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Reproducibility of cine displacement encoding with stimulated echoes (DENSE) in human subjects

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Abstract

Objectives—To test the hypothesis that two-dimensional (2D) displacement encoding via stimulated echoes (DENSE) is a reproducible technique for the depiction of segmental myocardial motion in human subjects.

Materials and methods—Following the approval of the institutional review board (IRB), 17 healthy volunteers without documented history of cardiovascular disease were recruited. For each participant, 2D DENSE were performed twice (at different days) and the images were obtained at basal, midventricular and apical levels of the left ventricle (LV) with a short-axis view. The radial thickening strain (Err), circumferential strain (Ecc), twist and torsion were calculated. The intra-, inter-observer and inter-study variations of DENSE-derived myocardial motion indices were evaluated using coefficient of variation (CoV) and intra-class correlation coefficient (ICC).

Results—In total, there are 272 pairs of myocardial segments (data points) for comparison. There is good intra- and inter-observer reproducibility for all DENSE-derived measures in 17 participants. There is good inter-study reproducibility for peak Ecc (CoV = 19.64%, ICC = 0.8896, $p < 0.001$), twist (CoV = 33.11%, ICC = 0.9135, $p < 0.001$) and torsion (CoV = 13.96%, ICC = 0.8684, $p < 0.001$). There is moderate inter-study reproducibility for Err (CoV = 38.89%, ICC = 0.7022, $p < 0.001$).

Conclusion—DENSE is a reproducible technique for characterizing LV regional systolic myocardial motion on a per-segment basis in healthy volunteers.

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Keywords

MR imaging; DENSE; reproducibility

Introduction

The existence of myocardial dysfunction, a common manifestation of various cardiovascular diseases, is a reliable predictor for future cardiovascular events and poor prognosis (1, 2). In its early stage, however, impaired myocardial motion can be confined to a limited number of myocardial segments. Global cardiac function parameters, such as the left ventricular ejection fraction (LVEF), may not be able to sensitively present such a subtle change. Therefore, accurately tracking abnormal myocardial motion by quantifying the movement of individual myocardial segments is arguably critical for the monitoring of the progression of cardiovascular diseases and for the assessment of individual responses to therapy. Various quantitative magnetic resonance imaging (MRI) techniques can be used to provide an operator-independent description of myocardial motion and deformation. *Displacement encoding with stimulated echoes* (DENSE) is a phase-based MRI technique that encodes the myocardial tissue's instantaneous displacement into the image phase. Regional displacement and strain can be quantified over the cardiac cycle (CINE mode), and the measured strain tensors can be decomposed into longitudinal, circumferential and radial components based on a polar cardiac coordinate system (3).

To date, the reliability of CINE DENSE for the evaluation of human cardiac motion patterns has not been systematically evaluated in human subjects. Specifically, studies evaluating the test-retest reproducibility as well as observer variability of CINE DENSE-derived cardiac indices such as peak myocardial strain and twist on a per-segment basis are lacking. This knowledge gap may potentially limit the application of the CINE DENSE in future clinical or epidemiology studies for cardiovascular risk estimation because multiple factors of image acquisition and analysis of cardiac MRI examinations may directly or indirectly affect the quality of cardiac measurements. The aim of this study was therefore to test the hypothesis that two-dimensional (2D) CINE DENSE is a reproducible technique for the quantification of segmental myocardial motion by assessing intra-, inter-observer and inter-study variability of CINE DENSE-derived cardiac measures, mapped on a standard American Heart Association (AHA) 16-segment left ventricle (LV) model, in 17 human subjects.

Materials and methods

Study Cohort

This study complied with HIPAA regulations. Following the approval of the institutional review board (IRB), 17 healthy volunteers without documented history of cardiovascular disease were recruited. All participants provided written informed consent before the study. The volunteers are 49.9 ± 12.3 years old (11 men and 6 women). All participants underwent two MRI examinations on different days (scan and rescan) on a 1.5 T scanner (MAGNETOM, Aera, Siemens AG, Germany). The heart rate and blood pressure of all participants were recorded before each scan.

MRI protocol

A 3-plane, rapid localization sequence was employed to provide anatomic orientation for the entire scan. A black-blood half-Fourier acquisition single-shot turbo spin-echo sequence was used to obtain images using standard 4-chamber and short-axis display orientations (4). 2D spiral CINE DENSE datasets were obtained at basal, midventricular and apical levels of the LV in short-axis orientation. Two in-plane displacement encoding directions were obtained in a single breath hold per slice. DENSE imaging parameters included: Repetition time(TR)/ Echo time(TE)/ Flip angle = 15.0 ms/ 1.0 ms/ 20°, spiral interleaves = 6, interleaves per heartbeat = 2, slice thickness = 8 mm, matrix = 192 × 192, field of view (FOV) = 380 × 380 mm, bandwidth = 1002 Hz/Pixel, displacement encoding frequency (ke) = 0.1 cycles/mm, temporal resolution = 30 ms, breath-holds duration = 14 heart beats per 2D slice. For each participant, a baseline scan (#1) and a repeat rescan (#2) was performed at different days, separated by a time interval (less than 20 days) using identical imaging protocols.

Data processing

All DENSE data were analyzed by using a software package developed in MATLAB (Mathworks, Inc., Natick, MA) (4, 5). The epicardial and endocardial borders of the LV wall were manually traced on the magnitude-reconstructed DENSE images by one observer (reader #1, KL, with 9 years of experience in cardiovascular imaging). A dedicated motion-guided segmentation algorithm was used to better define myocardial contours for motion analysis (6). A 2D phase-unwrapping algorithm was applied to convert the phase of each pixel on the LV wall to two-dimensional displacements (7, 8). Regional Lagrangian strain E , was then calculated by using the isoparametric formulation that was previously described (7). The directions and eigenvalues of the first and second principal strains (E_1 and E_2) were then determined (9). Using the center of the LV as the reference, E was decomposed into radial (E_{rr}) and circumferential (E_{cc}) components of strain (10). The twist (°, degree) of regional myocardial tissue, which is defined as the angle between lines connecting the center of LV and a moving myocardial pixel during the cardiac cycle, was also measured. The resulting LV torsion (°/mm) was defined as Φ/D , where Φ represents the difference of twist angles between the base and apex levels of the LV (averaged over 6 and 4 segments, respectively), and D denotes the longitudinal distance between two LV levels (11). All segmental measurements were averaged over three myocardial layers, including sub-endocardial, mid and sub-epicardial. Peak strains (E_{rr} and E_{cc}) and twist were calculated on a per-segment basis using a standard AHA 16-segment LV model as a reference. Peak torsion was calculated for each heart. To assess intra-observer reproducibility, reader #1 performed the myocardial strain analysis twice for all image data sets with a 1 month interval. To assess inter-observer reproducibility, a second reader (VC, with 2 year experience of clinical radiology) independently performed analysis for all subjects (either scan #1 or scan #2) using the same workflow.

Statistical analysis

All continuous variables were represented by mean $\pm$ one standard deviation (SD). The difference in segmental strain and twist variables between scans and rescans were compared using paired t -tests. The intra-class correlation coefficient (ICC) was applied to describe the

agreement of measurements acquired from two consequential scans. Reproducibility of duplicated measurements was also represented using coefficient of variation (CoV, defined as SD/Mean). An ICC value > 0.75 or a CoV $< 20\%$ between duplicated measurements was considered as good reproducibility. The distributions of ICCs and CoVs of individual myocardial segments were presented. Statistical analysis was performed using SPSS statistical software (Version 13.0, SPSS, inc, Chicago, IL). Statistical analyses were two-tailed. A p value < 0.05 was considered statistically significant. Bland-Altman plots were also used to visualize the intra-, inter-observer and inter-study variations of myocardial measurements.

Results

All 34 scans (17 scans and 17 rescans) were completed and all images are eligible for quantitative analysis. As summarized in table 1, the average time interval between scan #1 and #2 was 14 ± 6 days. For all participants, there were no significant variations of heart rates (68.4 ± 11.5 beats/minute vs. 68.9 ± 12.1 beats/minute), systolic blood pressure (SBP, 121.2 ± 28.4 mmHg vs. 122.3 ± 30.9 mmHg) and diastolic blood pressure (DBP, 79.3 ± 21.7 mmHg vs. 79.4 ± 19.8 mmHg) between scan #1 and scan #2. For each cardiac slice, the image processing time is 3.1 ± 0.8 minutes.

In total, there are 272 pairs of myocardial segments (from 2 consecutive scans on 17 subjects) for quantitative analysis and comparison. Figure 1 shows an example set of DENSE images demonstrating myocardial displacement maps from a single subject for scan #1 and #2. The images demonstrated similar image quality and systolic displacement patterns for both acquisitions. Demonstrated by high ICCs (> 0.75) and low CoVs ($< 20\%$), intra-observer and inter-observer reproducibility of DENSE-derived strain (per-segment), twist (per-segment) and torsion (per-subject) is good. There is good inter-study reproducibility for peak Ecc (CoV = 19.64%, ICC = 0.8896, $p < 0.001$), twist (CoV = 33.11%, ICC = 0.9135, $p < 0.001$) and torsion (CoV = 13.96%, ICC = 0.8684, $p < 0.001$). There is moderate inter-study reproducibility for Err (CoV = 38.89%, ICC = 0.7022, $p < 0.001$). Individual myocardial segments (based on AHA 16-segment LV model) have various ICCs and CoVs for duplicated strain and twist measurements. See figure 2 and figure 3.

Tables 2a, 2b and 2c summarize the side-by-side comparisons of the measures between two scans and two observers. Figures in supplement data present the Bland-Altman plots of intra-observer, inter-observer and inter-study variations of major measures (including 272 myocardial segments) for the description of segmented myocardial motions.

Discussion

In the present study, we demonstrated the overall reproducibility of DENSE for the description of LV motion behavior on a per-segment basis for human subjects. In-plane myocardial motion indices (calculated at the short-axis view), including Err, Ecc, twist and torsion demonstrated good intra- and inter-observer reproducibility. Ecc, twist and torsion had good inter-study reproducibility while Err showed moderate inter-study reproducibility.

Currently, DENSE has been applied to evaluate LV dyssynchrony in patients with congestive heart failure (CHF) and individual response to resynchronization therapy (12, 13). A previous study further demonstrated that DENSE is able to detect impaired global cardiac motion in asymptomatic patients with type 2 diabetes mellitus (T2DM), which is considered as a prominent equivalent of subclinical cardiovascular diseases (14). In clinical practice, a rigorous and precise scientific method must be reproducible. That means independent examiners should be able to replicate the results using the same testing protocol. Therefore, the reproducibility of the strain measurements should be evaluated before DENSE could be selected as an appropriate cardiac imaging method for regular clinical use. Recently, Haggerty et al. demonstrated the reproducibility of DENSE for the quantification of LV motion in 9 mice using a 7.0 T MRI scanner (15). However, data from animal studies may not fully predict the reliability of DENSE for describing myocardial motion patterns in human subjects. Actually, existing results of human studies on similar MRI techniques, such as traditional MRI tagging or feature tracking, were unable to show optimal inter-study reproducibility for strain and its related measurements in all myocardial planes in human subjects (11, 16). We thought this variability may originate from two major sources, image analysis and image acquisition. The selection of imaging analysis tools (with different algorithms) and the accuracy of manual region of interest (ROI) analysis can significantly affect the readings of measurements. Demonstrated by low intra- and inter-observer variability, we used a single software package and a well-defined workflow for data analysis to successfully control such adverse factors. On the other hand, inter-study variations seem to be more difficult to eliminate because other adverse factors are added to jointly affect the measures. Between scans, technical, operational and physiological conditions may change, including the selection of scan platforms, the different orientation of target organ by operators, changes of physiological status, such as heart rates and the locations of the diaphragm after breath holding in subjects. To minimize inter-study variations, we used the same MRI scanner for all examinations. Two experienced staff technicians who are also working for other ongoing cardiovascular imaging studies in our institutions performed all MRI scans. In a previous study, the location of the short-axis slice has been considered as a source of scan-rescan variances of measurements (11). Although the technicians are blinded to the previous orientations for imaging acquisition, they can usually identify reasonable locations for “base”, “mid” and “apex” levels at the LV without definite anatomic landmarks. Future work could also incorporate commercially available automated view planning for more consistent slice positioning (17). In addition, there were minimal changes of major physiological conditions between scan #1 and #2. As a result, we observed overall good reproducibility for cardiac motion measurements between two consecutive scans for all 16 myocardial segments. Although the moderate reproducibility of Err was found, DENSE still demonstrates its reliability in myocardial motion tracking as compared with MRI techniques, such as tagging (11). In a similar study conducted by Young et al, DENSE was found to have a lower inter-observer variation for measuring strain in healthy volunteers as compared with tagging (18).

In addition, we noticed that most previous studies assessing the reproducibility of cardiac motion measurements (using tagging, tissue phase mapping, DENSE, feature tracking [FT] or heart deformation analysis [HDA]), either in animal models or in human subjects were

performed on a per-slice basis (11, 15, 19, 20). As a methodology advance, our study demonstrated better reproducibility of DENSE-derived measures at the level of individual myocardial segments. Such a capability is considered to be essential in describing subtle cardiac motion abnormalities on local ventricular wall areas.

Our study has limitations. First, we were unable to comprehensively track myocardial motion patterns in diastole for those subjects with a long R-R interval because of signal decays (3). Therefore, only the data acquired in systole was enrolled for quantitative analysis. This is an inherent technical limitation of DENSE and similar techniques, such as MRI tagging, for clinical application in human subjects at 1.5 T. Second, all subjects are healthy volunteers without documented history of cardiovascular diseases. Therefore, the reproducibility and accuracy of DENSE for the description of regional myocardial motion needs to be further evaluated on patient groups with various cardiovascular diseases for describing characteristic myocardial motion patterns. Third, our study only included short-axis images and the motion analysis was limited to the LV. Images right ventricle (RV) were not included because of unsatisfactory visibility of the RV wall. Performing DENSE at a higher magnetic field or using a variable flip angle may be a potential solutions for these problems related to the 1.5 T magnet (21).

Conclusions

DENSE is a reproducible technique for characterizing LV regional systolic myocardial motion on a per-segment basis in healthy volunteers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Cho GY, Marwick TH, Kim HS, Kim MK, Hong KS, Oh DJ. Global 2-dimensional strain as a new prognosticator in patients with heart failure. *Journal of the American College of Cardiology*. 2009; 54(7):618–24. [PubMed: 19660692]
2. Cicala S, de Simone G, Roman MJ, et al. Prevalence and prognostic significance of wall-motion abnormalities in adults without clinically recognized cardiovascular disease: the Strong Heart Study. *Circulation*. 2007; 116(2):143–50. [PubMed: 17576870]
3. Aletras AH, Ding S, Balaban RS, Wen H. DENSE: displacement encoding with stimulated echoes in cardiac functional MRI. *Journal of magnetic resonance*. 1999; 137(1):247–52. [PubMed: 10053155]
4. Zhong X, Spottiswoode BS, Meyer CH, Kramer CM, Epstein FH. Imaging three-dimensional myocardial mechanics using navigator-gated volumetric spiral cine DENSE MRI. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 2010; 64(4):1089–97.
5. Kramer SP, Powell DK, Haggerty CM, et al. Obesity reduces left ventricular strains, torsion, and synchrony in mouse models: a cine displacement encoding with stimulated echoes (DENSE)

- cardiovascular magnetic resonance study. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2013; 15:109. [PubMed: 24380567]
6. Spottiswoode BS, Zhong X, Lorenz CH, Mayosi BM, Meintjes EM, Epstein FH. Motion-guided segmentation for cine DENSE MRI. *Medical image analysis*. 2009; 13(1):105–15. [PubMed: 18706851]
 7. Kim D, Gilson WD, Kramer CM, Epstein FH. Myocardial tissue tracking with two-dimensional cine displacement-encoded MR imaging: development and initial evaluation. *Radiology*. 2004; 230(3): 862–71. [PubMed: 14739307]
 8. Spottiswoode BS, Zhong X, Hess AT, et al. Tracking myocardial motion from cine DENSE images using spatiotemporal phase unwrapping and temporal fitting. *IEEE transactions on medical imaging*. 2007; 26(1):15–30. [PubMed: 17243581]
 9. Gilliam AD, Epstein FH. Automated motion estimation for 2-D cine DENSE MRI. *IEEE transactions on medical imaging*. 2012; 31(9):1669–81. [PubMed: 22575669]
 10. Epstein RS. The hypothetical migraine drug comparative effectiveness study: a payer's recommendations for obtaining more useful results. *Health affairs*. 2012; 31(10):2225–30. [PubMed: 23048101]
 11. Donekal S, Ambale-Venkatesh B, Berkowitz S, et al. Inter-study reproducibility of cardiovascular magnetic resonance tagging. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2013; 15:37. [PubMed: 23663535]
 12. Budge LP, Helms AS, Salerno M, Kramer CM, Epstein FH, Bilchick KC. MR cine DENSE dyssynchrony parameters for the evaluation of heart failure: comparison with myocardial tissue tagging. *JACC Cardiovascular imaging*. 2012; 5(8):789–97. [PubMed: 22897992]
 13. Bilchick KC, Kuruvilla S, Hamirani YS, et al. Impact of mechanical activation, scar, and electrical timing on cardiac resynchronization therapy response and clinical outcomes. *Journal of the American College of Cardiology*. 2014; 63(16):1657–66. [PubMed: 24583155]
 14. Ernande L, Thibault H, Bergerot C, et al. Systolic myocardial dysfunction in patients with type 2 diabetes mellitus: identification at MR imaging with cine displacement encoding with stimulated echoes. *Radiology*. 2012; 265(2):402–9. [PubMed: 22929334]
 15. Haggerty CM, Kramer SP, Binkley CM, et al. Reproducibility of cine displacement encoding with stimulated echoes (DENSE) cardiovascular magnetic resonance for measuring left ventricular strains, torsion, and synchrony in mice. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2013; 15:71. [PubMed: 23981339]
 16. Wu L, Germans T, Guclu A, Heymans MW, Allaart CP, van Rossum AC. Feature tracking compared with tissue tagging measurements of segmental strain by cardiovascular magnetic resonance. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2014; 16:10. [PubMed: 24450803]
 17. Lu X, Jolly MP, Georgescu B, et al. Automatic view planning for cardiac MRI acquisition. *Medical image computing and computer-assisted intervention : MICCAI International Conference on Medical Image Computing and Computer-Assisted Intervention*. 2011; 14(Pt 3):479–86.
 18. Young AA, Li B, Kirton RS, Cowan BR. Generalized spatiotemporal myocardial strain analysis for DENSE and SPAMM imaging. *Magnetic resonance in medicine*. 2012; 67(6):1590–9. [PubMed: 22135133]
 19. Lin K, Collins JD, Chowdhary V, Markl M, Carr JC. Heart deformation analysis: measuring regional myocardial velocity with MR imaging. *The international journal of cardiovascular imaging*. 2016
 20. Lin K, Collins JD, Lloyd-Jones DM, et al. Automated Assessment of Left Ventricular Function and Mass Using Heart Deformation Analysis:: Initial Experience in 160 Older Adults. *Acad Radiol*. 2016; 23(3):321–5. [PubMed: 26749328]
 21. Wehner GJ, Suever JD, Haggerty CM, et al. Validation of in vivo 2D displacements from spiral cine DENSE at 3T. *Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance*. 2015; 17(1):5. [PubMed: 25634468]

Figure 1.

A case shows reproducibility of DENSE in describing regional myocardial motion.

a An AHA 16-segment model for the quantitative measurements.

b A magnitude-reconstructed DENSE image of LV (short-axis) on a 71 years old male at the scan #1.

c Lagrangian displacement field (yellow arrows) at the end of systole.

d A magnitude-reconstructed DENSE image of LV (short-axis) on the same subject at the scan #2 (14 days later).

e Lagrangian displacement field (yellow arrows) at the end of systole.

Figure 2.
 ICCs of segmental strain and twist measures on the LV. For each segment, ICC was calculated based on two scans of 17 subjects.

Figure 3.
CoVs of segmental strain and twist measures on the LV. For each segment, Cov was calculated based on two scans of 17 subjects.

Table 1

Description of subjects

		Volunteers (N = 17)
Male (%)		11 (65)
Age (years old)		49.9 ± 12.3
Weight (kg)		85.2 ± 13.7
Height (cm)		174.3 ± 9.1
Heart rate (beats/minute)	scan 1	68.4 ± 11.5
	scan 2	68.9 ± 12.1
Systolic blood pressure (mmHg)	scan 1	121.2 ± 28.4
	scan 2	122.3 ± 30.9
Diastolic blood pressure (mmHg)	scan 1	79.3 ± 21.7
	scan 2	79.4 ± 19.8

Table 2

Overall reproducibility of DENSE-derived measurements. Variations of Err, Ecc and twist were calculated based on 272 myocardial segments. Torsion was calculated based on 17 subjects.

a Inter-study variability					
	Scan 1	Scan 2	CoV(%)	ICC [*]	95%CI of ICC
Err	0.390±0.221	0.347±0.191 ▼	38.89	0.7022	0.6219-0.7654
Ecc	-0.135±0.06	-0.135±0.06	19.64	0.8896	0.8598-0.9130
Twist(°)	5.50±4.77	5.63±4.45	33.11	0.9135	0.8902-0.9318
Torsion(°/mm) ◇	0.365±0.06	0.360±0.07	13.96	0.8684	0.6366-0.9523

b Intra-observer variability					
Table 3 Intra-observer variability of DENSE-derived indices					
	Analysis 1	Analysis 2	CoV (%)	ICC [*]	95%CI of ICC
Err	0.390±0.221	0.385±0.220	5.53	0.9954	0.9941-0.9964
Ecc	-0.135±0.06	-0.135±0.06	4.41	0.9945	0.9930-0.9957
Twist(°)	5.50±4.77	5.50±4.77	3.65	0.9991	0.9989-0.9993
Torsion(°/mm) ◇	0.365±0.06	0.365±0.06	0.49	0.9872	0.9648-0.9954

c Intra-observer variability					
Table 4 Inter-observer variability of DENSE-derived indices					
	Reader #1	Reader #2	CoV (%)	ICC [*]	95%CI of ICC
Err	0.390±0.221	0.380±0.215	22.28	0.9164	0.8939-0.9342
Ecc	-0.135±0.06	-0.138±0.06	10.67	0.9663	0.9572-0.9734
Twist(°)	5.50±4.77	5.36±4.41	14.17	0.9859	0.9822-0.9899
Torsion(°/mm) ◇	0.365±0.06	0.369±0.08	5.02	0.8897	0.6953-0.9600

* p < 0.001 for the correlation/reproducibility of duplicated measurements

▼ p < 0.05 for comparing measures between two groups using paired t-tests

◇ Only 17 data points were included for comparisons