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E. K. Walsh, H. Huang, Z. Wang, J. Williams, R. de Crom, R. van Haperen, C. I. Thompson, D. J. Lefer and T. H. Hintze

Am J Physiol Heart Circ Physiol, November 1, 2004; 287 (5): H2115-H2121.

[Abstract] [Full Text] [PDF]

Effects of anesthetics on systemic hemodynamics in mice

B. J. A. Janssen, T. De Celle, J. J. M. Debets, A. E. Brouns, M. F. Callahan and T. L. Smith

Am J Physiol Heart Circ Physiol, October 1, 2004; 287 (4): H1618-H1624.

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special communication

Chronic measurement of cardiac output in conscious mice

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Janssen, B., J. Debets, P. Leenders, and J. Smits. Chronic measurement of cardiac output in conscious mice. *Am J Physiol Regulatory Integrative Comp Physiol* 282: R928–R935, 2002; 10.1152/ajpregu.00406.2001.—We describe the feasibility of chronic measurement of cardiac output (CO) in conscious mice. With the use of gas anesthesia, mice >30 g body wt were instrumented either with transit-time flow probes or electromagnetic probes placed on the ascending aorta. Ascending aortic flow values were recorded 6–16 days after surgery when probes had fully grown in. In the first set of experiments, while mice were under ketamine-xylazine anesthesia, estimates of stroke volume (SV) obtained by the transit-time technique were compared with those simultaneously obtained by echocardiography. Transit-time values of SV were similar to those obtained by echocardiography. The average difference \pm SD between the methods was $2 \pm 7 \mu\text{l}$. In the second set of studies, transit-time values of CO were compared with those obtained by the electromagnetic flow probes. In conscious resting conditions, estimates (\pm SD) of cardiac index (CI) obtained by the transit-time and electromagnetic flow probes were 484 ± 119 and $531 \pm 103 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg body wt}^{-1}$, respectively. Transit-time flow probes were also implanted in mice with a myocardial infarction (MI) induced by ligation of a coronary artery 3 wk before probe implantation. In these MI mice ($n = 7$), average (\pm SD) resting and stimulated (by volume loading) values of CO were significantly lower than in noninfarcted mice ($n = 15$) (resting CO 16 ± 3 vs. $20 \pm 4 \text{ ml/min}$; stimulated CO 20 ± 5 vs. $26 \pm 6 \text{ ml/min}$). Finally, using transfer function analysis, we found that, in resting conditions for both intact and MI mice, spontaneous variations in CO ($>0.1 \text{ Hz}$) were mainly due to those occurring in SV rather than in heart rate. These data indicate that CO can be measured chronically and reliably in conscious mice, also in conditions of heart failure, and that variations in preload are an important determinant of CO in this species.

heart function; echocardiography; myocardial infarction; spectral analysis

MICE ARE INCREASINGLY USED in cardiovascular research. Whereas continuous long-term arterial pressure mea-

surements are feasible in conscious conditions in this species (4, 11, 14), accurate continuous measurements of cardiac output (CO) and stroke volume (SV) are missing. Up to now murine cardiac performance has been evaluated by 1) traditional thermodilution or indicator-dilution methods (3, 24), 2) conductivity-dilution techniques (20), 3) radioactive or fluorescent microspheres (1, 17, 18, 22), 4) left ventricular (LV) conductance measurements (8, 9, 26), 5) echocardiography (Doppler) (8, 21, 23, 27), or 6) ultrasonic epicardial crystals (8, 12). Recently magnetic resonance imaging (MRI) also has been used for this purpose (25). Values for CO and SV, as reported by these papers, vary considerably. Cardiac index (CI) estimates (CO/kg body wt) ranging from 209 ± 23 (26) up to $1,300 \pm 60$ (27) were found. The diversity in the animal preparation, varying from open chest conditions up to measuring in a conscious restrained state, is most likely responsible for this. However, with none of these aforementioned techniques is it possible to monitor CO continuously over time in unrestrained conditions. Whereas most dilution-based methods need multiple blood sampling, which is limited in the mouse, the noninvasive imaging techniques cannot be applied continuously and are, in many cases, performed under anesthesia. Because, in mice, most anesthetic drugs reduce heart rate (HR) by >200 beats/min, true estimates of CO (product of SV and HR) in conscious conditions have not been achieved yet.

The first aim of this study was to evaluate recently miniaturized electromagnetic as well as transit-time perivascular flow probes that can be implanted chronically on the ascending aorta. We previously used electromagnetic flow probes to evaluate cardiac function in acute experiments in transgenic mice (6, 10). The electromagnetic flow estimate is proportional to the volume flow through the probe lumen and the magnetic field strength (5). Ultrasonic transit-time flow probes are a comparable cuff-type flow probe for direct volume

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flow measurement. Transit-time flow probes transmit a wide beam of ultrasound across the width of a vessel alternately in upstream and downstream directions. The difference in the transit times required for the upstream and downstream signals, which are affected by the flow, is calculated. The volume flow is proportional to the difference in the integrated transit times (7). Transit-time flow probes are loose fitting on the vessel compared with electromagnetic flow probes and require use of acoustic gel or tissue ingrowth for stable transmission of the ultrasound signal. For this reason, a few days are allowed before measurements are made and the sensor is properly fixed after connective tissue has grown between the vessel and the sensor. To validate the transit flow values *in vivo* we compared in a group of mice the transit-time flow readings with those simultaneously determined by echocardiography.

The second aim of the study was to test whether these flow probes are useful instruments for monitoring murine heart function in pathophysiological conditions. We compared electromagnetic and transit-time flow readings in mice with heart failure induced by a ligation of the left anterior descending coronary artery.

The third aim of the study was to quantify to what extent spontaneous fluctuations in CO are caused by variations in HR or by variations in SV. To this end, the coherence function between HR and CO as well as SV and CO was calculated and compared (11).

METHODS

The study protocol was performed according institutional guidelines and approved by the Animal Care and Use Committee of the University of Maastricht. Male Swiss mice (>10 wk of age; body weight >30 g) were obtained from Charles River, Someren, The Netherlands. Depending on the protocol, surgery was performed in two or three stages under aseptic conditions.

Procedure for myocardial infarction. Details of this procedure have been published previously (13). Briefly, mice were anesthetized by an intraperitoneal injection of pentobarbital sodium (100 mg/kg) or ketamine (100 mg/kg im)-xylazine (5 mg/kg sc), intubated, and ventilated using a small rodent ventilator (Hugo Sachs Elektronik-Harvard Apparatus type 845). The pump's SV was set at 250 μ l and the respiration rate at 4–5 Hz. After the skin was incised and the pectoral muscles were dissected free, the thorax was entered between the left second to third rib about 2 mm lateral of the sternum. The opening was widened to 6 mm with a mouse retractor (Fine Science Tools). The pericardium was opened, and a prolene 6–0 ligature was used to occlude the left descending coronary artery just proximal to the site where the artery splits into two smaller branches. This results in an infarct size of ~40% (13). After the chest was closed and negative pressure restored, the skin was closed with 5–0 silk. When mice awakened from the anesthesia, buprenorphine (0.5 mg/kg) was intraperitoneally injected for analgesia. Twenty-four hours later the buprenorphine injection (2 mg/kg sc) was repeated. In general, ~30% of all mice die within the first 24 h after myocardial infarction (MI). Surviving mice were allowed to recover for 2–3 wk. Cardiac wound healing is completed in this period (13).

Procedure for the implantation of flow probes. Mice were anesthetized with halothane or isoflurane and quickly intubated. Gas anesthesia was maintained with halothane (2% in

a 1:1 mixture of NO₂-O₂, at 1,000 ml/min) or isoflurane (1.5–2% in normal air at 150 ml/min) for the duration of the surgery. The mice were artificially ventilated using the Hugo Sachs rodent ventilator and kept on a heated operating table to maintain body temperature at 37°C. Under aseptic conditions, the second left intracostal space was identified and the thorax was opened 2 mm from the sternum. The opening was enlarged using a small retractor. The ascending aorta was carefully dissected free and either an electromagnetic flow probe (1.0 mm Skalar Medical, Delft, The Netherlands, weight of the probe: 835 mg) or transit-time flow probe (type: 1.5 SL, Transonic, weight of the probe: 385 mg) was gently placed over the aorta with fine forceps. The flow signals were tested, and the position of the flow probes was optimized. For transit-time probes, gel (Surgilube) was inserted between the probe body and the artery. The wound was closed in layers, and animals were allowed to breath spontaneously. The connector of the probe was tunneled subcutaneously to the back of the neck, extended (~2 cm), and secured with two ligatures. At the end of the surgery, buprenorphine 0.5 mg/kg was injected subcutaneously for analgesia. The following day, the buprenorphine injection (2 mg/kg sc) was repeated and mice were allowed to recover for another 3 days. No antibiotics were applied. When successfully implanted, probes can function for several weeks. We have obtained recordings up to 6 wk ($n = 2$). The success rate of the implantation is largely dependent on the surgical skills of the investigator. Our present success rate is ~70%.

Procedure for the implantation of catheters. Mice were instrumented with catheters as described in detail previously (11). In short, under ketamine (100 mg/kg im) and xylazine (5 mg/kg sc) anesthesia, a heat-stretched piece of polyethylene (PE-25) tubing (OD/ID 0.15/0.1 mm at the tip) was inserted (1.5 cm) into the right femoral artery and subcutaneously guided to the neck of the mouse. Here the catheter was fixed, extended, filled with heparinized saline (10 U/ml), and plugged. A venous catheter (Silastic OD/ID 0.25/0.12 mm) was inserted into the right jugular vein and similarly guided and exteriorized in the neck to allow for intravenous injections of drugs without disturbing hemodynamics. The weight of the catheters was 60 mg. After surgery, animals were injected intraperitoneally with 1.5 ml of a Ringer solution to improve recovery, as well as buprenorphine (0.5 mg/kg sc) as an analgesic agent. The mice were allowed to recover for 48 h before measurements were made.

Protocols. CO measurements were made between days 6 and 15 after implantation of the flow probes in intact as well as in MI mice. Mice were kept in their home cages. The flow probe was connected to the recorder (electromagnetic: type MDL1401 Skalar Medical, Delft, The Netherlands; transit-time: type T206, Transonic Systems). The arterial catheter was connected to a low-volume pressure transducer. Pressure and flow signals were sampled at 2 kHz (12 bits) using data-acquisition software (HDAS) developed by the instrument services of our university. Beat-to-beat values of mean arterial pressure (MAP) and ascending aortic flow were determined using the end-diastolic flow value to determine the interbeat interval (IBI). Potential baseline drift of the aortic flow signal was corrected by a module in the acquisition software that identified the end-diastolic phase of each beat as zero flow. For each beat, the integrated value of ascending aortic flow equals the SV minus the coronary flow. Here we refer to CO as the integrated aortic flow signal per minute. The integrated flow signal per beat is defined as SV. HR was calculated as 60,000/IBI. SV index (SI) and CI were calculated as SV per kilogram and CO per kilogram body weight,

respectively. Total peripheral resistance was calculated as MAP/CO. Values were stored on hard disk for later analysis.

Effect of volume loading. Cardiac function was measured at baseline conditions and under stimulated conditions. Baseline values were obtained by averaging the hemodynamic parameters over a 10- to 15-min period when the mouse was not moving through the cage. Stimulated values of CO were obtained by volume loading. In this case, mice were infused intravenously with a Ringer solution (warmed to 37°C) at a rate of 2.5 ml/min for ~40–50 s. Considering the ~2 ml blood volume in mice, this is an acute doubling of the circulating volume. Stimulated values of CO were taken as the average maximal aortic flow over a 10-s period. The resting and maximal values of SI and CI were compared in intact and MI mice.

Comparison of SV measurements with volume estimates by echocardiography. SV readings of the transit-time flow probe were compared with those obtained in the same minute by echocardiography. To this end a 20-MHz probe connected to an AU4 Idea machine (Esaote Biomedica, Firenze, Italy) was used. Measurements were performed during anesthesia with ketamine-xylazine 1–2 wk after implantation of the Transonic flow probe. Body temperature was maintained at 37°C throughout the experiment using a Hugo Sachs thermostat. During anesthesia, HR is considerably lower (~400 beats/min) than in conscious conditions (~650 beats/min). This has the advantage that the echocardiographic resolution is enhanced because more pictures per cardiac cycle are taken per time unit. Cardiac dimensions were determined from a 4- to 5-s recording (~17 Hz) made in B-mode. From this recording, three pictures of the working heart in end-diastolic and three in the peak systolic phase were selected by looking for the widest and narrowest cross-sections of the LV, respectively. Then, in each picture, the area of the LV cross-section was measured as well as the length of the short axis. The LV cavity was assumed to have an ellipsoid shape and hence LV volume was estimated as $\frac{4}{3} \times \text{area} \times \text{radius}$. The three readings of end-diastolic and peak-systolic volumes were averaged, and SV was calculated as the difference between average end-diastolic and peak systolic volume. This procedure was repeated three times, and the average value over these three measurements was taken as the volume estimate made by echocardiography. This value was then compared with the average SV value obtained by the transit-time flow probe during the echocardiographic recording. Data were sampled in eight mice in different experimental conditions to obtain a wide range of SV estimates. Data in the low range of SV estimates were obtained in two mice with an MI, whereas

data in the high range were obtained during volume loading according to the procedure described above.

Calculation of coherence. The relationships between the spontaneous variations of CO, SV, and HR were evaluated from 10 min beat-to-beat recordings sampled in resting conditions in both intact and MI mice using transit-time flow probes. With the use of transfer function analysis the coherence was calculated between SV and CO as well as between HR and CO. The coherence is a frequency domain estimate of how well two variables are correlated. If coherence is zero, then there is no relation at all between CO and HR or CO and SV. If the coherence is one, then CO fluctuations can be fully explained by those in HR or SV. Coherence values were averaged over two frequency bands (0.03–0.1 Hz and 0.1–3 Hz). We used the same mathematical techniques before to examine the baroreflex-mediated coupling between blood pressure and HR changes in mice (11).

At the end of the studies, mice were anesthetized with pentobarbital sodium (100 mg/kg). The thorax was opened to ensure that the position of the probes did not cause any mechanical limitation on the function of the heart. Then the flow probes were dissected free, cleaned, and sterilized before they were reused. The hearts of the MI mice were taken out to determine the infarct size as described before (13).

Statistics. All data are means \pm SD unless specified otherwise. The data obtained with the echocardiography and transit-time flow probe were compared according to the method described by Bland and Altman (2). The hemodynamic data obtained with the transit-time and electromagnetic probes were compared using unpaired *t*-tests. The effects of volume loading obtained in intact and MI mice were analyzed with a two-way ANOVA. Statistical significance was accepted at $P < 0.05$.

RESULTS

The mice recovered quickly from the surgery. Water and food intake were normal. At the time of the experiments, average body weight (\pm SD) was slightly reduced from 38.7 ± 3.1 (at entrance of study) to 36.9 ± 3.1 g (including the weight of the implanted materials). Tracings of original signals of ascending aortic flow and arterial blood pressure are shown in Fig. 1. Note that the flow signals for both type of probes reach peak values up to 100 ml/min in these chronic preparations. Figure 2 summarizes the average hemodynamics as measurements in the resting conditions with the elec-

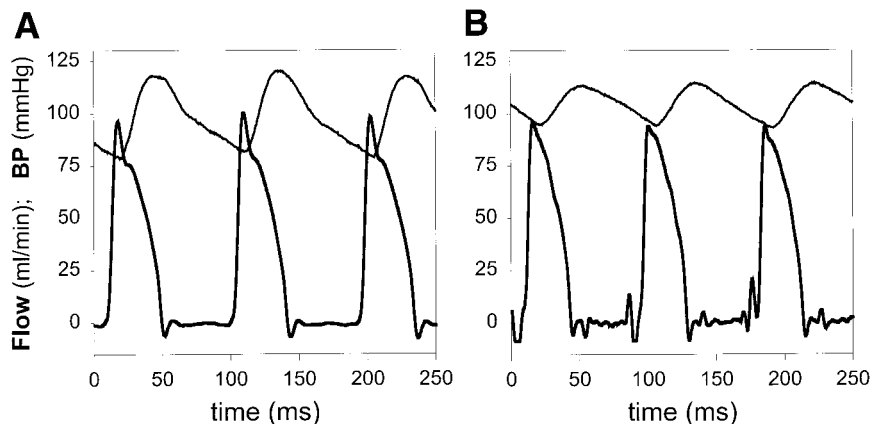


Fig. 1. Original signals of arterial blood pressure (BP; thin line) and aortic ascending flow (thick line) as measured (sampled at 2 kHz) in conscious mice chronically instrumented with a transit-time flow probe (A; signal was filtered at 100 Hz) or an electromagnetic flow probe (B; unfiltered signal). Note that peak flow values go up to 100 ml/min.

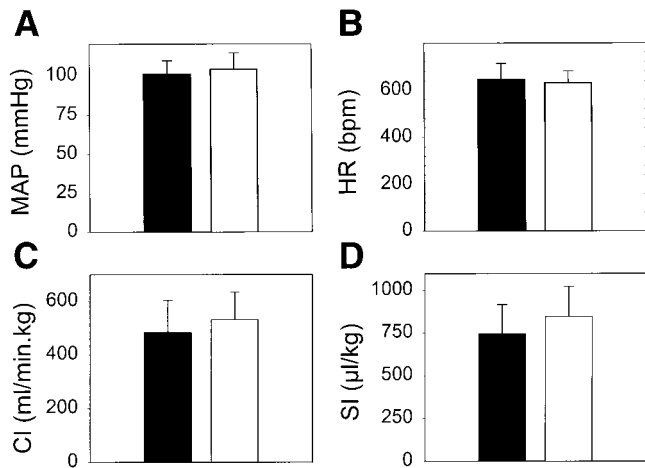


Fig. 2. Comparison of baseline hemodynamics of mean arterial pressure (MAP; A), heart rate (HR; B), and cardiac index (CI; C) and stroke index (SI; D) as obtained with electromagnetic flow probes ($n = 22$, solid bars) and transit-time flow probes ($n = 18$, open bars) in conscious mice. Values are means \pm SD. bpm, Beats/min.

tromagnetic and transit-time flow probes. For both probes, resting values for SV and CO were found in the range of 20–46 μ l and 12–27 ml/min, respectively. The mean (\pm SD) SI and CI estimated by the transit-time flow probes ($n = 17$, SI: $846 \pm 173 \mu$ l/kg and CI: $532 \pm 103 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) were not significantly different from those obtained by the electromagnetic probes ($n = 22$, SI: $744 \pm 173 \mu$ l/kg, CI: $484 \pm 120 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$), respectively.

SV values obtained by echocardiography are compared with those obtained with the transit-time flow probe in Fig. 3. The slope (1.02) of the linear regression line (forced through zero) indicates that the two SV estimates are in agreement ($R^2 = 0.51$, Fig. 3A). In Fig. 3B the SV readings are compared according to the method described by Bland and Altman (2). In this plot it can be observed that echocardiographic readings and transit-time flow probe readings vary substantially. On the other hand, there is no fixed pattern or systematic error in this variation. The average difference between the transit-time flow probe readings and echocardiographic readings was $+1.7 \pm 7.2 \mu$ l (mean \pm SD), which is not significantly different from zero. Further-

more, the magnitude of this difference was similar for low- and high-range values.

Figure 4 compares the hemodynamic parameters obtained in conscious intact and MI mice. The average infarct size in the MI mice was $41 \pm 8\%$. In resting conditions, average values (\pm SD) for SV and CO were significantly lower in MI mice ($n = 7$) than in intact ($n = 15$) mice (SV: 25 ± 4 vs. $31 \pm 7 \mu$ l; CO: 16 ± 3 vs. 20 ± 4 ml/min), respectively. Also resting MAP was slightly (~ 10 mmHg) but significantly lower in the MI mice. The hemodynamic changes obtained during the volume loading by the intravenous Ringer infusion are shown in Fig. 5. In this figure, measurements from a (responsive) intact and (unresponsive) MI mouse are presented. The average hemodynamic changes induced by volume loading are given in Fig. 4. In intact mice, volume loading increased SV (to $41 \pm 9 \mu$ l) and CO (to 26 ± 6 ml/min). However, in MI mice, the average (\pm SD) increase in SV and CO was lower than in intact mice (Δ SV: 6.1 ± 3.5 vs. $10.4 \pm 4.5 \mu$ l, $P = 0.03$, Δ CO: 4.0 ± 2.4 vs. 6.6 ± 3.6 ml/min, $P = 0.07$).

The spontaneous beat-to-beat variation of hemodynamic parameters is illustrated in Fig. 6. As can be seen from this figure, variations in SV and HR are different and the fluctuations in SV resemble those occurring in CO more than those in HR. This can be quantified by calculating the coherence. Coherence values for SV and CO as well as for HR and CO are compared for both intact and MI mice in Fig. 7. The figure shows that in all preparations for frequencies >0.1 Hz the spontaneous variations in SV are much better coupled (significantly higher coherence) to those in CO than the variations in HR.

DISCUSSION

The present study shows that CO can be reliably measured on a chronic basis in conscious mice by means of both miniaturized electromagnetic and transit-time flow probes. In addition, we show that these techniques can also be applied to characterize the pathophysiological hemodynamic state induced by MI in this species. Finally, we report that in the mouse species the spontaneous fluctuations in CO are due to those in SV rather than those in HR.

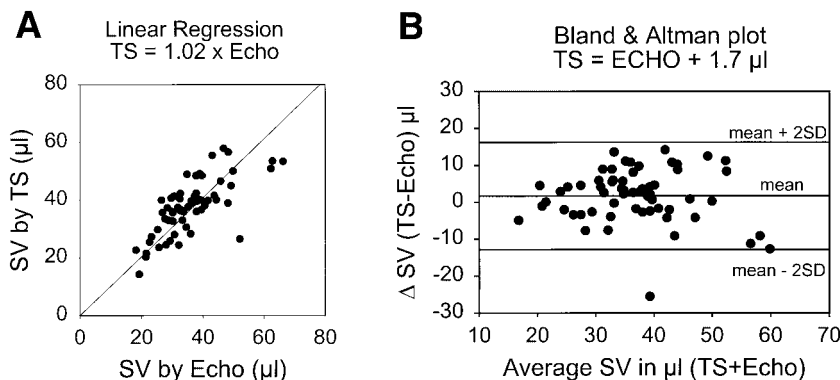
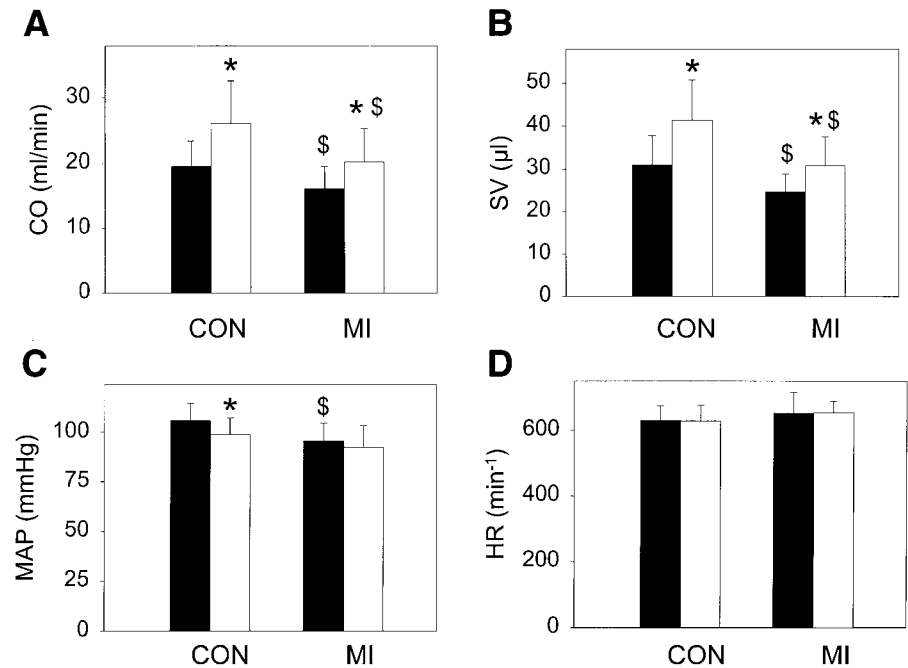


Fig. 3. Comparison of simultaneously obtained estimates of stroke volume (SV) obtained with echocardiography (Echo) and transit-time flow probes (TS) in chronically instrumented mice. Measurements were made in anesthetized conditions in 8 different animals (62 readings).

Fig. 4. Comparison of values of CO (A), SV (B), MAP (C), and HR (D) in resting (solid bars) and stimulated (open bars) conditions. Data were obtained in conscious mice chronically instrumented with transit-time flow probes ($n = 15$). Values are means \pm SD. * $P < 0.05$ between resting and stimulated values. \$ $P < 0.05$ between intact control (Con) and myocardial infarcted mice (MI).



After chronic (>6 days) implantation of either type of flow probe, mice appeared to be healthy and did not lose much body weight. The probes were implanted in mice with body weights >30 g. However, adult species of many transgenic mice strains have lower body weights. Using transit-time flow probes, we are also now capable of measuring CO in mice with body weights of 20 g (data not shown). We have not systematically assessed how long these probes can be kept

functional. In the present study, measurements were made between 6 and 16 days after implantation. Experiments were ended because of catheter failure.

Indexes of CO and SV obtained by the transit-time method were comparable to those indicated by the electromagnetic method. It should be noted, however, that the actual values of SV and CO are probably ~10% greater because coronary flow is not included. The SV values obtained by the transit-time method

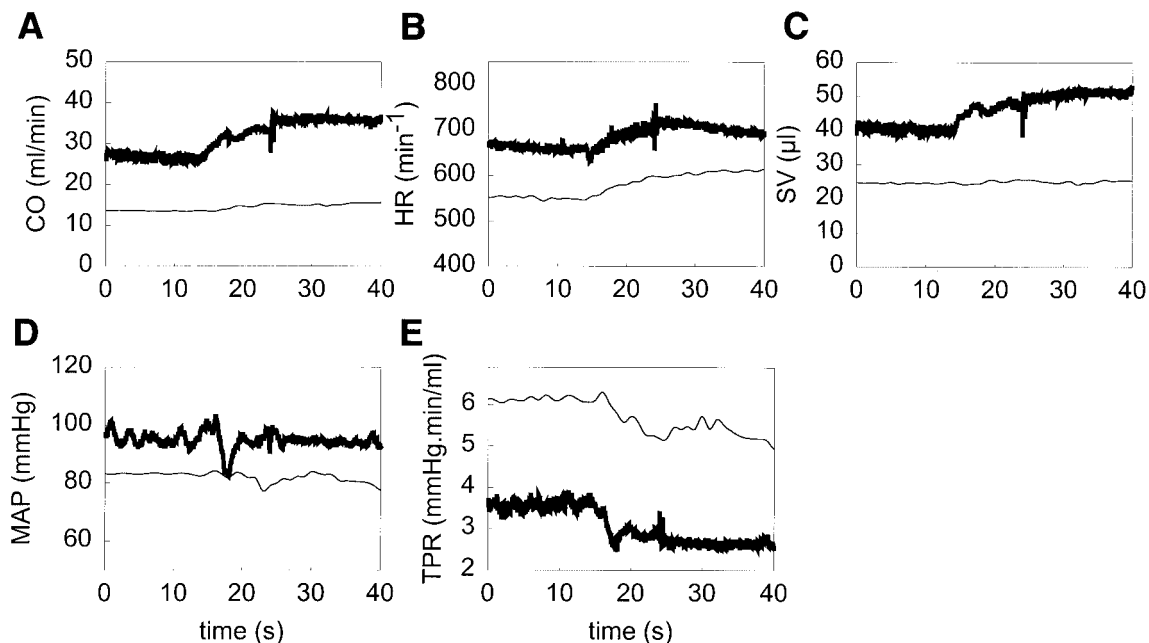


Fig. 5. Tracings of hemodynamic changes obtained in an intact responsive mouse (thick lines) and MI unresponsive mouse (thin lines) during volume loading with a 2.5 ml/min Ringer infusion. The infusion started at $t = 12$ s. The data are compared specifically for these 2 animals to illustrate the potential wide diversity in the cardiac response to this stimulus. Average changes are compared in Fig. 4. TPR, total peripheral resistance. Data were obtained in mice chronically instrumented with transit-time flow probes.

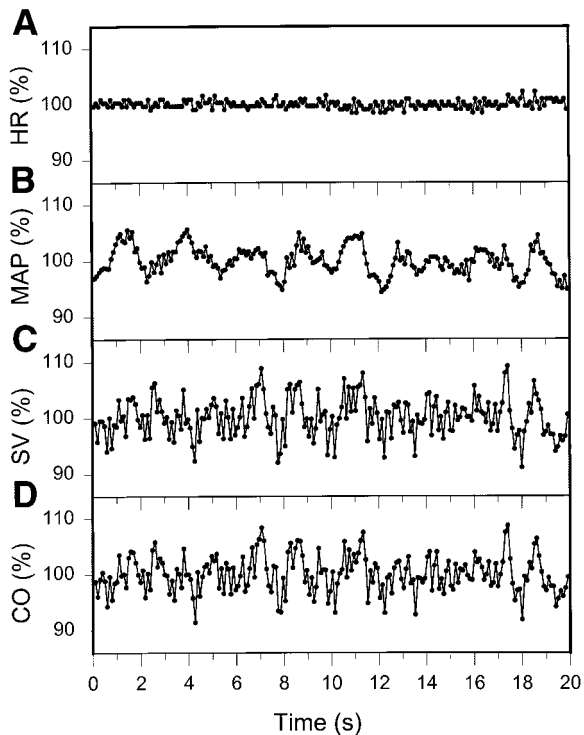


Fig. 6. Representative example of a tracing of beat-to-beat values of HR, MAP, CO, and SV plotted relative to their mean value. Note that the fluctuations in SV, but not of HR, resemble those of CO. Data were obtained in mice chronically instrumented with transit-time flow probes.

were also validated against those obtained by echocardiography. We found that the SV estimates varied considerably (see Fig. 3). Several factors account for this. First, it is difficult to estimate the exact end-diastolic and peak-systolic area in the echocardiographic B-mode recordings. Especially the peak-systolic area may be somewhat overestimated, because, with the ~ 17 -Hz sample frequency, it is unlikely that the smallest LV volume is depicted in the B-mode at its exact peak systolic volume. Second, there is an $\sim 10\%$ error in estimating the LV area and diameter. Thus the echocardiographic SI (end-diastolic volume – peak systolic volume) may be underestimated. Nevertheless, it

appeared that there was no systematic error when they were compared with the transit-time values of SV in either the low or in high range. On average, the volumes indicated by the transit-time flow probe were $\sim 2 \pm 7 \mu\text{l}$ higher than those estimated by echocardiography. Given the underestimation of true SV values by echocardiography and, on the other hand, the underestimation of true SV by the ascending aortic probe (because coronary flow is not included), these effects may compensate for each other. The true deviations of each method cannot be obtained by the present methods. However, we presume that the present errors are within the biological variation between animals.

The absolute values of SV and CO are in agreement with those obtained before with microspheres (22), echocardiography (21), and MRI (25). Thus, in an adult mouse with a blood volume of ~ 2.5 – 3 ml (70 ml/kg) and a CO of 20 ml/min, the blood volume is circulating seven to eight times per minute. This is nearly twice the value found in adult rats (blood volume of ~ 20 – 25 ml; CO of 90 ml/min). After volume loading in mice, maximal values of SV and CO were $\sim 33\%$ greater than the resting values. Thus, compared with humans, in whom CO can increase four to five times, maximal increments of CO are rather limited in the mouse. Also, in the rat, the capacity to increase CO is quite lower than in other mammals ($\sim 50\%$) (19). Thus, in the resting mouse, the amount of cardiac reserve is limited. This may also explain why the spontaneous relatively fast (>0.1 Hz) fluctuations of CO depend so much on SV and not on HR. At lower frequencies, coherence values between SV-CO and HR-CO fluctuations were comparable, suggesting that HR fluctuations also contribute to the variations in CO. The interpretation of this finding is that baroreflex-mediated adjustments of HR have a limited role in adjusting fast (>0.1 Hz) pressure fluctuations. Recently, using autonomic blocking agents, we reached the same conclusion (11).

Four to five weeks after MI in mice, resting values of CO were reduced by $\sim 20\%$. This was entirely due to a reduction in SV. HR was not different in MI and intact mice. After ligation of the left descending coronary artery in the mouse $\sim 40\%$ of the LV wall, including the apex, has lost functional myocytes (13). Given the

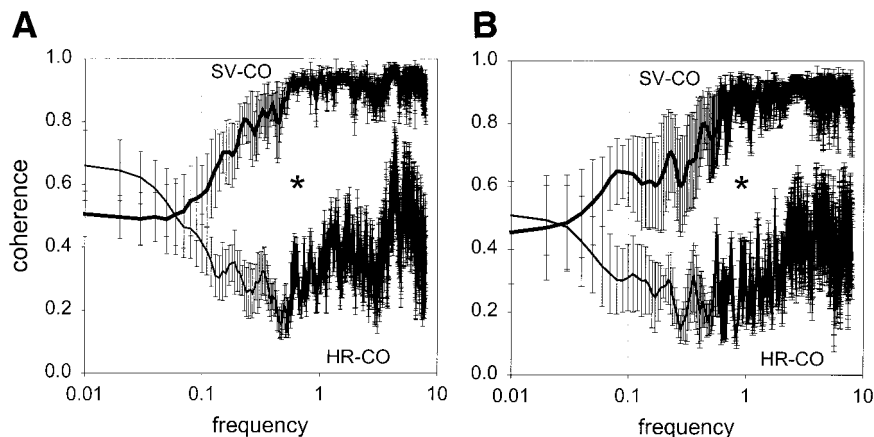


Fig. 7. Comparison of coherence functions between SV and CO (thick lines) and HR and CO (thin lines) in intact ($n = 7$) and MI mice ($n = 7$). $*P < 0.01$ for average coherence values >0.1 Hz between SV-CO and HR-CO. Data were obtained in mice chronically instrumented with transit-time flow probes.

amount of contractile tissue lost, the reduction in CO seems rather small. We assume that CO is maintained through enlargement of the ventricular volume and by enhanced contraction of the septum, which becomes hypertrophic (13, 16). Similarly after volume loading, maximal values of CO were 20–25% lower in MI than intact mice, which was again due to smaller SV in the MI group. Relatively, the reduction of the cardiac function curve is comparable to values observed in rats (19).

In conclusion, using miniaturized electromagnetic or transit-time flow probes, CO can be measured reliably and chronically in conscious unrestrained mice. These techniques allow novel approaches to assess cardiac function in this species.

Perspectives

The feasibility of chronically measuring CO in conscious unrestrained mice is a further step in the miniaturization process that has been set in motion by the need to phenotype genetically altered mice. The present study has been conducted in mice weighing >30 g. We are currently examining genetically altered mice (C57BL6 background) with body weights ranging between 20 and 25 g. The new technique is particularly useful to test for genetically induced changes in cardiovascular function, because it can discriminate between cardiac and peripheral causes of deranged hemodynamic function. In addition, the new technique allows for detailed monitoring of within-animal changes induced by investigator interventions. At the moment, patency of the arterial catheter is limiting the recording period. When combined with electrocardiographic electrodes, additional parameters, such as the time delay between electrical and mechanical activation of the heart, can be measured. We have considerable experience with 24-h CO measurements in rats (15). Whether continuous 24-h recordings are feasible in the mouse species, in the absence of the investigator, is not clear yet. The development of small reliable swivels that do not hamper mice in their locomotor behavior is needed. Presently, the recording period is mainly limited by the endurance of the investigator.

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