Intrinsic Myoarchitectural Differences Between the Left and Right Ventricles of Fetal Human Hearts: An Ultrasonic Backscatter Feasibility Study

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Objective: Embryologically, cardiac chambers differ in their morphologic and contractile properties from the beginning. We hypothesized that a noninvasive ultrasonic backscatter investigation might illustrate the fundamental differences in myocardial morphologic properties of the 2 ventricles during heart development. The goals of this investigation were to 1) explore the feasibility of measuring the magnitude of cyclic variation of ultrasonic backscatter from the left and right ventricular free walls of fetal hearts; 2) compare measurements of the magnitude of cyclic variation from the left and right sides of the heart; and 3) determine if the observed results are consistent with predictions relating the overall backscatter level and the magnitude of cyclic variation.

Methods: Cyclic variation data from the left and right ventricular free walls were generated from analyses of the backscatter from echocardiographic images of 16 structurally normal fetal hearts at mid-gestation.

Results: The magnitude of cyclic variation was found to be greater for the left ventricular free wall than for the right ventricular free wall (4.5 $\pm$ 1.1 dB vs 2.3 $\pm$ 0.9 dB, respectively; mean $\pm$ standard deviation; $P <$ .0001, paired *t* test).

Conclusion: Measurements of the cyclic variation of backscatter can be obtained from both the left and right sides of fetal hearts demonstrating a significant difference between the measured magnitude of cyclic variation in the left and right ventricular myocardium. This observation is consistent with predictions relating the overall backscatter level and the magnitude of cyclic variation. The results of this study suggest cyclic variation measurements may offer a useful approach for characterizing intrinsic differences in myocardial properties of the 2 ventricles in assessing fetal heart development. (J Am Soc Echocardiogr 2009;22:170-176.)

Keywords: Backscatter, Cyclic variation, Echocardiography, Fetus, Heart, Tissue characterization

Formation of the heart involves an orchestrated series of morphogenetic events. Although individual cardiac chambers do not become morphologically distinguishable until after cardiac looping, each cardiac chamber differs in its morphologic and contractile properties from the beginning.[1] How chamber identities are established is unknown, but identification of morphologic properties may provide a window to appraise perturbations in the developmental process that can have consequences in the form of myopathic or congenital heart disease. In both developing and developed hearts, global and local structural anisotropy of myocardial fibers and other ventricular wall constituents (eg, the types and concentrations of proteins) produce mechanical and electrical properties that may be anisotropic, time varying, and spatially inhomogeneous, and may differ between left and right ventricles.[2-7] Understanding and establishing the normal patterns of these intrinsic properties in normally developing hearts will be a necessary prerequisite to their use in discerning the evolution of myopathic or congenital heart disease.

We hypothesized that a noninvasive ultrasonic backscatter investigation might illustrate the fundamental differences in myocardial morphologic properties of the 2 ventricles during heart development. The rationale that underlies this prospective study is that the intrinsic myoarchitectural properties of the developing heart are reflected in the ultrasonic properties, and thus an assessment of myoarchitecture can be achieved through echocardiography-based analyses. Several investigations have demonstrated the relationship between measured ultrasonic parameters (attenuation, backscatter, speed of sound) and the inherent properties of myocardial tissue. The nature of the intrinsic myocardial properties (eg, the types and concentrations of proteins present resulting in specific intra- and extracellular viscoelastic properties) and the geometric properties (structural morphology) of the myocardium combine to produce the observed ultrasonic parameters.[8-13] For example, several studies have been published illustrating the relationship between the measured ultrasonic backscatter properties and collagen content in myocardial tissue.[8,14-21] The results demonstrate an increase in both the measured ultrasonic

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attenuation and backscatter correlates well with increased collagen concentration determined biochemically or histologically.

Measurement of the systematic variation of backscattered ultrasonic energy from the myocardium over the heart cycle (ie, the *cyclic variation of backscatter*) is a validated approach for investigating intrinsic myocardial characteristics in vivo[13,22] and has been successfully applied to characterize a number of cardiac pathologies.[23] However, relatively few studies have explored the feasibility of measurements of cyclic variation to characterize myoarchitecture in the developing fetal heart, which may be relevant to postnatal ventricular structure and function.[24,25]

Measurements of the cyclic variation of backscatter represent a clinically useful approach for characterizing the nature of backscatter properties from a myocardial region because each heart serves as its own reference. In this approach only the relative change in myocardial backscatter over the heart cycle is measured, not the absolute level of backscatter. Estimates of the absolute level of ultrasonic backscatter require explicit compensations for imaging system-dependent contributions (eg, system gain and beam volume) and ultrasonic field propagation effects (eg, effects of overlying attenuation). By measuring the cyclic variation of myocardial backscatter, the effects of imaging system gain, body habitus, and other effects that can influence the overall backscattered signal are largely mitigated as long as the acquired images are not saturated.

The *objectives* of this initial investigation were 3-fold: 1) to explore the feasibility of measuring the magnitude of cyclic variation of backscatter from the left and right ventricular free walls from the hearts of human fetuses at mid-trimester; 2) to compare measurements of the magnitude of cyclic variation from the left and right sides of the heart as an approach to understand and establish the normal patterns of the morphologic properties in normally developing hearts; and 3) to determine if the observed results are consistent with predictions based on a previously described model[26] demonstrating a relationship between overall backscatter level and the magnitude of cyclic variation using recently published backscatter measurements[27] in a fetal animal model.

MATERIALS AND METHODS

Subjects

Data from 16 human fetuses ranging in age from 17 to 29 weeks gestation were included in this study. Eligible fetuses were those whose mothers had been referred for a comprehensive fetal echocardiogram at St Louis Children's Hospital, Washington University School of Medicine because of a family history of congenital heart defects or suspected maternal exposure to teratogens. A full fetal echocardiographic examination was performed, and only fetuses exhibiting structurally normal hearts were included in the study. Signed informed consent for participation was obtained from one of the parents under a human studies protocol approved by the Washington University Investigation Review Board.

Image Acquisition

Fetal echocardiographic images to be used for this study were obtained with a Siemens/Acuson Sequoia C256 imaging system (Mountain View, CA) using a 6C2-S curvilinear array using an approach similar to that previously described by our laboratory in which the imaging system is configured to provide grayscale images linearly proportional to the measured level of backscatter.[28-30] Images were acquired in the harmonic imaging mode at 5.0 MHz with the imaging system configured to provide an approximately linear relationship between a change in backscattered ultrasound intensity (expressed in decibels, dB) and a change in the displayed grayscale value (ie, a linear compression curve) over the useful dynamic range of the imaging system. For this study, the Siemens/Acuson Sequoia C256 imaging system was configured with settings: "space/time" = T1; "edge" = 0; "persistence" = 0; "post-process" = 1; "delta" = 1; "dynamic range" = 40 dB.

Transverse, long-axis, cross-sectional images of each fetal heart were acquired with the imaging system in the "Zoom" mode, and the overall (2-dimensional) receive gain and time-gain compensation controls were adjusted to provide relatively strong backscatter (unsaturated, mid-level grayscale values) from the mid-myocardial regions. Approximately 6 heart cycles worth of images were acquired and stored as 8-bit digital cineloops (AVI format) for subsequent analyses. The transverse, long-axis, cross-sectional echocardiographic view provides images with the insonifying ultrasonic field perpendicular to the predominant myocardial fiber orientation and thus reduces the confounding effects of tissue anisotropy on measurements.[29,31-35]

Generation of Cyclic Variation Data

Cyclic variation of backscatter data were generated by analyzing the acquired echocardiographic images offline using the NIH ImageJ (National Institutes of Health, Bethesda, MD) software package. The acquired cineloops were opened as Quicktime (Apple Inc, Cupertino, CA) movies, and separate regions of interest were placed in the myocardial free walls of the left and right ventricles. The position of each region of interest was manually adjusted in every acquired image frame such that approximately the same 2-dimensional area of myocardium was measured over the heart cycles. The mean grayscale value within each region of interest was measured for each image frame, thus producing a trace of mean backscatter values as a function of frame number (ie, time). These data represent the measured cyclic variation of backscatter waveforms. Changes in measured mean grayscale values representing the cyclic variation of backscatter were converted to changes in ultrasonic backscatter values expressed in dB using a previously described system calibration procedure.[28-30,36] Figure 1 shows the typical placements of regions of interest in a fetal echocardiogram and a representative cyclic variation of backscatter curve generated from the region of interest in the left ventricular free wall.

Measurement of the Magnitude of Cyclic Variation of Backscatter

We define the magnitude of cyclic variation as the difference between the average peak and average nadir values of backscatter.[31,37-39] Individual heart cycles were delineated by determining the image frames closest to end diastole from analysis of the wall motion in the acquired echocardiogram. Similarly, the end-systolic frames for each heart cycle were identified. For each set of cyclic variation data, a composite cyclic variation waveform was constructed by averaging the individual cyclic variation data for each heart cycle. This was accomplished by plotting the measured backscatter as a function of the percent of the individual heart cycle, linearly interpolating values between measured frames, and averaging the individual cyclic variation data. The composite cyclic variation data were expressed as a zero-mean waveform, and low-pass binomial filtering was applied to reduce noise. The magnitude of cyclic variation was determined using a previously described automated algorithm based on Fourier decomposition of the cyclic variation data and the average systolic and diastolic intervals.[37,38] Figure 2 shows