Myocardial contractility by strain echocardiography: comparison with physiological measurements in an in vitro model

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Myocardial contractility by strain echocardiography: comparison with physiological measurements in an in vitro model. Am J Physiol Heart Circ Physiol 285: H2599–H2604, 2003. First published August 7, 2003; 10.1152/ajpheart.00994.2002.—Strain echocardiography (SE) provides the rate and extent of myocardial segment shortening and lengthening. Thus we hypothesized that SE will noninvasively provide estimates of shortening velocity (SV) and length change (ΔL). We compared SE-derived strain rate (SR) and strain (ε) to force/length transducer-derived SV and percent ΔL in isolated muscle strips at multiple load levels and under varying conditions. Electrically stimulated contractions in left ventricular muscle strips (n = 20) were simultaneously recorded with a force/length transducer (to measure SV and percent ΔL) and an ultrasound transducer (to measure SR and ε). Measurements were made at baseline, after inotropic stimulation, and during hypoxia at multiple load clamp levels (simulating multiple levels of afterload). Despite a difference in absolute numbers, there was a close correlation between SR and SV at baseline (R² = 0.95), with dobutamine treatment (R² = 0.99), and during hypoxia (R² = 0.99). SR was load dependent at baseline (r = 0.98), with dobutamine treatment (r = 0.99), and during hypoxia (r = 0.92). Similarly, there was a close correlation between ε and ΔL at baseline (R² = 0.99), with dobutamine treatment (R² = 0.96), and during hypoxia (R² = 0.87). Percent ε was load dependent at baseline (r = 0.98), with dobutamine treatment (r = 0.98), and during hypoxia (r = 0.94). Bland-Altman analysis revealed a systematic overestimation of SV by SE-derived SR at baseline and with dobutamine treatment. There was no bias with SR measurements during hypoxia or with ε measurements. SE closely tracks standard physiological parameters of regional contractile function, such as SV and ΔL, under conditions of varying afterload.

myocardial contraction; strain rate; echocardiography

CURRENT INVASIVE AND NONINVASIVE ASSESSMENT of myocardial contractile function involves the characterization of left ventricular pressure and volume changes. However, it is the cumulative effect of myocardial segment shortening and the development of force that results in a rise in chamber pressure, an expulsion of ventricular contents, and a reduction in ventricular volume. Therefore, in muscle physiology experiments, shortening velocity (SV), percent length change (ΔL), and peak developed tension are the standard parameters of contractile function and are given by the force, length, velocity, and time relations, usually assessed in isolated muscle strips. Pressure and volume changes are related to, but not exactly the same as, these classic parameters (10). Furthermore, evaluation of the pressure-volume relationship only assesses changes in global function.

Ejection fraction (EF) and wall motion analysis are the predominant forms of current clinical echocardiographic assessment of global and regional contractile function. However, EF and wall motion analysis are predominantly visual, subjective, and widely variable (7). Quantitative echocardiographic assessment of cardiac function would greatly enhance the quality, reproducibility, and potentially the clinical value of the echocardiographic examination. Novel echocardiographic techniques such as tissue Doppler imaging (TDI) and TDI-derived strain echocardiography (SE) measure myocardial displacement and deformation, respectively, and have been shown to reliably quantify systolic and diastolic function (6, 12, 14). Because SE is less susceptible to translation and tethering artifacts, it is more accurate than TDI in assessing regional myocardial dysfunction (1). Shortening and lengthening rates measured by SE have been validated in gel phantoms and in an animal model using sonomicrometric crystals (3, 13). More recently, systolic strain rates were found to more closely correlate with invasive measures of global contractility than systolic tissue velocity (5). However, a major advantage of SE lies in its ability to depict regional myocardial function by calculating the rate and extent of myocardial deformation. SE provides the rate and extent of myocardial shortening and lengthening. Thus we hypothesized that SE would noninvasively provide estimates of physiological parameters of contractile function, such as SV and percent ΔL. Although strain rates and strain have been validated through comparison with global hemodynamic parameters (elastance) and regional deformation (strain) derived from sonomicrometric crys...
tals, there are no data on how SE-derived parameters compare to standard physiological parameters of contractile function.

We therefore compared the SE-derived strain rate (SR) and strain (ε) with force/length transducer-derived SV and percent ΔL in isolated muscle strips at multiple load levels and under varying conditions.

MATERIALS AND METHODS

This protocol was approved by the Institutional Animal Care and Use Committee.

Tissue Harvest

Male Sprague-Dawley rats (n = 20) weighing an average of 300 g were euthanized using pentobarbital (100 mg/kg) intraperitoneally. Hearts were rapidly excised, placed in a petri dish containing modified Krebs-Ringer bicarbonate solution [containing (in mM) 120 NaCl, 5 KCl, 5.5 dextrose, 25 NaHCO3, 1.2 MgCl2, 1 CaCl2, and 1.2 NaH2PO4 at pH 7.4], and continuously bubbled with 95% O2-5% CO2 at room temperature.

A custom Plexiglas tissue chamber was fabricated to facilitate artifact-free simultaneous force-length and ultrasound measurements (Fig. 1).

Force/Length Measurements

A slender muscle strip (mean length 13 ± 2 mm, width 1.5 ± 0.6 mm, and thickness 1.3 ± 0.4 mm) was dissected from the left ventricle and suspended vertically between a force/length transducer (Gould Instruments; Cleveland, OH) and a stationary plastic rod at the bottom of the tissue chamber. We attempted to selectively dissect out the endocardial layer with a preponderance of longitudinal muscle fibers. The initial dissection was performed at room temperature, and the muscle strip was promptly transferred to the tissue chamber. The chamber contained modified Krebs-Ringer bicarbonate solution (containing (in mM) 120 NaCl, 5 KCl, 5.5 dextrose, 25 NaHCO3, 1.2 MgCl2, 1 CaCl2, and 1.2 NaH2PO4 at pH 7.4) and was continuously bubbled with 95% O2-5% CO2 and maintained at ~36°C. The muscle was supramaximally stimulated (Grass Instruments) using 5-ms duration pulses delivered via platinum plate electrodes. The muscle was stretched to the length at which maximal force developed [optimal length (L0) measured using a Vernier caliper] and then allowed to equilibrate for 30 min while being stimulated at 0.5 Hz. Maximum isometric twitch force and ΔL were recorded digitally at sampling rate of 500 Hz (LabView 5.0 software, National Instruments; Austin, TX).

Load clamps. A load clamp applies load to the muscle after the initiation of contraction (afterload). By measuring SE and physiological parameters at various load clamp levels, we attempted to simulate varying levels of afterload. Load clamp levels were determined by the peak isometric force at L0 and applied at the following percentages of peak force: 0, 2, 4, 6, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, and 90%. Only data points of acceptable quality obtained at baseline, with dobutamine treatment, and during hypoxia were included in the analyses.

Ultrasound Measurements

Each muscle contraction was simultaneously imaged using a 10-MHz ultrasound transducer coupled with a Vivid FiVe GE Vingmed ultrasound machine (GE Medical Systems; Milwaukee, WI). The transducer was adjusted such that the entire length of the suspended muscle strip was visualized in

Fig. 1. Left: schematic representation of the experimental setup showing the ventricular muscle strip suspended between a force/length transducer and a stationary plastic rod in the custom Plexiglas tissue chamber containing oxygenated Krebs-Ringer solution. The muscle is stimulated using platinum electrodes, and the contraction is recorded simultaneously by the transducer and a 10-MHz ultrasound transducer. E+, positively charged electrode; E−, negatively charged electrode. A: representative graphic recording of the length change recorded by the force/length transducer. ΔL, change in length (the percent length change is ΔL normalized to initial length); ΔL/ΔT, shortening velocity (SV). *Stimulus time point. B: representative strain rate (SR) signal tracing. C: representative strain tracing (integral of SR). Arrow, peak systolic SR or strain.
Hypoxia. We induced hypoxia in the tissue bath by substituting oxygenated with nitrogenated Krebs solution. The chamber was bubbled with 95% N2 and 5% CO2 and contamination with room air minimized with paraflm applied over the chamber aperture. Force-length measurements were obtained before and after hypoxia. We measured solution PO2 and pH before and after hypoxia and defined hypoxia as PO2 < 20 mmHg.

Analysis

We analyzed force/length transducer traces (Fig. 1A) using a custom program (LabView 5.0 software, National Instruments). δL was measured in volts and converted to distance (1 V = 0.5 cm). We normalized the absolute length change to L0 to yield the percent δL. We calculated SV by dividing δL by the change in time and normalized SV to L0 (normalized SV expressed in s−1).

We analyzed SR images offline using a custom analysis package (Echopac version 6.3b). We arbitrarily selected a strain distance of 10 mm for analysis. This strain distance covered a majority of the muscle strip length and potentially minimized the influence of variations in SRs within the length of the muscle strip. Echocardiographic SR and ε analysis have been previously described (1, 6, 8, 11). By convention, tissue shortening is represented by the negative SR trace (Fig. 1B), which is integrated to yield percent ε (Fig. 1C). We averaged three raw measurements at each load clamp level to obtain the final value.

Statistics

Pearson’s correlation coefficient was used to test the relationship between load clamp level and SR/ε or SV/percent δL. Bland-Altman analysis was used to test the agreement between SE variables and measurements obtained from the force transducer (4).

RESULTS

A total of 20 muscle strips from an equal number of animals was examined. The initial six experiments were used to finalize the study design, and data from 14 experiments are presented. There was a correlation between SR and SV, and ε and δL, respectively, at all load clamp levels at baseline (n = 14), with dobutamine treatment (n = 10), and during hypoxia (n = 10). For hypoxia experiments, mean prehypoxia and hypoxia PO2 were 220 ± 38 and 18 ± 6 mmHg, respectively.

Strain Rate and Shortening Velocity

Although there was a difference in absolute values, SR and SV progressively decreased with increasing load clamp level at baseline (Fig. 2A). SR appropriately tracked the SV increase with dobutamine treatment (Fig. 2B) and decrease with hypoxia (Fig. 2C) at all load levels. SR was load dependent at baseline (r = 0.98), with dobutamine treatment (r = 0.99), and during hypoxia (r = 0.92). Bland-Altman analysis revealed a positive systematic bias for mean difference for SR at baseline [95% confidence interval (CI), 0 to 0.16 s−1; Fig. 2D] and with dobutamine treatment (95% CI, 0.08 to 0.4 s−1; Fig. 2E) and no systematic bias for SR with hypoxia (95% CI, 0.02 to −0.01 s−1; Fig. 2F).

Strain and Length Change

Percent δL and ε progressively decreased with increasing load clamp level (Fig. 2G). SE appropriately tracked the increase in percent δL with dobutamine treatment (Fig. 2H) and the decrease with hypoxia (Fig. 2I) at all load clamp levels. Percent ε was load dependent at baseline (r = 0.98), with dobutamine treatment (r = 0.98), and during hypoxia (r = 0.94). Bland-Altman analysis showed no bias for strain measurements at baseline (mean difference −3.0%, 95% CI, 0.4 to −6.4%) or with dobutamine treatment (mean difference 4.1%, 95% CI, 10.3 to −2.1%) and a negative bias with hypoxia (mean difference −0.8%, 95% CI, −2.1 to −7.6%).

Correlation Between Force/Length Transducer and Ultrasound Measurements

The correlation between force/length transducer and ultrasound measurements is shown in Fig. 3. There was a close correlation between SR and SV at baseline (R2 = 0.95), with dobutamine treatment (R2 = 0.99), and during hypoxia (R2 = 0.99). Similarly, there was a close correlation between ε and δL at baseline (R2 = 0.99), with dobutamine treatment (R2 = 0.96), and during hypoxia (R2 = 0.87).

DISCUSSION

Our data indicate that SE-derived SR and ε correlate closely with force/length transducer-derived SV and percent δL, respectively, at baseline, with dobutamine stimulation, and during hypoxia. Akin to the physiological measurements, SE-derived SR and ε are load dependent. SE-derived measurements appear to non-invasively quantify contractile function by providing parameters similar to those used in muscle physiology. Accurate knowledge of myocardial function is essential in the management of cardiac disease, and the quest continues for an optimal, quantitative technique to assess myocardial contractile function (2). The non-invasive determination of EF is probably the most common parameter of cardiac function. Although echocardiographic EF measurements are widely used to help guide clinical practice, they tend to be subjective and variable (9). In addition, EF does not provide any information regarding regional function, which is assessed by systolic wall motion analysis. However, data suggest that visual wall motion analysis is not accurate or reproducible even in large academic centers (7). Furthermore, in current clinical practice, echocardiographic EF and wall motion analysis are both primarily subjective measurements.
Fig. 2. Strain echocardiography (SE)-derived SR and strain appropriately track changes in force/length transducer-derived SV and percent ΔL over a wide range of load clamp levels at baseline (A and G, respectively; n = 14), with dobutamine treatment (B and H, respectively; n = 10), and during hypoxia (C and I, respectively; n = 10). ●, SE-derived SR or strain; ○, force/length transducer-derived normalized SV or percent ΔL; and ■, data points for mean difference between SR and SV (D–F) and mean difference between strain and ΔL (J–L). Bland-Altman plots demonstrate a positive systematic bias for SR estimation of SV at baseline (D) and with dobutamine (E) and hypoxia (F), a negative systematic bias for strain estimation of ΔL at baseline (J), and no systematic bias for strain estimation of ΔL with dobutamine (K) and hypoxia (L). The thick solid line indicates the mean difference and the thin solid line indicates the zero mean difference. Dashed lines are means ± 2SD.
Additionally, there is an obvious disconnect between clinical and basic laboratory measures of cardiac function. In a classic muscle physiology laboratory, systolic function is measured by suspending strips of ventricular muscle in a tissue bath and measuring SV, $\delta L$, and force. These methods obviously preclude clinical application. Tissue Doppler-derived SE is able to quantitatively calculate rates of tissue deformation and yield local SR and $\varepsilon$. If the line of insonation is along the muscle fiber, the change in muscle length would approximate $\varepsilon$, whereas SV would approximate the rate of change of the muscle length, which if corrected for its original length (normalized SV) would yield SR. Thus it is possible that echocardiographic SR and $\varepsilon$ may allow direct yet noninvasive measurements of normalized SV and $\delta L$.

Our data indicate that there is indeed a close relation between echocardiographic and physiological measurements of myocardial function. To test whether these correlations held under conditions of hyper- and hypofunction, we repeated measurements during dobutamine stimulation and hypoxia. Echocardiographic SR and $\varepsilon$ closely the tracked the appropriate increase in normalized SV and $\delta L$ with dobutamine treatment and the decrease with hypoxia. Although the absolute values of $\varepsilon$ and $\delta L$ were quite similar, there was a systematic overestimation of normalized SV by SE. There are several possible explanations for this difference: 1) the series elastic component in the force transducer system is not accounted for in SE; 2) the force/length transducer measures SV and percent $\delta L$ in the entire muscle strip, but SE measures $\varepsilon$ in a particular segment of the muscle; and 3) there is a difference in the calculation of the parameters between the two systems. The transducer measurements are normalized to the true muscle length (measured by a caliper), but SR measurements are normalized to “strain distance” (an arbitrary length, adjusted by the interpreter).

**Limitations**

SR is calculated from a point along the ultrasound beam rather than a point in the myocardium. Signal noise may be an issue in clinical imaging. Tissue hypoxia was not confirmed. However, demonstration of significantly low solution PO2 is probably sufficient for the purposes of this study. We did not evaluate whether SE measurements correlate with peak developed force. Force is measured during an isometric muscle twitch (muscle contraction with no change in length). Because SE depicts changes in length, it would be challenging to establish a correlation between SE and force. As mentioned in the DISCUSSION, there were differences in absolute values between the SR and normalized SV measurements. Although SE tends to overestimate physiological parameters, our data dem
onstrate that SE closely tracked the changes in standard physiologic parameters. This may be adequate in clinical settings, where complex fiber orientation in the myocardium will often yield nonparallel measures of $\varepsilon$ and SR.

In conclusion, SE measurements appear to closely track classic physiological parameters of systolic function under varying conditions.

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DISCLOSURES

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