changes in ventricular diastolic filling patterns

In the mouse embryo, the diastolic function of both ventricles significantly improved during prenatal development from E14.5 to E17.5 as indicated by the increase in peak E velocity (from 10 to 18 cm/s), peak A velocity (from 33 to 38 cm/s) and peak E/A ratio (from 0.25 to 0.4). These results are similar to prior studies over the same age range in mice but in which the left and right ventricular inflow tracts were not differentiated and/or were both contained within the relatively large Doppler sample volume of the clinical ultrasound systems (peak E velocity increased from 5-10 to ~13 cm/sec, peak A velocity was ~33 cm/sec, and peak E/A ratio increased from 0.1-0.3 to ~0.4) (12;41). The peak E/A ratio of the embryonic heart in late gestation in the mouse (0.25 to 0.4) was lower than that of human embryos in late gestation where the peak E/A ratio increases from ~0.60 at mid-gestation to ~0.85 at late gestation for both left and right ventricles (29;34). Thus our data suggest that, as in human embryos in late gestation, both ventricles in the mouse embryo display similar ventricular diastolic function and both show similar functional changes with gestational age. In mice, the peak E/A ratio is lower at birth than in humans suggesting that diastolic function is less mature at birth in mice.

In postnatal mice, the mitral peak E/A ratio increased rapidly in the first 3 weeks after birth and reached relatively high values of ~2.0 to 2.5 in the adult period. The mitral peak E/A ratio became greater than one between 3 and 5 days after birth. In contrast, in humans, the mitral peak E/A ratio is greater than one from the first day after birth (17;47). The tricuspid peak E/A ratio also significantly increased during development in mice to reach ~0.8 in adulthood but, although it changed with a time course similar to that of the mitral peak E/A ratio, increments in peak E were much smaller and the peak E/A ratio remained less than one into adulthood. Comparatively, in humans, the tricuspid peak E/A ratio becomes greater than one at about six months of age (5). In human young adults, the mitral and tricuspid peak E/A ratios are similar, with a value of ~2 for both ventricles (18). This contrasts with mice where mitral and tricuspid peak E/A ratios differ in young adults (8 weeks) being ~2 and 0.7 respectively.

In the current study, developmental changes in E/A ratios were primarily due to the ~7 fold increase in mitral peak E (from ~10 to 70 cm/s) and ~3 fold increase in tricuspid peak E (from ~10 to 30 cm/s) whereas peak A velocity was similar between ventricles and relatively constant throughout development (~35 cm/s). In humans, over a similar developmental period (fetus to young adult), mitral peak E increases 2.7 fold (from ~30 cm/s to 80 cm/s) and tricuspid peak E
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increases ~1.4 fold (from ~35 cm/s to 50 cm/s) whereas peak A velocity remains relatively constant in the mitral orifice (~40 cm/s) but decreases in the tricuspid orifice (from ~45 cm/s to <30 cm/s) (34;34;47;50). Thus, in both species, there is a maturational increase in peak E velocity which is greater in the left ventricle than the right, and peak A velocity is relatively constant with development in the left ventricle. The species differ more markedly in the right ventricle. In mice, tricuspid peak E velocity is much lower than that in humans throughout development and peak A velocity does not decrease with development as in humans.

The period of rapidly increasing peak E velocity (which ended at ~3 weeks) did not coincide with the period of rapidly increasing heart rate (ended at ~1 week) or body weight (ended at ~8 weeks). Nor did it coincide with the period of rapid growth of the mouse heart which ends at about 8 to 10 weeks of age (2;34). The early ventricular filling wave (E wave) is mainly generated by active myocardial relaxation and by ventricular recoil. Thus, the developmental increase in peak E velocity may be related to the developmental increase in the capacity of the sarcoplasmic reticulum to sequester Ca\(^{2+}\) and thereby expedite ventricular active relaxation. Both phospholamban (which increases the rate of Ca\(^{2+}\) uptake by sensitizing the sarcoplasmic reticulum ATPase to Ca\(^{2+}\) when phosphorylated) and Ca\(^{2+}\)-ATPase mRNAs were approximately 40% of adult levels at birth and gradually increased to approach adult levels by day 15 of development in the mouse heart (14). In cats, the volume fraction of myofibrils in myocardial fibres significantly increased from neonates, to infants, and then to adults (35). Higher myofibril content may increase ventricular recoil following systole and thereby augment peak E velocity. Thus, a maturational enhancement in myocardial active relaxation and ventricular recoil likely contributes to the increase in peak velocity of the early ventricular filling wave that occurs during development in mice.

The late ventricular filling wave (A wave) is generated by atrial contraction and its amplitude depends primarily on the strength of atrial contraction, atroventricular orifice area, and on ventricular compliance. Peak A velocity was relatively constant throughout development in the mitral and tricuspid orifices suggesting that these factors remain in balance from late gestation to adulthood in mice.

The peak E/A ratio is a commonly used index for evaluating ventricular diastolic function but it is sensitive to heart rate and loading conditions (17;25;29). In the transitional circulation from fetus to newborn, there are changes in the cardiovascular system that affect preload and
afterload of both ventricles and consequently would be anticipated to affect peak E/A ratios. Changes include decreased pulmonary vascular resistance and increased pulmonary venous return, increased systemic vascular resistance and decreased inferior vena cava blood flow, and closure of the foramen ovale and ductus arteriosus (1). Furthermore, there are more gradual but marked changes in heart rate, and in pulmonary and systemic arterial pressures during development. As suggested by human studies, the peak E/total TVI is a sensitive index of ventricular diastolic function that is not affected by heart rate, preload or afterload (17;25;29). In the current study, the peak E/A ratio and peak E/total TVI for both ventricles showed similar changes with age suggesting that the effect of maturation on ventricular diastolic function dominated effects caused by changes in heart rate and loading conditions during development.

Ventricular IVRT is inversely related to the rate of decline in ventricular pressure during early diastole and therefore depends in part on the rate of myocardial relaxation. Because heart rate increased markedly during development, we expressed the IVRT as a percentage of the R-R interval to make the parameters from different age points comparable and to facilitate comparisons with the human heart. In the current study, left ventricular %IVRT was ~14% in the mouse embryo in late gestation, a value that is similar to prior reports in mouse embryos in which the ventricles were not differentiated (12% to 20%) (12;41). We showed that left ventricular %IVRT decreased with advancing development from 14% at birth to 10% at 4 weeks then remained stable into adulthood in mice. In humans, left ventricular %IVRT is ~16% at 6 weeks gestation and decreases to ~12% in the last trimester of pregnancy then stabilizes at 8-9% in children and young adults (13;22;42;44). Thus, at birth, the %IVRT of the heart is greater in mice than humans again suggesting that the mouse heart is less mature at birth.

Comparison between left and right ventricular diastolic filling patterns

The left and right ventricular diastolic filling patterns, which were similar in the embryo, started to differ within 1 day after birth and continued to gradually diverge to adulthood. As found in our study and previously reported, both ventricles of the embryonic heart at E14.5 are similar in shape and size (20;21), while on the first day after birth, the left ventricle has already started to become morphologically dominant (figure 7). The right-ward shift of the interventricular septum, caused by the establishment of an interventricular pressure gradient after birth, might be responsible at least in part for the rapid postnatal change in morphological appearance of the two
ventricles. Subsequently, structural differences between the ventricles of the newborn mouse heart are enhanced by the differences in the rates of apoptosis and proliferation between left and right ventricles (8). Structural remodelling presumably plays an important role in the continuing postnatal divergence in the diastolic filling patterns of the two ventricles in mice. However, a similar morphologic divergence occurs postnatally in humans, yet left and right ventricular diastolic filling patterns are similar in human adults whereas they differ markedly in mice. Thus, further study is required to explore the mechanisms responsible for the difference between mice and humans in right ventricular diastolic filling patterns during adulthood.

In the early neonate, similar total TVIs in the mitral and tricuspid orifices (figure 4C) suggests that the areas of the mitral and tricuspid orifices were similar, given that stroke volume (which is equal to TVI x orifice area) is the same for left and right ventricles after closure of the embryonic shunts. That the tricuspid TVI did not change postnatally suggests that the tricuspid orifice area increased in proportion with the developmental increase in right ventricular stroke volume. However, total TVI of mitral flow increased significantly with postnatal development to ~3 weeks of age (figure 4C, table 2) suggesting a slower rate of growth in mitral orifice area than in left ventricular stroke volume. The difference in TVI’s in adult mice suggests that the mitral orifice area is ~65% that of the tricuspid orifice area. This finding is similar to that of adult humans in which the mitral orifice area is estimated to be about 70% that of the tricuspid orifice (46).

Methodological considerations in evaluating ventricular diastolic function

Anesthetic agents have cardiac depressant actions that may introduce confounding factors in hemodynamic assessment. Isoflurane was reported to depress the left ventricular systolic and diastolic functions in dogs (30;31). However, other studies in healthy children (11) and in dogs (49) and chick embryos (48) found that isoflurane at low dose did not significantly change the ventricular relaxation and myocardial compliance, and isoflurane caused less myocardial depression than other volatile anesthetics. On the other hand, isoflurane may change the loading conditions (52) and consequently affect the ventricular filling patterns (28). However, as observed in the present study, the peak E/A ratios for both ventricles in the mice under anesthesia were similar to those of the mice without anesthesia. Velocities were slightly higher in conscious mice possibly due to the stress of restraint (tables 4). No significant changes were found in most
parameters of mitral and tricuspid flows during anesthesia and at the time when the mouse awakened (table 5). These data suggest that in the current study, isoflurane anesthesia had minimal effect on the diastolic filling pattern of either ventricle.

The heart rate of isoflurane-anesthetized adult mice included in the current study was ~450 beats/min and thus was lower than that of awake, nonrestrained adult mice of the same strain (~540 beats/min) obtained by chronic catheterization (6). When the mouse heart rate was higher than ~500 beats/min, we found that the E and A waves often merged so peak E and peak A velocity could not be measured. The fusion of the E and A waves was relatively more common in adult mice and resulted in exclusion of ~10% of mice from analysis in this study. On the other hand, exclusion of mice with higher heart rates would tend to reduce the mean heart rate reported for our adult groups. Compared to other commonly used injectable anesthetics, isoflurane had the least effect on the heart rate of ICR mice (52). And also, the anesthetic level was kept as light as possible in our experiments. Previous studies in normal adult mice report mitral peak E/A ratios from 2.0 to 4.5 (16;33;38;40). Higher peak E/A ratios in some prior reports may be partially explained by lower heart rates (~230 to 275 beats/min) than in the current study (~450 beats/min) due to differences in anesthetics. Heart rate in embryonic mice from E14.5 to E17.5 in the current study increased from 175 to 230 beats/min and thus was similar to prior reports where heart rate increased from ~150 to 230 beats/min over a similar age range (12;41).

**Summary**

The current study reports the developmental changes in both left and right ventricular diastolic filling patterns from late gestation to adulthood in mice. The mouse body weight continued to increase to ~8 wk, while the heart rate increased rapidly from E14.5 to 1 week after birth, followed by further slight increase to adulthood. Based on the Doppler diastolic flow parameters including peak E/A ratio, peak E/total TVI and left ventricular %IVRT, the diastolic function of both ventricles improved during late gestation and the early postnatal period and became mature at ~3 weeks after birth. The maturation of ventricular diastolic function primarily resulted in an increase in early ventricular filling likely due to maturation of ventricular recoil and active relaxation mechanisms. Late ventricular filling produced by atrial contraction was relatively constant throughout the study period. Left and right ventricular diastolic filling patterns were similar in embryos with peak E/A ratio less than 1, but diverged markedly during postnatal
development, with the mitral peak E/A ratio increasing to more than 2 but the tricuspid peak E/A ratio remaining less than one. Considering the lower peak E/A ratio in mouse embryo, the later reversal of left ventricular peak E/A ratio in mouse neonates, and the higher %IVRT in mice at birth, mice are born with less mature ventricular diastolic function than are humans. As in humans, in mice the growth of the mitral orifice appears to lag that of the tricuspid orifice during postnatal development, and the mitral peak E/A ratio increases during late gestation and postnatal development to reach ~2 in adulthood. However, in the mouse, the tricuspid peak E/A ratio remains less than one into adulthood in contrast with humans where it increases postnatally to ultimately become similar to that of the mitral orifice.

ACKNOWLEDGEMENTS

We thank the Richard Ivey Foundation for funding the purchase of the ultrasound biomicroscope, the Canadian Institutes of Health Research for operating grant support and the Ontario Research and Development Challenge Fund for fellowship support for YQ Zhou.

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Reference List


52. Zuurbier, C. J., V. M. Emons, and C. Ince. Hemodynamics of anesthetized ventilated mouse 
models: aspects of anesthetics, fluid support, and strain. *Am.J.Physiol Heart Circ.Physiol*
FIGURE LEGENDS

**Figure 1.** Image-guided Doppler flow measurement in a mouse embryo heart at 14.5 days of gestation (E14.5) using the ultrasound biomicroscope. (A) Transverse view of the heart showing the left and right ventricular inflow tracts. Blood flowing into the ventricles was clearly visible because of the echogenic properties of embryonic blood, and flow direction was easily evaluated for the angle correction of Doppler flow velocity. The Doppler sample volume (white box) was located in the mitral orifice to sample the flow velocity. (B) Doppler spectrum obtained from the mitral orifice. The biphasic waveforms in the downward direction are the diastolic inflow spectra. In most Doppler recordings from the mitral valve, the outflow spectra was also visible as in this example. The monophasic waveforms in the upward direction were caused by the Doppler sample volume including at least a portion of the adjacent left ventricular outflow tract. This waveform was used to measure the left ventricular isovolumic relaxation time (IVRT) (i.e. the time interval from the end of outflow (arrow a) to the start of mitral inflow (arrow b)). (C) Transverse image of the heart with the Doppler sample volume (white box) located in the tricuspid orifice. (D) Doppler spectrum obtained from the tricuspid orifice. In A and C, the smallest division on the scale bar is 100 μm. In B and D, the ordinate shows blood velocity in cm/s. LA: left atrium; LV: left ventricle; RA: right atrium; RV: right ventricle; E wave: early diastolic filling wave caused by ventricular relaxation; A wave: late diastolic filling wave caused by atrial contraction.

**Figure 2.** Typical Doppler flow spectra obtained from the mitral orifice (A, B, C, D and E in the upper panel) and from the tricuspid orifice (a, b, c, d and e in the lower panel) in mouse neonates on the 1st, 3rd, 5th and 7th day after birth, and in a mouse at weaning age (4 weeks old). Arrows in e mark tricuspid waves acquired near the end of inspiration. Note the changes in waveform shape and amplitude. Time and velocity scales for all Doppler flow spectra are the same. MV: mitral valvular orifice; TV: tricuspid valvular orifice.

**Figure 3.** Two-dimensional image guided pulsed Doppler flow sampling from the mitral and tricuspid orifices in an adult mouse using a clinical ultrasound system (Acuson Sequoia C256). (A) Apical four chamber view of the heart with Doppler sample volume located in mitral orifice (as indicated by the equal sign). (B) The Doppler flow spectrum obtained from mitral orifice. (C)
Apical four chamber view of the heart with Doppler sample volume located in tricuspid orifice (as indicated by the equal sign). (D) The Doppler flow spectrum obtained from tricuspid orifice. Arrows in B and D mark waveforms acquired near the end of inspiration. Note that inspiration decreases inflow velocities in the mitral orifice, but increases those in the tricuspid orifice. LA: left atrium; LV: left ventricle; RA: right atrium; RV: right ventricle.

**Figure 4.** Changes of mouse body weight (A), heart rate (B) and total time-velocity integrals (total TVI) of mitral and tricuspid flow (C) with development. The abscissa represents age in weeks where zero denotes the time of birth. No significant difference was found between the heart rate derived from mitral versus tricuspid flow in (B). In (C), a significant difference (p<0.05) was found between the total TVI of mitral flow and that of tricuspid flow at all time points except for those before birth and at day 1, 3 and 7 after birth. The vertical dashed lines divide data obtained from each of the four age groups.

**Figure 5.** Peak velocities of E and A waves for mitral flow (A) and tricuspid flow (B) as a function of age in weeks where zero denotes the time of birth. The peak E and peak A velocities were significantly different (p<0.05) at all time points for both mitral and tricuspid flow spectra except for the mitral flow spectrum on day 3 and 5 after birth. The vertical dashed lines divide data obtained from each of the four age groups.

**Figure 6.** Developmental changes of the peak E/A ratio (A) and peak E velocity to total time-velocity integral ratio (peak E/total TVI) (B) for both mitral and tricuspid orifices, and the left ventricular isovolumic relaxation time expressed as a percentage of the cardiac cycle length (%IVRT) (C). The abscissa represents age in weeks where zero denotes the time of birth. Significant differences (p<0.05) were found in the peak E/A ratio and peak E/total TVI between the mitral and tricuspid values at all time points except for the embryonic period. The vertical dashed lines divide data obtained from each of the four age groups.

**Figure 7.** Histological sections of the ventricles in the short-axis cross-section from a mouse embryo at gestational day 14.5 (E14.5) (A) and day 17.5 (E17.5) (B), a neonate one day after birth (C), and an adult mouse at 8 weeks (D). LA: left atrium; LV: left ventricle; RV: right ventricle.
Table 1. The numbers of studied mice and of the included measurements at different time points throughout development.

<table>
<thead>
<tr>
<th></th>
<th>Fetal group</th>
<th>Neonatal group</th>
<th>Pre-weaning group</th>
<th>Post-weaning group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E14.5</td>
<td>E17.5</td>
<td>1d</td>
<td>3d</td>
</tr>
<tr>
<td>Number of studied mice</td>
<td>16</td>
<td>16</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Number of measurements*</td>
<td>16</td>
<td>16</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>Number of measured LV IVRT</td>
<td>15</td>
<td>13</td>
<td>14</td>
<td>21</td>
</tr>
</tbody>
</table>

E14.5 and E17.5 represent the day 14.5 and day 17.5 of gestation, respectively. 1d, 3d, 5d and 7d represent the 1st, 3rd, 5th and 7th day after birth, respectively. 1w, 2w, 3w, 4w, 8w and 12w represent 1, 2, 3, 4, 8 and 12 weeks of age, respectively. * The number of included measurements for all parameters except for the left ventricular isovolumic relaxation time (LV IVRT).
### Table 2. Comparison of left and right ventricular diastolic inflow parameters of mice at four different ages of development (mean ± SEM).

<table>
<thead>
<tr>
<th></th>
<th>Fetal group at E14.5</th>
<th>Neonatal group at 1 day</th>
<th>Pre-weaning group at 1 week</th>
<th>Post-weaning group at 4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of measurements</td>
<td>16</td>
<td>21</td>
<td>18</td>
<td>26</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>----</td>
<td>1.57 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.64 ± 0.11&lt;sup&gt;B&lt;/sup&gt;</td>
<td>14.32 ± 0.41&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Mitral flow</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>180 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>244 ± 9&lt;sup&gt;B&lt;/sup&gt;</td>
<td>382 ± 11&lt;sup&gt;C&lt;/sup&gt;</td>
<td>454 ± 10&lt;sup&gt;D&lt;/sup&gt;</td>
</tr>
<tr>
<td>Peak E (cm/s)</td>
<td>9.8 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.7 ± 1.3&lt;sup&gt;B&lt;/sup&gt;</td>
<td>48.6 ± 2.3&lt;sup&gt;C&lt;/sup&gt;</td>
<td>73.4 ± 1.7&lt;sup&gt;D&lt;/sup&gt;</td>
</tr>
<tr>
<td>Peak A (cm/s)</td>
<td>34.4 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.4 ± 1.6&lt;sup&gt;A&lt;/sup&gt;</td>
<td>35.9 ± 1.4&lt;sup&gt;D&lt;/sup&gt;</td>
<td>35.3 ± 1.6&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Peak E/A ratio</td>
<td>0.28 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.76 ± 0.02&lt;sup&gt;B&lt;/sup&gt;</td>
<td>1.36 ± 0.03&lt;sup&gt;C&lt;/sup&gt;</td>
<td>2.20 ± 0.10&lt;sup&gt;D&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total TVI (cm)</td>
<td>1.4 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6 ± 0.1&lt;sup&gt;B&lt;/sup&gt;</td>
<td>1.9 ± 0.1&lt;sup&gt;C&lt;/sup&gt;</td>
<td>2.2 ± 0.1&lt;sup&gt;D&lt;/sup&gt;</td>
</tr>
<tr>
<td>Peak E/total TVI (/s)</td>
<td>7.0 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.7 ± 0.5&lt;sup&gt;B&lt;/sup&gt;</td>
<td>25.1 ± 0.6&lt;sup&gt;C&lt;/sup&gt;</td>
<td>33.2 ± 0.7&lt;sup&gt;D&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Tricuspid flow</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>174 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>241 ± 9&lt;sup&gt;B&lt;/sup&gt;*</td>
<td>381 ± 9&lt;sup&gt;C&lt;/sup&gt;*</td>
<td>449 ± 11&lt;sup&gt;d&lt;/sup&gt;*</td>
</tr>
<tr>
<td>Peak E (cm/s)</td>
<td>8.9 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.6 ± 1.0&lt;sup&gt;b&lt;/sup&gt;*</td>
<td>23.2 ± 0.9&lt;sup&gt;c&lt;/sup&gt;*</td>
<td>28.2 ± 1.1&lt;sup&gt;d&lt;/sup&gt;*</td>
</tr>
<tr>
<td>Peak A (cm/s)</td>
<td>33.4 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.3 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.2 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.1 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Peak E/A ratio</td>
<td>0.27 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.49 ± 0.03&lt;sup&gt;b&lt;/sup&gt;*</td>
<td>0.65 ± 0.03&lt;sup&gt;c&lt;/sup&gt;*</td>
<td>0.82 ± 0.03&lt;sup&gt;d&lt;/sup&gt;*</td>
</tr>
<tr>
<td>Total TVI (cm)</td>
<td>1.5 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5 ± 0.1&lt;sup&gt;a&lt;/sup&gt;*</td>
<td>1.4 ± 0.1&lt;sup&gt;a&lt;/sup&gt;*</td>
</tr>
<tr>
<td>Peak E/total TVI (/s)</td>
<td>5.9 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.8 ± 0.6&lt;sup&gt;b&lt;/sup&gt;*</td>
<td>15.6 ± 0.5&lt;sup&gt;c&lt;/sup&gt;*</td>
<td>20.0 ± 0.4&lt;sup&gt;d&lt;/sup&gt;*</td>
</tr>
</tbody>
</table>

Peak E: peak velocity of the early ventricular filling wave (E wave); Peak A: peak velocity of the late ventricular filling wave due to atrial contraction (A wave); Peak E/A ratio: the ratio of peak E to peak A; Total TVI: the time–velocity integral (or total area) under E and A waves. Peak E/total TVI: the ratio of peak E velocity to the total TVI; LV IVRT: left ventricular isovolumic relaxation time; LV %IVRT: LV IVRT normalized to R-R interval and expressed as a percentage.

Along each row, the same superscript letter means no significant difference among the values of the same parameter, while different superscript letter indicates significant difference (p<0.05) among the values of the same parameter. Along each column, * indicates significant difference (p<0.05) compared to the corresponding value of mitral flow waveform at the same age.
Table 3. Comparison of left and right ventricular diastolic flow parameters between male and female adult mice (mean ± SEM).

<table>
<thead>
<tr>
<th></th>
<th>8 weeks</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Number of mice</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>34.5 ± 0.6</td>
<td>26.7 ± 0.5*</td>
</tr>
<tr>
<td>Mitral flow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>427 ± 13</td>
<td>429 ± 14</td>
</tr>
<tr>
<td>Peak E (cm/s)</td>
<td>70.3 ± 2.6</td>
<td>65.8 ± 2.6</td>
</tr>
<tr>
<td>Peak A (cm/s)</td>
<td>39.4 ± 3.1</td>
<td>32.9 ± 2.3</td>
</tr>
<tr>
<td>Peak E/A ratio</td>
<td>1.93 ± 0.21</td>
<td>2.07 ± 0.12</td>
</tr>
<tr>
<td>Total TVI (cm)</td>
<td>2.5 ± 0.1</td>
<td>2.1 ± 0.1*</td>
</tr>
<tr>
<td>Peak E/total TVI (/s)</td>
<td>28.7 ± 1.1</td>
<td>31.8 ± 1.4</td>
</tr>
<tr>
<td>Tricuspid flow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>426 ± 1.6</td>
<td>415 ± 10</td>
</tr>
<tr>
<td>Peak E (cm/s)</td>
<td>26.1 ± 1.6</td>
<td>24.0 ± 1.8</td>
</tr>
<tr>
<td>Peak A (cm/s)</td>
<td>39.3 ± 2.7</td>
<td>33.5 ± 3.1</td>
</tr>
<tr>
<td>Peak E/A ratio</td>
<td>0.68 ± 0.03</td>
<td>0.73 ± 0.03</td>
</tr>
<tr>
<td>Total TVI (cm)</td>
<td>1.6 ± 0.1</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>Peak E/total TVI (/s)</td>
<td>16.4 ± 0.7</td>
<td>17.4 ± 0.8</td>
</tr>
<tr>
<td>Number of mice</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>LV IVRT (ms)</td>
<td>14.5 ± 0.7</td>
<td>13.7 ± 0.9</td>
</tr>
<tr>
<td>LV %IVRT</td>
<td>10.2 ± 0.3</td>
<td>9.8 ± 0.8</td>
</tr>
</tbody>
</table>

For abbreviations, see Table 2. * indicates significant difference (p<0.05) compared to the corresponding value of the males at the same age.
Table 4. Comparison of the ventricular diastolic flow patterns between 10 one-week-old mice without anesthesia and neonatal group at 7th day after birth (N=19) under anesthesia (mean ± SEM).

<table>
<thead>
<tr>
<th></th>
<th>Mitrail flow</th>
<th>Tricuspid flow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No anesthesis</td>
<td>Aneasthesia</td>
</tr>
<tr>
<td>Number of measurements</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>3.5 ± 0.1</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>423 ± 7</td>
<td>388 ± 10*</td>
</tr>
<tr>
<td>Peak E (cm/s)</td>
<td>63.1 ± 3.4</td>
<td>47.3 ± 2.2*</td>
</tr>
<tr>
<td>Peak A (cm/s)</td>
<td>41.0 ± 2.2</td>
<td>35.2 ± 1.0*</td>
</tr>
<tr>
<td>Peak E/A ratio</td>
<td>1.55 ± 0.10</td>
<td>1.35 ± 0.07</td>
</tr>
<tr>
<td>Total TVI (cm)</td>
<td>2.1 ± 0.1</td>
<td>1.7 ± 0.1*</td>
</tr>
<tr>
<td>Peak E/total TVI (/s)</td>
<td>30.4 ± 0.6</td>
<td>27.9 ± 0.7*</td>
</tr>
</tbody>
</table>

For the abbreviations, see Table 2. * indicates significant difference (p<0.05) compared to the corresponding value without anesthesia. In the group without anesthesia, 2 mice were excluded from analysis because of the fusion of E and A waves.
Table 5. Comparison of the ventricular diastolic flow parameters during anesthesia and at the time of waking up in 10 mice of 2 weeks old (mean ± SEM; n=10).

<table>
<thead>
<tr>
<th></th>
<th>Mitral flow</th>
<th>Tricuspid flow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>During anestheisa</td>
<td>Waking up</td>
</tr>
<tr>
<td>Number of measurements</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>406 ± 32</td>
<td>416 ± 22</td>
</tr>
<tr>
<td>Peak E (cm/s)</td>
<td>60.8 ± 8.9</td>
<td>60.4 ± 5.5</td>
</tr>
<tr>
<td>Peak A (cm/s)</td>
<td>32.1 ± 5.4</td>
<td>33.2 ± 4.5</td>
</tr>
<tr>
<td>Peak E/A ratio</td>
<td>1.92 ± 0.32</td>
<td>1.84 ± 0.15</td>
</tr>
<tr>
<td>Total TVI (cm)</td>
<td>2.1 ± 0.3</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>Peak E/total TVI (/s)</td>
<td>29.1 ± 1.8</td>
<td>32.0 ± 3.0*</td>
</tr>
</tbody>
</table>

For the abbreviations, see Table 2. * indicates significant difference (p<0.05) compared to the corresponding value during anesthesia. In the measurement of mitral flow, 2 mice were excluded from analysis because proper Doppler waveform could not be obtained at the time of waking up.
Table 6. The inter-observer and inter-session variabilities in the measurement of tricuspid flow parameters in 10 mice (6 females at 6 weeks old and 4 males at 8 weeks old) (mean ± SEM).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Inter-observer error within session</th>
<th>Inter-session error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate</td>
<td>1.3% ± 0.4% (0.2%~3.6%)</td>
<td>3.7% ± 1.0% (0.6%~9.1%)</td>
</tr>
<tr>
<td>Peak E/A ratio</td>
<td>6.0% ± 1.7% (2.1%~16.8%)</td>
<td>12.6% ± 2.4% (1.6%~22.5%)</td>
</tr>
<tr>
<td>Total TVI</td>
<td>6.8% ± 1.4% (1.6%~15.3%)</td>
<td>10.0% ± 1.7% (1.0%~19.3%)</td>
</tr>
<tr>
<td>Peak E/total TVI</td>
<td>3.7% ± 1.3% (0.5%~10.9%)</td>
<td>10.5% ± 1.8% (1.6%~18.7%)</td>
</tr>
</tbody>
</table>

The inter-observer error within session and inter-session error were calculated as the absolute value of the difference between two measurements divided by the mean of two measurements and expressed in percentage. For the abbreviations, see Table 2.
Figure 1
Figure 3
Figure 4
Age (week)

Peak velocity of tricuspid flow (cm/s)

Age (week)

Peak velocity of mitral flow (cm/s)

A

- Peak E velocity
- Peak A velocity

B

- Peak E velocity
- Peak A velocity
Figure A: Graph showing the peak E/A ratio over age (weeks). The graph includes two lines: one for mitral flow (solid line) and one for tricuspid flow (open circles). The x-axis represents age in weeks, ranging from -1 to 12, and the y-axis represents the peak E/A ratio.

Figure B: Graph showing the peak E/total TVI (liters per second) over age (weeks). Similar to Figure A, it includes lines for mitral flow (solid line) and tricuspid flow (open circles). The x-axis represents age in weeks, and the y-axis represents the peak E/total TVI.

Figure C: Graph showing LV %IVRT over age (weeks). The graph includes a line for LV %IVRT (solid line). The x-axis represents age in weeks, ranging from -1 to 12, and the y-axis represents LV %IVRT.