Left ventricular volume measurement in mice by conductance catheter: evaluation and optimization of calibration

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The conductance catheter (CC) allows thorough evaluation of cardiac function because it simultaneously provides measures of pressure and volume. Calibration of the volume signal remains challenging. With different calibration techniques, in vivo left ventricular volumes (VCC) were measured in mice (n = 52) with a Millar CC (SPR-839) and compared with MRI-derived volumes (VMRI). Significant correlations between VCC and VMRI [end-diastolic volume (EDV); R² = 0.85, P < 0.01; end-systolic volume (ESV): R² = 0.88, P < 0.01] were found when injection of hypertonic saline in the pulmonary artery was used to calibrate for parallel conductance and volume conversion was done by individual cylinder calibration. However, a significant underestimation was observed [EDV = −17.3 μl (−22.7 to −11.9 μl); ESV = −8.8 μl (−12.5 to −5.1 μl)]. Intravenous injection of the hypertonic saline bolus was inferior to injection into the pulmonary artery as a calibration method. Calibration with an independent measurement of stroke volume decreased the agreement with VMRI. Correction for an increase in blood conductivity during the in vivo experiments improved estimation of EDV. The dual-frequency method for estimation of parallel conductance failed to produce VCC that correlated with VMRI. The dual-frequency method for estimation of parallel conductance and volume conversion was done by individual cylinder calibration. However, a significant underestimation was observed [EDV = −17.3 μl (−22.7 to −11.9 μl); ESV = −8.8 μl (−12.5 to −5.1 μl)]. Intravenous injection of the hypertonic saline bolus was inferior to injection into the pulmonary artery as a calibration method. Calibration with an independent measurement of stroke volume decreased the agreement with VMRI. Correction for an increase in blood conductivity during the in vivo experiments improved estimation of EDV. The dual-frequency method for estimation of parallel conductance failed to produce VCC that correlated with VMRI. We conclude that selection of the calibration procedure for the CC has significant implications for the accuracy and precision of volume estimation and pressure-volume loop-derived variables like myocardial contractility. Although VCC may be underestimated compared with MRI, optimized calibration techniques enable reliable volume estimation with the CC in mice.

MICE ARE INCREASINGLY USED for research in genetically engineered models of human cardiac disease. This has increased the need for optimized techniques to evaluate the functional cardiac phenotype. The development of miniaturized conductance catheters (CC) has enabled evaluation in the pressure-volume plane and allowed quantification of left ventricular (LV) volumes and load-independent indexes of contractility.

However, because of methodological limitations the calibration of the volume signal remains challenging. Calibration is required not only for evaluation of LV volumes but also for indexes of contractility, because these are dependent on absolute volume. Recently CC volumes were reported to be underestimated and inaccurate compared with magnetic resonance imaging (MRI) (9). However, the potential for improving CC calibrating procedures was not evaluated. The aim of the present study was to evaluate volume calibration procedures for optimizing measurement of volume data with CC, using MRI as a reference method.

MATERIALS AND METHODS

Theoretical Background

The CC technique has previously been described in detail (1, 4, 8). As proposed by Baan et al. (1) in 1984, the calibrated time-varying volume [V(t)] can be calculated as:

\[ V(t) = \left( t/a \right) \times \left( L \cdot \sigma_b \right) \times \left[ G_i(t) - G_p \right] \]  

(1)

where \( a \) is the ratio of stroke volume (SV) measured by CC and by an independent method, \( L \) is the distance between the sensing electrodes, \( \sigma_b \) is the specific conductivity of the blood, \( G_i \) is the instantaneous conductance, and \( G_p \) is the parallel conductance.

Three issues are of major importance for calibration of the volume signal: 1) \( G_p \), which leads to volume overestimation; 2) a nonuniform electric field generated by the CC, which may influence the conversion from conductance signal to volume (1, 4, 20, 21); and finally 3) the signal processing by the pressure-volume system.

Parallel conductance. Injection of a bolus of hypertonic saline into the bloodstream is frequently used to estimate \( G_p \) (1). During the wash-in phase of saline into the LV \( \sigma_b \) increases, and the relation between recorded conductance at end diastole (\( G_{cd} \)) and end systole (\( G_{cs} \)), respectively, can be described as:

\[ G_{cs} = (m \times G_{cd} + b) \]

(2)

where \( m \) is the slope and \( b \) is the intercept of the regression line. At the point where the extrapolated linear regression of multiple \( G_{cs} \) and \( G_{cd} \) intercepts the line of identity, \( \sigma_b = 0 \) and the remaining conductance represents a single value of \( G_p \). The saline bolus may be injected into the pulmonary artery (PA method) or into a peripheral vein [intravenous (IV) method]. Because the pulmonary artery is difficult to access in mice, the IV method is most frequently used. However, by this method the diluted bolus of hypertonic saline passes through the blood pool in the right side of the heart, which may temporarily change \( G_p \). Whether this influences the result is unresolved in mice.

The hypertonic saline method poses a potential risk to mice because of volume loading and contractility changes (7). Consequently, the dual-frequency (DF) method was introduced as an alternative method to estimate \( G_p \) (6). While myocardial conductivity increases with higher excitation frequency, \( \sigma_b \) is unchanged (16, 24). From the change in conductance measured at two different frequencies, estimation of \( G_p \) is possible.
The percent rise in conductance with frequency change is proportional to \( G_2 \) as a percentage of total conductance (6), which enables calculation of \( G_p \) by

\[
\left( \frac{1}{\beta - 1} \right) \times \left( \frac{G_{20kHz} - G_{2kHz}}{G_{2kHz}} \right) = \frac{G_p}{G_{2kHz}} \tag{3}
\]

where \( \beta \) is a proportionality factor, \( G_{20kHz} \) is total conductance at 20 kHz, \( G_{2kHz} \) is total conductance at 2 kHz, and \( G_p \) is the parallel conductance at 20 kHz. When Eq. 3 is solved with respect to \( \beta \) in a set of animals, \( G_p \) is determined with the saline dilution method estimation of \( G_p \) by the DF method is possible. The relation between \( G_p \) at 2 kHz and 20 kHz is

\[
G_{2kHz} = \beta \times G_{2kHz} \tag{4}
\]

Conversion from conductance to volume. When \( \sigma_b \) is known, volume can be calculated according to Eq. 1. Since SV may be underestimated because of a nonuniform electric field produced by the CC and the geometric conditions of the heart, calibration by \( \alpha \) is used (1, 21). When \( \alpha \)-calibration is included, Eq. 1 is reduced to:

\[
V(t) = \frac{(1/\alpha) \times [G(t) - G_p]}{\sigma_b} \tag{5}
\]

where \( \alpha \) under these conditions represents the ratio of the amplitude of the conductance signal \( (G_2) \) and the SV estimated by an independent method. In this study, SV was estimated by a flow probe on the ascending aorta.

In vitro cylinder calibration has been proposed as an alternative method to convert conductance-derived data into volume (22, 23). By loading a series of cylindrical holes of volumes spanning expected in vivo volumes with heparinized blood the relation between measured conductance and volume is established. A volume calibration formula is constructed and used to convert all in vivo-acquired conductances into volumes.

An increase in the amplitude of the conductance signal during in vivo CC studies has been observed (1). This is mainly due to injection of hypertonic saline, which contributes to increase \( \sigma_b \) (5). With cylinder calibration, drifting of \( \sigma_b \) during the in vivo protocol may influence the final volume estimates. However, the significance for volume measurements in mice is unknown.

In this study we estimated and corrected for the drifting that occurred during the in vivo protocol. When only SV is considered, Eq. 1 is reduced to Eq. 5 and it becomes apparent that calibrated SV and the conductance signal amplitude \( (G_2) \) are proportional to \( \sigma_b \) and independent of parallel conductance.

\[
SV = \frac{1}{\alpha} \times \left( \frac{L}{\sigma_b} \right) \times G_2 \tag{5}
\]

Assuming that the in vivo SV is unchanged, a constant \( c_d \) that represents the ratio of change in conductivity can be defined.

\[
c_d = \frac{\sigma_{b_{end}}}{\sigma_{b_{measurement}}} = \frac{G_{b_{end}}}{G_{b_{measurement}}} \tag{6}
\]

From Eq. 1 it is apparent that the constant applies to all volume data that have been corrected for parallel conductance. Consequently, conductance data corrected for parallel conductance can be adjusted for drift in \( \sigma_b \) during the protocol by multiplying with \( c_d \).

Signal processing. The voltage difference on the sensing electrodes is amplified and inverted by the pressure-volume system to reflect conductance \( (G) \). As a result, the volume signal is linearly correlated to the input conductance with a fixed gain and offset. Calibration is necessary to measure absolute values of conductance and to compare conductances at different excitation frequencies, as with the DF method. A series of frequency-independent resistors integrated in the pressure-volume system facilitate the calibration.

Experimental Strategy

The experimental strategy addressed two specific aims: 1) to establish the agreement of in vivo absolute LV volume measurements with CC compared to MRI and 2) to evaluate the influence of different calibration procedures associated with the conductance technique on the accuracy and precision of LV volume measurements.

Male C57BL/6J mice (M&B Taconic, Ry, Denmark) were used in the study \( (n = 52) \). In the main study, different-sized animals (21.8–34.6 g) were included \( (n = 11) \). All types of calibrating procedures and measurements were performed in each animal, which made direct comparison between methods possible. MRI was performed on day 1 and constituted the independent reference. The open-chest procedures were made on day 3 and included CC and flow probe measurements.

In a separate study \( (n = 12) \) we estimated drifting of the conductivity of the blood during the protocol according to Eq. 5, by continuously measuring the cardiac output with a flow probe. In another separate study \( (n = 6) \) we studied the influence of opening the chest on the CC measurements. Finally, we investigated the agreement between volumes measured with CC and MRI in an independent series of animals \( (n = 23) \).

Animal preparation. The investigation conformed with the Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Pub. No. 85-23, Revised 1996), and the experimental protocol was approved by the Institutional Animal Care Committee and the Ethical Review Committee. During all procedures the animals were intubated and mechanically ventilated (Inpira AVS, Harvard Apparatus, Holliston, MA) with 2% isoflurane in 50% O2 and 50% room air at 100 breaths/min and a tidal volume of 10 μl/g. All data were digitalized and stored on a PC with acquisition software (Notocord system, SAS, Paris, France).

MRI. After establishment of mechanical ventilation, isotonic saline (0.2 ml) was administered subcutaneously and the animals were positioned supine on a tray in the magnet (Varian 7 Tesla, Varian, Palo Alto, CA). Rectal temperature was monitored, and body temperature was kept constant at 37°C through an air heating system. ECG was monitored by subdermal needles and respiration by a pneumatic pillow. Measurements were transmitted through a monitoring and gating system (model 1025 Monitoring and Gating System; SA Instruments, Stony Brook, NY) to the MR system. In the magnet a dedicated Helmholtz-type coil was positioned around the heart. Cardiac images were acquired with a fast spoiled gradient echo cine sequence in a number of adjacent short- and long-axis slices. A slice thickness of 1.0 mm, a matrix of 256×256, and a field of view of 30×30 mm were used, yielding pixel size of 117×117 μm (Fig. 1B). The echo time was 3.2 ms, and the repetition time (the temporal resolution) was 7 ms.

Open-chest procedure. The pressure-volume system consisted of a 1.4-F four-electrode CC for mice (SPR-839; Millar Instruments, Houston, TX) and a signal conditioning box (MPCU-200; Millar Instruments). A 20-kHz excitation frequency was used, and data were sampled at 1,000 Hz.

Temperature was maintained at 37°C by a rectal temperature-controlled heating pad and a heating lamp. The left jugular vein was cannulated with a 27-gauge needle connected to a pressure gauge and an automated infusion pump. The animals were supplemented with isotonic NaCl at an infusion rate of 0.1 ml·g⁻¹·h⁻¹ during the initial phase until stabilization of LV systolic pressure and maximal derivative of change in pressure over time (dP/dt), subsequently reduced to 0.05 ml·g⁻¹·h⁻¹ throughout the remaining protocol. The infusion of NaCl was discontinued for 1–2 min, and the ventilator was stopped at end expiration for 2–3 s before recording pressure-volume loops (Fig. 1A).

The chest was entered via an anterior thoracotomy, and the ascending thoracic aorta was exposed. The CC was inserted into the LV through the apex and positioned along the cardiac longitudinal axis. A 0.1-mm PVC tube was inserted in the lateral wall of the right ventricle and advanced to the pulmonary valve.

We randomly assigned each animal to start with either the PA method or the IV method for bolus injection of 30% hypertonic saline.
A bolus of 2 or 5 μl was delivered for the PA and IV methods, respectively, over ~0.5 s. In between, the DF method was performed. A stabilizing period of 2–3 min was allowed between each measurement. At the end of the open-chest procedure, SV was measured with a transit time flow probe (1PR; Transonic Systems, Ithaca, NY) positioned on the ascending aorta. Finally, the CC was removed and 0.5 ml of blood was drawn from the LV into a heparinized syringe. A plastic block maintained at 37°C with two 15-mm-deep cylindrical holes with diameters of 2.0 and 4.0 mm was filled with blood, and the conductance of each cylinder was measured.

Closed- vs. open-chest procedure. The CC was inserted through the right carotid artery and advanced to the LV. Steady-state SV was recorded before and after opening of the thorax.

Calculations

Maximal and minimal LV volumes were used as end-diastolic volume (EDV) and end-systolic volume (ESV).

MRI. Cine sequences during the cardiac cycle recorded in five to seven slices (depending on heart size) in a short-axis view were used to calculate EDV and ESV of blood and myocardium by planimetry (Fig. 1B).

\[ V_i = \sum_{i=1}^{N} (A_i \times h) \]  

(7)

where \( V_i \) is the total volume, \( A_i \) is the area of a slice, \( h \) is the height of the slice, and \( N \) is the number of slices. The density of myocardium was set to 1.05 g/cm² (12), and the LV weight \( \left(V_{myo, MRI}\right) \) was calculated as \( V_{myo, MRI} = 1.05 \times V_{myo, MRI} \).

Conductance catheter. Figure 2 illustrates different ways to obtain calibrated CC volumes. The method that provided the most precise volume estimates is indicated by bold arrows. Initially, the acquisition software was calibrated to display measured conductance directly. For volume estimates is indicated by bold arrows. Initially, the acquisition software was calibrated to display measured conductance directly. For calibration by the PA method, a data sequence containing conductance waveforms from steady state and the subsequent wash-in phase of the saline bolus into the LV (~4–5 cardiac cycles) was used. \( G_p \) was estimated according to Eq. 2 and subtracted from steady-state data, before multiplying with \( G_d \) according to Eq. 6 to correct for drift in \( G_d \). Cylinder calibration data from each animal were used to construct individual volume calibration formulas to enable conductance-to-volume conversion. However, data were only multiplied with the gain factor, because the offset volume is included in the \( G_p \) already subtracted.

Temporally averaged volume waveforms from five consecutive cardiac cycles at 2 and 20 kHz were used to evaluate the DF method according to Eq. 3. Plots of LV volume at 2 kHz vs. LV volume at 20 kHz were made to check for nonlinearity and phase difference.

Transit time flow probe. The SV estimation was based on the mean flow integral of five consecutive cardiac cycles. The slope factor \( \alpha \) was calculated as the ratio to the mean conductance SV of the corresponding five cardiac cycles.

Statistics. Data are presented as means (SD). All statistical analysis was conducted with Intercooled Stata 8.0 for Windows (Stata). No substantial deviation of the measured volumes from a normal distribution was found. Mean values were compared by a paired t-test. Pitman’s test of difference in variance for paired data was used to compare correlations (13). Agreements between methods were evaluated with difference vs. average plots (Bland-Altman plot) with 95% limits of agreement given by the average ± 2SD of the differences (2).

RESULTS

Hemodynamics

The open-chest and MRI procedure times were ~35 and 60 min, respectively. The average heart rates during MRI [519 (SD 39) min⁻¹] and the open-chest procedure [556 (SD 56) min⁻¹] did not differ significantly (P = 0.09). The average rectal temperature was in the range 36.1–36.6°C. Rate-pressure product (RPP) and central venous pressure (CVP) did not change from the beginning [RPP 52,232 (SD 8,852) mmHg/min, CVP 5.9 (SD 2.0) cmH₂O] to the end [RPP 50,018 (SD 10,118) mmHg/min, CVP 5.7 (SD 1.6) cmH₂O] of the study.
(RPP $P = 0.26$, CVP $P = 0.75$). No change in $dP/dt$ and RPP was observed during the three to five cardiac cycles representing the wash-in phase of hypertonic saline in the LV.

**Conductance-to-Volume Signal Processing**

Input conductance correlated with output voltage from the pressure-volume system at both 2- and 20-kHz excitation frequencies (both $R^2 > 0.999$, $P < 0.001$). However, the signal amplification was characterized by a difference in offset and gain between the two excitation frequencies. The difference in signal processing was calculated to be equal to $U_{20kHz} = 0.785 \times U_{2kHz} + 0.128$, where $U_{2kHz}$ is output voltage at 20 kHz and $U_{2kHz}$ is output voltage at 2 kHz.

The correlation between volume and conductance was linear only up to volumes of $\sim50 \, \mu L$, when measured in a series of cylindrical holes. Within the range of volumes used for two-point calibration in this study the correlation was strong ($R^2 = 0.99$, $P < 0.01$).

**Left Ventricular Volumes**

$G_p$ estimated with the PA method correlated with $G_p$ estimated with the IV method ($R^2 = 0.69$, $P < 0.01$). However, CC volumes calibrated with the PA method correlated more closely to MRI volumes than those calibrated with the IV method (Table 1). Consequently, the range defined by the limits of agreement was larger in the comparison between the IV method-calibrated volumes and MRI [EDV $-18.1 \, (-28.1$ to $-8.1)$, ESV $-9.6 \, (-16.9$ to $-2.2)$] than in the comparison between the PA method and MRI (see Fig. 5). We observed that passage of the saline bolus through the right side of the heart temporarily affected the $G_p$ measured in the LV with the IV method (Fig. 3). A similar injection of a bolus of saline with a conductivity matching that in blood did not produce any significant increase of the conductance signal.

PA method and cylinder-calibrated CC volumes correlated with MRI volumes with (EDV $R^2 = 0.85$; ESV $R^2 = 0.88$) and without (EDV $R^2 = 0.58$; ESV $R^2 = 0.86$) drifting correction of $\sigma_p$. The correlation between EDVs with correction was superior to EDVs without correction ($P < 0.05$).

**Table 1. Correlation matrix ($R^2$) between MRI and cylinder-calibrated CC volumes**

<table>
<thead>
<tr>
<th></th>
<th>PA Method</th>
<th>IV Method</th>
<th>$P$ Values (PA vs. IV method)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual volume calibration formulas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDV</td>
<td>0.85†</td>
<td>0.54*</td>
<td>0.004</td>
</tr>
<tr>
<td>ESV</td>
<td>0.88†</td>
<td>0.54*</td>
<td>0.025</td>
</tr>
<tr>
<td>Mean volume calibration formula</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDV</td>
<td>0.73†</td>
<td>0.40*</td>
<td>0.009</td>
</tr>
<tr>
<td>ESV</td>
<td>0.86†</td>
<td>0.51*</td>
<td>0.040</td>
</tr>
<tr>
<td>$P$ values (individual vs. mean)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDV</td>
<td>0.31†</td>
<td>0.44†</td>
<td></td>
</tr>
<tr>
<td>ESV</td>
<td>0.540</td>
<td>0.767</td>
<td></td>
</tr>
</tbody>
</table>

Parallel conductance is estimated by the pulmonary artery (PA) method or the intraventricular (IV) method, and conversion to volume is based on a mean volume calibration formula or on individually constructed volume calibration formulas. MRI, magnetic resonance imaging; CC, conductance catheter; EDV, end-diastolic volume; ESV, end-systolic volume. *$P < 0.05$, †$P < 0.01$.

Flow probe-calibrated CC volumes ($\alpha$-calibrated) correlated with MRI volumes (EDV $R^2 = 0.70$, $P < 0.01$; ESV $R^2 = 0.64$, $P < 0.01$). However, cylinder- and PA method-calibrated volumes were more closely correlated to MRI volume (Table 1) than flow probe-calibrated CC volumes (EDV $P = 0.038$; ESV $P = 0.018$).

Average values of EDV and ESV estimated with MRI were larger than volumes estimated with CC regardless of the method for estimating $G_p$ (IV or PA method) and conductance-to-volume conversion (flow probe or cylinder) (all $P < 0.001$; Fig. 4).

The closest correlation between CC and MRI was observed for PA method- and cylinder-calibrated volumes. Of these volumes, a Bland-Altman plot showed a systematic underestimation of CC volumes (Fig. 5). Linear regression analysis resulted in the following equations: $V_{MRI} = 0.89 \times V_{CC} + 20.1 \, (EDV)$ and $V_{MRI} = 1.08 \times V_{CC} + 8.0 \, (ESV)$, where $V_{MRI}$ and $V_{CC}$ are MRI-measured and CC-measured volume,
respectively. These equations applied to CC volume measurements from an independent series of 23 mice and compared with MRI volume measurements confirmed the consistency of CC measurements without any significant offset (Fig. 6).

SV measured with CC and calibrated with flow probe or cylinder technique correlated equally to MRI SV ($R^2 = 0.63; P = 0.01$; Fig. 7). Evaluation of agreement demonstrated a systematic underestimation of CC SV compared with MRI ($8.5 \pm 12.5$ to $-2.4$; both $P < 0.01$).

In the substudy including six mice, opening of the thorax resulted in a significant increase in SV of 18.2% (9.3–27.2%, $P = 0.01$).

**Dual-Frequency Method**

A scatter plot according to Eq. 3 demonstrated no correlation between percent change from $G_{2kHz}$ to $G_{20kHz}$ at end systole and $G_{p,2kHz}$ found by the PA method as a percentage of $G_{2kHz}$ ($R^2 = 0.17, P = 0.20$). However, with the $y$-intercept set at zero, $\beta$ was calculated to 1.139 by linear regression, and a formula for estimating parallel conductance by the DF method ($G_{p,DF}$) was constructed (Eq. 3). The calculated numerical $G_{p,DF}$ values did not correlate with $G_{p,PA}$ values ($R^2 = 0.01, P = 0.81$).

**Myocardial Weight**

The wet weight of the explanted LVs ranged from 86 to 125 mg and correlated to the MRI estimates of LV weights ($R^2 = 0.68, P < 0.01$). With a diastolic measurement, MRI underestimated the weight by $10.8 \pm 16.9$ to $4.7$; with a systolic measurement, a nonsignificant overestimation of $0.7 \pm 6.4$ to $7.9$; $P = 0.20$ was observed.

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**Fig. 4.** Relation between CC volumes and MRI. Individual constructed volume calibration formulas were used for the cylinder calibrated volumes. $\times$, Cylinder and IV method calibrated; $\bullet$, cylinder and PA method calibrated; $\triangle$, $\alpha$ (flow probe) and PA method calibrated; ESV, end-systolic volume; EDV, end-diastolic volume.

**Fig. 5.** Bland and Altman plot showing difference of MRI and CC volumes (PA method and cylinder calibrated) against their geometric average. The CC systematically underestimated volumes [ESV $-8.8 \mu l$ ($-12.5$ to $-5.1 \mu l$); EDV $-17.3 \mu l$ ($-22.7$ to $-11.9 \mu l$)]. Solid lines represent mean values. Dashed lines represent 95% limits of agreement.

**Fig. 6.** Bland and Altman plot illustrating the consistency of CC volume measurements observed in an independent series of 23 mice. Equations found by linear regression analysis on CC and MRI volumes from the main study were used on CC volumes before comparing with MRI volumes. No significant offset was found. ESV $= 0.4 \mu l$ ($-8.6$ to $9.3 \mu l$); EDV $= -2.9 \mu l$ ($-11.6$ to $5.7 \mu l$). Solid lines represent mean values. Dashed lines represent 95% limits of agreement.

**Fig. 7.** Correlation between stroke volume (SV) measured with CC and MRI. $\blacksquare$, Flow probe calibrated ($R^2 = 0.71, P < 0.01$); $\bigcirc$, cylinder calibrated ($R^2 = 0.58, P < 0.01$).
**Conductivity of Blood**

Calibration with individually constructed volume calibration formulas compared with calibration with a common formula did not improve the correlation with MRI significantly (Table 1). In the substudy in which the drifting of \( \sigma_h \) was evaluated, we found an average 14 (SD 8.69)% increase in \( \sigma_h \) from the start to the end of the protocol \( (P < 0.01) \).

**DISCUSSION**

We studied the influence of the most frequently used calibration techniques for CC on estimation of cardiac volume measurements in vivo, with MRI as reference method. Our results indicate that the calibration procedure influences estimates of in vivo LV volumes. Moreover, we demonstrated that an offset in volume estimates may be present, but with an optimized protocol a high precision of LV volume estimates can be obtained with the CC in mice.

**Estimation of Offset**

**Dual-frequency method.** We found no correlation between \( G_p \) estimated with the hypertonic saline method and the DF method. In contrast to previous results \((5–7)\), this finding suggests that the DF method is not feasible in mice. Differences related to the pressure-volume system and the in vivo protocol could explain the variable results. Feldman et al. \((5)\) and Gawne et al. \((6)\) used a CC operating simultaneously at both frequencies and a wider range of frequencies \((10–100 \text{ kHz and } 3.3–30 \text{ kHz, respectively})\) than we did. These conditions provide a larger signal difference \((5)\) and a higher signal-to-noise ratio. In addition, Feldman et al. used a technique with epicardial measurement of the specific myocardial resistivity. However, this approach was not possible here because the commercially available pressure-volume systems do not support these features. We also tried to estimate \( G_p \) by a DF method as suggested by Georgakopoulos and Kass \((7)\), who used a system comparable to ours, but with the same result. In that study urethane and etomidate were used as anesthesia. We used isoflurane, which increases coronary blood flow and myocardial blood volume \((10)\). Since an essential part of the extracellular conductivity occurs through blood, the use of isoflurane may increase the frequency-independent proportion of the overall myocardial conductivity \((19)\). This would challenge the estimation of \( G_p \) because the signal from the frequency-dependent membrane resistivity is diminished. In addition, an overall increased conductivity of the myocardium might allow the electric field generated by the catheter to spread outside the walls of the LV. We found several indications that this was evident. First, the offset of the raw volume waveform was influenced by metal instruments or fluid in the thoracic cavity. Second, no correlation between \( G_{p,DF} \) and \( m_{myo, MRI} \) was found as otherwise expected. Finally, the blood pool at the right side of the heart appeared to contribute to \( G_p \), as illustrated in Fig. 3.

**Hypertonic saline dilution method.** To our knowledge the comparison of \( G_p \) estimated with the IV method and the PA method has not previously been conducted in mice. In accordance with findings in sheep \((18)\), we found a correlation between the methods. We extended our study by evaluating the significance of the methods on the final volume estimation compared with MRI and confirmed that the PA method was superior to the IV method. The IV method appears less robust than the PA method, because we observed that the procedure for injection of the hypertonic saline bolus influenced the estimate of \( G_p \). This was not caused by a simple volume loading because no influence on the conductance signal was observed when a liquid with a conductivity matching that in blood was injected. Because of the instability of \( G_p \) during the wash-in of the bolus in the LV, the criteria for calculating \( G_p \) are not met.

**Closed- vs. open-chest procedure.** Opening of the thorax has been shown to increase SV by \(~6–7.9 \text{ (EDV) and } 4.6–13.4 \text{ (ESV) } \mu l \text{ in mice} \) \((11)\). Consistent with this, we found an increase in SV when the thorax was opened. Consequently, the underestimation of SV measured with CC may be even larger than estimated here. In contrast, the apparent underestimation of absolute volumes by the CC \((\text{Fig. 5})\) may be well explained by the opening of the thorax.

**MRI volume estimation.** Evaluation of the agreement between CC and MRI volumes revealed a significantly higher offset between EDVs compared with ESVs. MRI may overestimate diastolic volumes because of an inflow effect during diastole that may increase the signal intensity from blood. Consequently, the visual border between blood and myocardium may be moved outward because of partial volume effect. We found support for this in our data. The diastolic estimate of LV myocardial volume demonstrated a 10.8-mg underestimation compared with the systolic estimate. Consequently, \(~10 \mu l \text{ of volume may be misclassified as a part of the LV cavity in diastole. This circumstance explains the difference in offset at end diastole and end systole (20.1 and 8 } \mu l \text{, respectively) of 12.1 } \mu l \). Accordingly, the underestimation of SV apparently related to the CC method is well explained.

**Conductance-to-Volume Conversion**

**Cylinder calibration.** We found that cylinder calibration provides reliable estimates of in vivo LV volumes compared with MRI, which is in accordance with previous results \((22)\). Furthermore, we estimated the significance of using individually constructed volume calibration formulas compared with a formula based on a mean value and found a tendency toward improved volume estimation. In this study we used a standardized fluid support protocol. However, within a study with heterogeneous fluid supply or between studies, construction of individual volume calibration formulas may improve volume calibration significantly, because the type and amount of intravenous fluid used influences \( \sigma_h \). As an example we have found that \( \sigma_h \) was up to \(~50\% \) lower in a study in which hetastarch was used instead of isotonic saline for fluid support.

A new method for nonlinear conversion of conductance to volume that overcomes the nonuniform electric field produced by the CC has been proposed \((20)\). Since we found a linear relation within the working range of the heart, we did not apply this method, which may yield only minor improvements of volume estimates in mice. However, in larger animals, such as rats, the new method seems promising.

In this study we demonstrated a significant drifting of \( \sigma_h \) during the in vivo protocol. We proposed a method to compensate for the drifting and demonstrated an improved preci-
sion of the estimation of EDV. It should be emphasized that the method is dependent on changes of in vivo SV. A decrease in in vivo SV could mask a coincident increase of σp. However, there is no a priori reason that the SV at the end of the protocol, which is the reference, is not representative. A potential approach to overcome these difficulties is to use a flow probe throughout the entire protocol.

Flow probe calibration. Our data suggest that the accuracy and precision of volume estimates are not improved by in vivo flow probe calibration compared with cylinder calibration.

Limitations

Day-to-day variation of in vivo LV volumes may have led to reduction of the estimated precision of the CC method. However, evaluation of the calibration methods against each other by comparing correlations of CC volumes to MRI volumes is still justified.

MRI is a well-established method for noninvasive assessment of LV volumes in mice, and inter- and intraobserver variability are estimated to be ~5% (14, 15). However, a systematic bias of MRI volumes may have been introduced by the tracing of LV volumes, even though ventricular cavities are easily outlined. Newer MRI methods for LV volume quantification without tracing and geometric approximations potentially could improve accuracy and reproducibility (3).

Conclusions

The calibration procedure has significant implications for the precision and accuracy of the volume estimates. We recommend that accurate descriptions of the calibration procedures and calculations of volumes be included in reports with CC-derived results. In this study we demonstrate a high precision of the CC method and provide evidence that an offset to CC volumes may be present. To clarify the existence of an offset, additional studies with simultaneous and accurate measurements of absolute volumes in vivo are required. However, by optimization of calibration procedures adjusted to the specific purpose of the individual study, the CC method yields reliable estimates of relative changes in LV volumes in mice and volume-derived indexes like contractility.

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REFERENCES