Impact of physiological variables and genetic background on myocardial frequency-resistivity relations in the intact beating murine heart

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Reyes, Maricela, Mark E. Steinhelper, Jorge A. Alvarez, Daniel Escobedo, John Pearce, Jonathan W. Valvano, Brad H. Pollock, Chia-Ling Wei, Anil Kottam, David Altman, Steven Bailey, Sharon Thomsen, Shuko Lee, James T. Colston, Jung Hwan Oh, Gregory L. Freeman, and Marc D. Feldman. Impact of physiological variables and genetic background on myocardial frequency-resistivity relations in the intact beating murine heart. Am J Physiol Heart Circ Physiol 291: H1659–H1669, 2006. First published May 12, 2006; doi:10.1152/ajpheart.00609.2005—Conductance measurements for generation of an instantaneous left ventricular (LV) volume signal in the mouse are limited, because the volume signal is a combination of blood and LV muscle, and only the blood signal is desired. We have developed a conductance system that operates at two simultaneous frequencies to identify and remove the myocardial contribution to the instantaneous volume signal. This system is based on the observation that myocardial resistivity varies with frequency, whereas blood resistivity does not. For calculation of LV blood volume with the dual-frequency conductance system in mice, in vivo murine myocardial resistivity was measured and combined with an analytic approach. The goals of the present study were to identify and minimize the sources of error in the measurement of myocardial resistivity to enhance the accuracy of the dual-frequency conductance system. We extended these findings to a gene-altered mouse model to determine the impact of measured myocardial resistivity on the calculation of LV pressure-volume relations. We examined the impact of temperature, timing of the measurement during the cardiac cycle, breeding strain, anisotropy, and intrameasurement and interanimal variability on the measurement of intact murine myocardial resistivity. Applying this knowledge to diabetic and nondiabetic 11- and 20- to 24-wk-old mice, we demonstrated differences in myocardial resistivity at low frequencies, enhancement of LV systolic function at 11 wk and LV dilation at 20–24 wk, and histological and electron-microscopic studies demonstrating greater glycogen deposition in the diabetic mice. This study demonstrated the accurate technique of measuring myocardial resistivity and its impact on the determination of LV pressure-volume relations in gene-altered mice.

gene-altered mouse; diabetes; left ventricular volume; dual-frequency conductance method; myocardial resistivity

FOR RESEARCHERS TO UNDERSTAND the effects of genetic perturbations on integrative cardiac physiology in the mouse, the use of in vivo preparations is necessary. Evaluation of left ventricular (LV) pressure-volume (PV) relations has provided a framework for understanding cardiac mechanics in larger experimental animals and humans (23). Extension of this technique to the mouse model would be useful. Unfortunately, determination of instantaneous volume in the murine LV has been problematic because of small heart size (160 mg) and rapid heart rate (500–700 beats/min). Although ultrasonic crystals (7), MRI (10, 11), and echocardiography (8) have been used to measure instantaneous LV volume in the mouse with some degree of success, these technologies have limitations, particularly during dynamic maneuvers, such as transient inferior vena cava (IVC) or aortic occlusion, which are required to generate load-independent indexes of contractility.

Conductance technology offers a more robust alternative for generation of instantaneous LV PV relations in the intact murine heart. Single-frequency conductance has been used in mice to generate measures of ventricular function (8, 13, 37). However, this traditional method is limited, because the instantaneous intraventricular LV conductance signal is derived not only from the blood but also from the LV myocardium, such that it is an overestimate of the true LV blood volume (3). Only the blood signal is desired. Investigators have applied the hypertonic saline technique developed for larger mammals to the mouse to determine a single value of steady-state parallel (muscle) conductance to derive absolute LV volume. The saline technique, however, is problematic in such small animals, because repeated administration of hypertonic saline alters blood resistivity and hemodynamics and, thus, violates the assumptions behind the conversion of conductance to volume (3).

Moreover, the traditional hypertonic saline technique and a more recent solution, i.e., dual frequency, as proposed by other investigators (12, 14, 36), determine a single value of steady-state parallel conductance. These methods assume that parallel conductance does not change throughout the cardiac cycle (18). A more recent study examining not only the magnitude of the conductance signal but also its phase angle, however, has demonstrated that parallel conductance does change between end diastole and end systole during steady-state conditions (20). Thus murine conductance (single frequency) technology presently available to the scientific community cannot calculate the instantaneous change in parallel conductance as the LV shrinks around the intracardiac electric field during occlusion of the IVC. During generation of absolute measures of ven-
tricular function via LV PV relations, such as end-systolic elastance, calculation of instantaneous parallel conductance is required.

Our laboratory recently developed a dual-frequency conductance system to address these problems in the mouse (9). The underlying concept for the development of our dual-frequency conductance system was that myocardial resistivity varies with frequency, whereas blood resistivity does not (9, 24, 25, 27–30). The greatest source of error in the use of this instrument is variation of myocardial resistivity in gene-altered mice, which is anticipated to differ from that in their wild-type littermates. For instance, when LV PV relations were generated for diabetic [BKS Cg-m^{−/−} Lep{db}^db] mice with our dual-frequency system and their own myocardial resistivity was used in the algorithms, physiological volumes were obtained (Fig. 1). When the same dual-frequency signals obtained in the murine LV were processed with the myocardial resistivity of a wild-type mouse, unphysiological (negative) volumes were obtained. Of even greater importance, the calculated volumes had different ranges. The difference between maximal and minimal values was 42 μL when resistivity from wild-type mice was used and 52 μL when resistivity from diabetic mice was used. Thus, not only will absolute values of volume differ, but measures of LV performance, such as ejection fraction and end-systolic elastance, will vary. Clearly, the use of the appropriate resistivity values is critical for accurate assessment of LV function.

Despite the importance of accurate measurements of myocardial resistivity for proper use of our dual-frequency conductance system, the factors associated with accurate measurement of myocardial resistivity and myocardial resistivity in a gene-altered mouse have not been determined.

**METHODS**

**Tetrapolar resistivity catheter and associated instrumentation.** The technology for accurate measurement of in vivo murine myocardial resistivity was developed in our laboratory and was modeled after a technique developed by Steendijk et al. (29) for the canine heart. However, Steendijk et al. designed their electrode spacing to sample only the epicardium. In contrast, we sought wider electrode spacing relative to the myocardial thickness to gain a greater depth of penetration by the electric field to minimize the effect of anisotropy. A tetrapolar resistivity catheter (Fig. 2) was custom fabricated to our specifications by Millar Instruments (Houston, TX). The catheter contains four parallel platinum electrodes aligned with electrode spacing of 0.25, 0.4, and 0.25 mm between electrodes 1 and 2, 2 and 3, and 3 and 4, respectively. The electrodes are contained within a 1-mm-diameter plastic tip, which also contains two holes, which act as ports for the application of a weak vacuum to enhance the contact between the resistivity catheter and the epicardium of the intact beating murine heart.

A function generator board (Data Translation) was used to produce the desired excitation frequency, which was applied to the two outer electrodes. The frequencies used for our study, 1, 2, 5, 10, 20, 40, 60, 80, and 100 kHz, were fed into a signal processor and then converted...
to a current signal. The two inner electrodes measured the instantaneous voltage signal, which was fed back into our instrument and acquired using Charts Acquisition software (AD Instruments).

**Conversion of instantaneous voltage to conductance.** Because instantaneous voltage was acquired from the mouse myocardium, a calibration procedure was developed to convert voltage to conductance. With use of saline of known conductivity (800–2,100 µS/cm), at 37°C, a calibration curve of voltage vs. conductance at frequencies of 1–100 kHz was used to convert voltage to conductance. Resistivity, the inverse of conductivity, was plotted as a function of frequency.

**Common experimental protocol.** The Institutional Animal Care and Use Committee at the University of Texas Health Science at San Antonio approved all experiments. A total of 96 mice were studied. Background strains included 8–10 wk-old CD-1 (n = 33) and C57B1Ks/J (n = 9) mice. The gene-altered mice were 11-wk-old (n = 30) and 20–24 wk-old (n = 24) female BKS Cg-m+/+ Leprdb (db/db) mice, as well as their nondiabetic female littermates [C57B1KS (db/+ or +/+)]. Glucose levels were obtained using a blood glucose meter (Lifescan, Milpitas, CA) where indicated. The mice were anesthetized by administration of urethane (1,000 mg/kg ip) and etomidate (25 mg/kg ip) and mechanically ventilated with a rodent ventilator set at 150 breaths/min (100% O₂). The mice were placed on a heated, temperature-controlled operating table for small animals (Vestavia Scientific). The experiments were performed at a murine body temperature of 37°C, except where indicated. The chest was entered via an anterior thoracotomy. The tetrapolar resistivity catheter mounted on a micromanipulator was placed on the LV epicardium of the intact beating mouse heart. Voltage outputs were acquired at excitation frequencies of 1–100 kHz using Charts Acquisition software. The mice were randomized to frequency increases from 1 to 100 kHz or decreases from 100 to 1 kHz.

**Temperature studies.** To determine the effect of temperature on murine myocardial resistivity measured at various frequencies, a group of CD-1 mice (n = 7) underwent the surgical procedures outlined above without initial heating on the operating table. Murine body temperature was measured with a rectal probe and recorded. In addition, mice, open-chest surface myocardial temperature was measured (n = 4). Voltage measurements were obtained at the frequencies noted above. The mice were heated to a body temperature of 37°C, and a second set of voltage measurements was obtained. Because of the additional time required to cool a heated mouse and the associated reduced survival, the temperature alterations were not randomized.

**Mouse strain.** To determine whether variation in the background strain of mice had an impact on myocardial resistivity measurement, we compared frequency-resistivity relations in 8–10-wk-old CD-1 mice (n = 6) with those in C57B1Ks/J mice (n = 6).

**Variability throughout the cardiac cycle.** The LV mass at end diastole has been estimated to be 12.8% blood (16). During end systole, the percentage of blood in the LV myocardium is reduced. The resistivity of murine blood is lower than that of murine myocardium (9). Thus instantaneous myocardial resistivity is anticipated to vary between end systole and end diastole. The instantaneous end-diastolic, end-systolic, and mean myocardial resistivity was determined in CD-1 mice (n = 6) at 100 kHz.

**Anisotropy.** We sought to overcome the effect of fiber orientation on murine myocardial frequency-resistivity relations with our resistivity probe electrode spacing. A group of CD-1 mice (n = 6) underwent the surgical procedures outlined above, the resistivity catheter was stabilized longitudinal to the cardiac axis on the LV epicardium, and voltage was measured at the various frequencies. The resistivity device was removed from the epicardial surface, and frequency-voltage measurements were repeated after stabilization of the device transverse to the cardiac axis. The same protocol was repeated 45° and 135° from the longitudinal axis. The direction of application of the resistivity device on the epicardium was random. Four orientations were chosen to ensure placement both longitudinal and transverse to the fiber orientation, because the epicardial fiber orientation could be visualized, even with a ×10 microscope.

**Intrameasurement and interanimal variability.** To characterize the variability in measurement between mice and between observations in a given mouse, we performed repeated measurements in a set of gene-altered mice. Diabetic (BKS Cg-m+/+ Leprdb) mice (n = 4) underwent surgical procedures as outlined above. Myocardial resistivity was measured in each mouse from 1 to 100 kHz. The resistivity device was removed and replaced at the identical location on the epicardium, and the resistivity measurements were repeated. These steps were repeated four times.

**Isolated balloon-filled murine hearts.** Each heart (n = 3) was suspended from a cannula that perfused the beating heart with Tyrode solution at 37°C. Via the left atrium, a balloon constructed of clear polyethylene tied to a needle was inserted into the LV. A pressure gauge via a side port allowed determination of pressure in the balloon as air was added, which was 60 mmHg. For measurements of epicardial resistivity, the epicardial probe was placed on the beating anterior LV myocardium while the isolated heart was held in place by the LV balloon and aortic cannula.

Determination of LV PV relations in diabetic and nondiabetic mice. Additional studies were performed to assess whether the myocardial resistivity measurements could be successfully applied in the determination of LV contractility in diabetic and nondiabetic mice. Female mice, including diabetic and nondiabetic controls at 11 (n = 17) and 20–24 (n = 12) wk of age, were examined. Body weight was recorded. The mice were anesthetized and intubated and subjected to anterior thoracotomy as described above. The apex of the heart was stabbed with a 30-gauge needle, and the miniatureaturized conductance catheter (Millar Instruments) was advanced retrograde into the LV along the long axis, with the proximal electrode just within the myocardial wall of the apex. The IVC was isolated immediately below the diaphragm for transient occlusion.

Baseline pressure-conductance relations at 10 and 100 kHz were acquired and stored for off-line conversion to PV relations as previously described (9). Data were acquired during transient occlusion of the IVC. To derive a correction for gain (α) at the end of the experiment, a small animal blood flowmeter (model T106, Transonic Systems, Ithaca, NY) was placed around the aorta. The flowmeter was placed on the ascending thoracic aorta, and simultaneous LV conductance at 10 and 100 kHz and aortic flow were recorded.

**Histology, transmission electron microscopy, and collagen biochemistry.** To determine whether differences in the myocardial resistivity of diabetic and nondiabetic mice at 11 (n = 14) and 20–24 (n = 14) wk of age were due to alterations in the composition of the myocardium, hearts undergoing resistivity and LV PV measurements were processed for light and transmission electron microscopy. The beating LV was cannulated via the apex and infused with saline and then immediately infused with 10% formalin after a surgical incision was made in the liver as a route for blood to escape. The LV was isolated and cut along the short axis. The apex was cut into 1-mm³ pieces in glutaraldehyde, and the cubes were embedded in plastic for transmission electron microscopy. The base portion was processed for light microscopy, and the sections were stained with Alcian blue/polarized acid-Schiff (PAS) for polysaccharides, PAS with and without diastase for glycogen, Weigert van Gieson for elastin, Masson’s periodic acid-Schiff (PAS) for polysaccharides, PAS with and without diastase for glycogen, Weigert van Gieson for elastin, Masson’s trichrome for collagen, and hematoxylin and eosin for general morphology.

**Biochemistry for collagen.** The quantitative determination of recently synthesized collagen was performed using the Sircol soluble collagen assay (Biocolor, Newtownabbey, N. Ireland) according to the manufacturer’s protocol. Briefly, tissues from diabetic (28–37 mg, n = 7) and nondiabetic (23–29 mg, n = 7) hearts that had been stored at −80°C were thawed on ice and incubated in 0.5 M acetic acid/pepsin buffer with gentle agitation at room temperature overnight (10:1 buffer-to-tissue wet weight ratio). The samples were centrifuged at 15,000 g for 1 h, and the supernatants were collected for Sircol.
The time-varying difference between the maximum and the minimum power spectral density of the signal was determined. The goal of this endpoint was to compare end-systolic and end-diastolic myocardial voltage measurements, and Dunnett’s post hoc test was used for multiple comparisons for the difference at each pairwise frequency. SAS (version 8.2, Statistical Analysis Software) was used to examine individual frequency-group combinations. If the group-frequency interaction was statistically significant, the main effect for frequency tests whether the frequency-resistivity relation (the slope) is the same across the groups of interest. A general linear mixed model, an extension of ANOVA, was used to compare the resistivity due to frequency (in kHz) and age or strain group status, along with an age-by-frequency interaction term for these two main effects. Each variable, including group (age or diabetic vs. wild-type), was analyzed with its individual values. The main effect for frequency tests whether there is no overall association between frequency and resistivity, whereas the main effect for group (age or diabetic vs. wild-type) tests whether there are overall differences attributable to this factor. If the group-frequency interaction was statistically significant, then individual frequency-group combinations were examined. The t test was used to perform multiple comparisons for the difference at each pairwise frequency. SAS (version 8.2, Statistical Analysis Software) was used for all analyses.

To demonstrate that there was a significant difference between end-systolic and end-diastolic myocardial voltage measurements, a power spectral density of the signal was determined. The goal of this approach is to prove that the signal is a sinusoidal wave with a time-varying difference between the maximum and the minimum values. The alternative explanation is that noise is responsible for the signal. Each frequency component of the signal was examined with the corresponding energy contained at that frequency. The signal-to-noise ratio was determined by finding the maximum energy contained in the spectrum and dividing by the sum of the energy at all the other frequency components. This analysis was performed on 10 consecutive epochs of 1,024 points each of instantaneous myocardial voltage data, and these 10 epochs were then averaged.

The LV PV relations were analyzed with software developed by us and licensed to and modified by Millar Instruments (PVAN, Conductance Technologies). The algorithms used for dual frequency were developed by us and published previously (9). We utilized the mean myocardial resistivity for 11- and 20- to 24-wk-old diabetic and wild-type mice at 10 and 100 kHz in the dual-frequency algorithms. Absolute volume measurements from the conductance catheter were calibrated with a correction for α, which was defined as the ratio of flow probe stroke volume to conductance stroke volume. Each mouse was analyzed with its individual α. Measures of ventricular function between diabetic and wild-type mice at 11 and 20–24 wk of age were compared using ANOVA.

**RESULTS**

### Resistivity changes as a function of excitation frequency

The results of our measurement of myocardial resistivity at various excitation frequencies are presented as plots of resistivity vs. log frequency. In studies of excitation frequencies of 1, 2, 5, 10, 20, 40, 60, 80, and 100 kHz (Fig. 3; see Fig. 6), there is a steady decrease in myocardial resistivity as frequency is increased (P < 0.01, by repeated-measures ANOVA). However, the decrease in resistivity at 1–2 kHz and 1–5 kHz was not significant in any of the measurements (P > 0.05). Resistivity at 1 kHz was significantly higher than resistivities at ≥40 kHz.

### Resistivity changes as a function of temperature

Frequency-resistivity results at 29–32°C and 37°C are compared in Fig. 3. At any given input frequency, the resistivity is greater at lower temperatures. Repeated-measures ANOVA revealed statistical significance in resistivity at each frequency between the two temperatures (P < 0.01). As mentioned above, murine myo-

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**Fig. 3.** Effect of temperature on myocardial resistivity measured by a tetrapolar electrode resistivity device in intact murine (CD-1) hearts (n = 7). Measurements were obtained at 29–32°C, then mice were heated to 37°C and measurements were repeated. Lower temperature increases resistivity at any frequency; physiological temperature (37°C) lowers resistivity at any frequency. P < 0.01 (by repeated-measures ANOVA).

**Fig. 4.** Effect of strain and gene alteration on myocardial resistivity in CD-1, C57BlkJ, and diabetic BKS Cg-m (“Leprdb” db/db) mice. There is a significant difference among all three groups (P < 0.0001). *Significant difference between diabetic (db/db) and corresponding control (C57BlkJ) at low frequencies (1, 2, 5, 10, and 20 kHz), P < 0.05. **Significant difference between background strains (CD-1 and C57BlkJ) at higher frequencies (20, 40, 60, 80, and 100 kHz), P < 0.05.
cardial resistivity decreases as a function of frequency ($P < 0.01$).

Body temperature was an accurate reflection of surface myocardial temperature. When body temperature was 37.0 ± 0.7°C, myocardial temperature was 36.5 ± 0.8°C ($n = 4$, $P$ not significant (NS)). When body temperature was 31.4 ± 0.8°C, myocardial temperature was 30.8 ± 0.9°C ($n = 4$, $P = \text{NS}$).

Resistivity changes as a function of background strain. The myocardial resistivity relations between two mouse background strains, CD-1 and C57BlkS/J, at 8–10 wk of age are compared in Fig. 4. There was a significant difference between strains at the higher frequencies ($P < 0.001$, by repeated-measures ANOVA). The difference in resistivities between individual frequencies was also significant but of lesser magnitude at 2, 5, 20, 40, 60, 80, and 100 kHz ($P < 0.01$).

Resistivity changes as a function of cardiac cycle. Representative raw data from an intact beating mouse heart at 100 kHz with simultaneously acquired LV pressure are shown Fig. 5, top, to illustrate the time points of end diastole and end systole during a voltage measurement by the tetrapolar resistivity catheter. The calibration procedure outlined above was used to convert maximum and minimum voltage, corresponding to end systole and end diastole, respectively, to corresponding resistivity values. A signal-to-noise ratio of 10 dB was determined, implying that the signal-to-noise ratio was 8:1. This is consistent with the myocardial voltage signal being a sinusoidal wave with a significant time-varying difference between the end-systolic and the end-diastolic values.

To demonstrate that no portion of the instantaneous myocardial voltage signal was derived from the underlying LV cavity blood, we repeated these studies using the murine probe on a thicker (3-mo-old Wistar-Kyoto rat) intact beating heart (Fig. 5, middle) and an isolated beating balloon-filled murine heart (Fig. 5, bottom). Similar results were found in these studies.

Table 1. Characteristics of mice and hearts used for myocardial resistivity and ventricular function studies

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetic (db/db)</th>
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<tbody>
<tr>
<td></td>
<td>11 wk 20–24 wk</td>
<td>11 wk 20–24 wk</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>22 ± 1</td>
<td>48 ± 3*</td>
</tr>
<tr>
<td>Heart wt, mg</td>
<td>113 ± 4</td>
<td>116 ± 12</td>
</tr>
<tr>
<td>LV wt, mg</td>
<td>64 ± 6</td>
<td>80 ± 4*</td>
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<tr>
<td>LV wt/body wt</td>
<td>2.9 ± 0.2</td>
<td>3.0 ± 0.4</td>
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<tr>
<td>Blood glucose, mg/ml</td>
<td>82 ± 19</td>
<td>246 ± 36*</td>
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</tbody>
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Values are means ± SE. LV, left ventricle. *$P < 0.05$ vs. 11-wk control; †$P < 0.05$ vs. 20- to 24-wk control (Student’s $t$-test).
representative LV PV relations in 11-wk-old (A and B) and 20- to 24-wk-old (C and D) diabetic (A and C) and nondiabetic (B and D) mice. $E_a$, effective arterial elastance; EF, ejection fraction; $\frac{dP}{dt}_{\text{max}}$ and $\frac{dP}{dt}_{\text{min}}$, maximum rate of LV pressure increase and decrease, respectively; $V_o$, volume intercept; $E_{\text{max}}$, end-systolic elastance; EDV, end-diastolic volume; PRSW, preload recruitable stroke work; $\tau$, time constant; PED, end-diastolic pressure.

Values are means ± SE. $E_a$, effective arterial elastance; EF, ejection fraction; $\frac{dP}{dt}_{\text{max}}$ and $\frac{dP}{dt}_{\text{min}}$, maximum rate of LV pressure increase and decrease, respectively; $V_o$, volume intercept; $E_{\text{max}}$, end-systolic elastance; EDV, end-diastolic volume; PRSW, preload recruitable stroke work; $\tau$, time constant; PED, end-diastolic pressure. Significant difference (by ANOVA): *P < 0.05 vs. 11-wk control; †P < 0.05 vs. 11-wk diabetic; ‡P < 0.05 vs. 20- to 24-wk control.

Fig. 8. Representative LV PV relations in 11-wk-old (A and B) and 20- to 24-wk-old (C and D) diabetic (A and C) and nondiabetic (B and D) mice. $E_{\text{max}}$, curvilinear end-systolic elastance; $V_o$, volume intercept; $a$, extent of concavity of the slope. LV systolic pressure is higher and contractility is enhanced in 11-wk-old diabetic mice. LV of 20- to 24-wk-old diabetic mice were dilated compared with other groups.
heart (Fig. 5, bottom) and obtained identical timing and waveform patterns.

Anisotropy. No differences in the myocardial frequency-resistivity relations were observed between the four angles at which these measurements were obtained in the intact murine (CD-1) heart (n = 6; Fig. 6). Repeated-measures ANOVA revealed no significant difference in resistivity between the four angles at any of the excitation frequencies (P = 0.20).

Intrameasurement and interanimal variability. Intrameasurement and interanimal variabilities (between mice and in a given mouse) in resistivity measurements between 1 and 100 kHz are shown in Fig. 7. Interanimal and intrameasurement variabilities were small in magnitude and similar (n = 4 diabetic mice).

Comparison of diabetic mice and hearts used for myocardial resistivity studies. Table 1 summarizes data from the mice in each group. Body mass at 11 and 20–24 wk of age was greater in the diabetic mice than in the wild-type controls. LV mass increased with age in control and diabetic mice. LV weight at 11 wk of age was greater in diabetic mice than in wild-type controls, but because of their elevated body weight, the LV weight-to-body weight ratios were lower at each age in the diabetic mice.

Myocardial resistivity studies in diabetic and nondiabetic mice. Differences in myocardial resistivity between diabetic and nondiabetic mice were limited to lower frequencies. Myocardial frequency-resistivity relations are shown in Fig. 4 for the control and diabetic mice at 11 wk of age. The overall relation was different between the two sets of animals (P < 0.0001), with significant differences in myocardial resistivity at 1, 2, 5, and 10 kHz (P < 0.01, P < 0.01, P < 0.05, and P < 0.05, respectively). A similar comparison was observed at 20–24 wk of age, where the overall relation was also different between diabetic and nondiabetic controls (P = 0.013), with significant differences in myocardial resistivity at 1 and 5 kHz (P < 0.01 and P < 0.05, respectively).

Impact of dispersion of myocardial resistivity and disease-appropriate myocardial resistivity on calculation of LV volume. The end-diastolic volume (EDV) varied between 36 ± 13 and 33 ± 14 μl (P = NS) and the end-systolic volume (ESV) between 22 ± 9 and 20 ± 9 μl (P = NS) when the dispersion (measured by the standard deviation) of diabetic myocardial resistivity was used to calculate LV volumes of the 11-wk-old diabetic mice. However, when the mean control murine myocardial resistivity was used to calculate the LV volume of these same diabetic mice, unphysiological volumes were obtained [−15 ± 9 μl (EDV) and −24 ± 10 μl (ESV), both P < 0.001 compared with diabetic murine resistivity; see example in Fig. 1].

LV PV relations in diabetic and nondiabetic mice. Myocardial resistivity measurements were used to determine measures of LV contractility in diabetic and nondiabetic mice. Although there is a well-known reduction in systolic function late in the course of diabetes, the impact of elevated glucose and free fatty acid levels, potential substrates for myocardial metabolism, on contractile performance early in diabetes is not known. We quantified the LV PV relations at 11 and 20–24 wk of age in control and diabetic mice. Results are summarized in Table 2. Although LV systolic pressures were higher in diabetic mice at 11 wk of age, the LV volumes were very similar in the diabetic and nondiabetic animals. Multiple measures of LV contractile function were enhanced in the diabetic mice at 11 wk of age compared with the control group. By 20–24 wk of age, the LV of the diabetic mice were dilated, and one measure of ventricular function (power/EDV^2) demonstrated reduced systolic function. Representative examples are shown in Fig. 8.

Histology and biochemistry. Intracellular glycogen deposition was greater in 11- and 20- to 24-wk-old diabetic mice than
in controls, as identified by positive PAS staining for intracellular glycogen (Fig. 9). This difference was quantified with a hue-saturation-intensity color analysis of the slides shown in Fig. 9, top, as well as in additional hearts to measure the visible color differences. We analyzed six slides of cardiac tissue from 11-wk-old mice: three from diabetic animals and three from nondiabetic animals. Hue analysis yielded $38 \pm 45$ and $107 \pm 95$ for nondiabetic and diabetic hearts, respectively ($P = 0.016$); saturation analysis yielded $9 \pm 9$ and $18 \pm 12$ for nondiabetic and diabetic hearts, respectively ($P = 0.067$); and intensity analysis yielded $246 \pm 4$ and $213 \pm 17$ for nondiabetic and diabetic hearts, respectively ($P = 0.002$).

Glycogen deposition was confirmed by removal of the Alcian blue/PAS-positive material with diastase (Fig. 9). Transmission electron microscopy also confirmed enhanced intracellular glycogen deposition between myofilaments in 11- and 20- to 24-wk-old diabetic mice (Fig. 10). Other histological studies showed no differences between diabetic and nondiabetic myocardium, including the extracellular stroma. The collagen content was also similar between diabetic and control hearts: $5.40 \pm 3.29$ and $4.92 \pm 3.12 \mu g/mg$, respectively ($P = \text{NS}$).

DISCUSSION

The present study focused on myocardial resistivity, because review of the algorithms used to generate an instantaneous volume signal with dual-frequency conductance (9) reveals that it is one of the largest sources of error in the use of the instrument. Thus the first goal of this study was to determine and minimize the impact of potential sources of error in the measurement of murine myocardial resistivity in the intact open-chest mouse. A resistivity catheter with an electric field depth of penetration sufficient to overcome anisotropy of the myocardial fibers was developed. Although temperature has an impact on the measurement of myocardial resistivity, the open-chest murine myocardium is able to maintain body temperature, ensuring accurate measurement in the open-chest preparation. Myocardial frequency-resistivity relations differ between control and genetically altered mice, as well as mice of different strains, establishing that, for the conductance method of volume calculation to be accurate, resistivity must be defined for each group of animals to be studied. Because of low intrameasurement and interanimal variability, four animals are needed to define the myocardial resistivity for a particular gene alteration and time point. Finally, when the measured frequency-resistivity relations were used in conjunction with measurement of instantaneous parallel conductance by the dual-frequency technique, 1) EDV and ESV did not change when calculated by the range (standard deviation) of myocardial resistivity, and 2) LV PV relations demonstrated enhanced LV contractility early in Type 2 diabetes (11 wk) and LV dilation by 20–24 wk of age. Histology and electron microscopy demonstrated increased intracellular glycogen deposition in the diabetic mice at both ages.

The present study demonstrates that in mice, as in other species, as the frequency of the input signal is increased,

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Fig. 10. Transmission electron micrographs of myocardium from 20- to 24-wk-old diabetic mice. Glycogen deposition (black arrows) was greater in diabetic (bottom) than in nondiabetic (top) mice. Findings were similar in 11-wk-old mice (not shown).
myocardial resistivity falls. Previous studies in other species (24, 28, 30) have shown a similar relation between resistivity and frequency that is unique to cardiac tissue. The magnitude of the impedance of the sarcolemma to low-frequency electric current is sufficiently high that current flows through the extracellular space. As the frequency is increased, the magnitude of the impedance to the flow of current through the sarcolemma decreases, and more current will flow through the sarcolemma but never enters the myocyte. This translates to a reduction in myocardial resistivity (Fig. 11). One example in the present study is our finding of differences in resistivity between strains of mice only at higher frequencies, which indicates differences in the electrical properties of the sarcolemma itself but not in the extracellular space.

Our ability to demonstrate differences in myocardial resistivity measurements between end systole and end diastole is consistent with studies in the canine heart. Steendijk and coworkers (30) demonstrated in the dog that myocardial resistivity was higher during end ejection and lower during late diastole. Our findings were the same in terms of resistivity measurement throughout the cardiac cycle in the mouse. Because blood is known to have lower resistivity than myocardium, these findings may reflect the impact of the greater blood content of the myocardium during late diastole, lowering myocardial resistivity. Additionally, we cannot rule out the contribution of other factors, such as alterations in the location of stored calcium and the configuration of the myofilaments, to myocardial resistivity varying with timing in the cardiac cycle. Clearly, consistency is important in measurement of myocardial resistivity, and, on the basis of our analysis, use of mean, rather than end-systolic or end-diastolic, voltage is recommended.

The similarity of the interanimal and intrameasurement variability implies that the simple act of placing the resistivity catheter on the myocardium, rather than making the measurement in different mice, is the major source of error in our measurement. Furthermore, the absolute sample-to-sample and mouse-to-mouse variability is low, indicating that results from a small sample of mice can be used with confidence in calibrating the conductance system. For each strain or gene-altered mouse to be used, the mean of four measurements of myocardial resistivity from four representative intact hearts should provide sufficiently detailed determination of resistivity for use in the dual-frequency algorithms (9). It is not necessary to measure myocardial resistivity on each mouse to obtain LV PV relations.

A practical strategy for use of the dual-frequency conductance system would be for investigators to set aside four mice at every time point to be examined by LV PV relations. For instance, a common experiment consists of measurement of ventricular function at baseline followed by aortic banding to produce concentric hypertrophy. The present study suggests that the resistivity would change over time, and the myocardial resistivity could be determined in just four representative mice and applied to other mice at the same time point. Such a strategy would also apply to mouse models of progressive disease, such as fibrosis, inflammatory infiltrates, and wall thinning.

Our results on the impact of tissue anisotropy on murine myocardium differed from those reported in larger animals. Using a nontraumatic epicardial electrode system, Steendijk et al. (29, 30) showed that longitudinal and transverse myocardial resistivity differ in the intact canine heart. Their electrode spacing resulted in relatively superficial penetration of the myocardium by the electric field. Streeter et al. (31, 32) showed that the orientation of myocardial fibers changes in a radial fashion by as much as 160° from epicardium to endocardium and that, in a beating heart, each muscle fiber changes direction and length uniquely as the left ventricular wall changes shape throughout the cardiac cycle. Because the myocardial penetration was limited in the studies of Steendijk et al., the impact of local fiber orientation would be maximized. In our study, on the other hand, electrode spacing was chosen to deliver current to a depth of 0.6 mm, which is approximately three-fourths of the murine end-diastolic thickness as determined by MRI (10, 11). Thus, in our study, the current would have passed through fibers arrayed over 70–80°, such that the relation of electrode placement to fiber orientation would be minimized. Similar results could be found in larger hearts, if the depth of penetration of the current was much deeper. Our results demonstrate that increasing the depth of electric field penetration limits the impact of anisotropy on tissue resistance in the murine heart, such that this factor can be discounted in the calibration process.

Comparison of the diabetic mice and their nondiabetic littermates at 11 and 20–24 wk of age showed differences at low frequencies but similar resistivity at higher frequencies. This suggests that differences in the extracellular space between these groups are responsible for the myocardial resistivity differences we found. Histological stains for extracellular matrix (Weigert van Gieson for elastin and Masson’s trichrome for collagen) revealed no differences between the diabetic and wild-type mice. However, extracellular glucose concentrations were higher in the diabetic mice than in their nondiabetic littermates (Table 1). Glucose is an insulator, lowering conductivity (or raising resistivity) as was found. At higher fre-
quencies, the current travels in part through the sarcolemma but never enters the myocyte. Thus, although histological studies (Alican blue/PAS-positive material removed with diastase) and electron microscopy demonstrated enhanced intracellular glycogen deposition surrounding the myofilaments, it did not impact the myocardial resistivity measurements at high frequency.

Our unexpected finding of enhanced LV function early in diabetes in the 11-wk-old mice, with LV dilatation and slight depression of one of multiple measures of ventricular function by 20–24 wk of age, is inconsistent with the accepted idea that diabetic mice develop a progressive cardiomyopathy. A previous histological study demonstrated structural myocardial damage, but it was performed in animals older than the 11- and 20- to 24-wk-old mice we examined (15). Studies showing impaired LV performance in the diabetic mouse were performed in ex vivo preparations in which insulin, free fatty acids, and glucose were added to the perfusion medium at concentrations considerably lower than those known to be present in vivo (4, 21). When these substrates have been used in more physiological concentrations in the isolated heart preparation, enhanced LV systolic performance was seen (21).

In addition, we showed improved performance with the PV analytic approach, which controls for the impact of potential differences in loading conditions. Echocardiographic assessment of load-dependent parameters such as ejection fraction may not provide valid comparisons between groups of animals when ventricular pressures are not known (26).

Glucose and free fatty acids serve as a source of ATP production in the heart. Both are ultimately converted to acetyl-CoA, which enters the citric acid cycle and generates reducing equivalents for oxidative phosphorylation. The supply of free fatty acids is increased in Type 2 diabetes, including our mouse model (33, 38). Although there is insulin resistance in peripheral muscles, there is not insulin resistance of cardiac muscle in Type 2 diabetes (34). Glucose was also increased in the mice we examined (Table 1), and glycogen was increased in the myocytes (Figs. 9 and 10). Hence, it can be hypothesized that there is an increase in the substrate supply available to the heart for use early in diabetes that may lead to enhancement of contractility before the onset of structural changes, which occurred as early as 20–24 wk with LV dilatation. Our findings support this hypothesis and may explain in part the increased glomerular filtration rate of the kidneys early in diabetes before the onset of structural damage (19) due to increased cardiac perfusion of the renal bed.

In summary, our methodology for accurate in vivo measurement of murine myocardial resistivity at various frequencies will simplify implementation of dual-frequency conductance determination of LV PV relations. To show the importance of this instrumentation, for the first time, we have demonstrated enhanced contractility in this diabetic mouse model, despite its description in the literature for 24 years (1, 4–6, 15, 17).

GRANTS

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