

Impaired Myocardial Reserve Unmasked by High-frequency Speckle-Tracking Stress Echocardiography During Late Recovery From Experimental Kawasaki Disease

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Abstract

Background

Residual myocardial injury after Kawasaki disease (KD) may not be detected by resting left ventricular ejection fraction (LVEF). Whether stress deformation imaging unmasks impaired myocardial reserve linked to residual fibrosis remains uncertain.

Methods

Forty male C57BL/6 mice were assigned to independent rest and stress cohorts, each comprising control and *Lactobacillus casei* cell-wall extract (LCWE)-treated groups (n = 10 per group). Eight weeks after LCWE or vehicle administration, the rest cohort underwent high-frequency echocardiography followed by histology; the stress cohort underwent paired baseline and peak-dobutamine speckle-tracking echocardiography followed by histology. Contractile reserve was calculated as peak stress minus baseline. The primary functional endpoint was global longitudinal strain (GLS) reserve. Strain values are reported as absolute magnitudes. Myocardial collagen volume fraction (CVF) was quantified by Masson trichrome staining.

Results

In the stress cohort, LVEF reserve was preserved after LCWE exposure (control, $14.5 \pm 2.8\%$; LCWE, $14.1 \pm 2.8\%$; $P = 0.764$), whereas GLS reserve was lower ($7.9 \pm 1.6\%$ vs $4.5 \pm 1.3\%$; $P < 0.001$). Global circumferential strain reserve and longitudinal and circumferential strain-rate reserves did not differ between groups. CVF was higher after LCWE exposure in both cohorts (both $P < 0.001$). Within LCWE-exposed animals, GLS reserve correlated inversely with CVF ($r = -0.876$; $P = 0.001$). In an exploratory analysis of the full stress cohort, GLS reserve remained associated with CVF after adjustment for resting GLS ($B = -0.717$ per percentage point; 95% CI, -1.005 to -0.428 ; standardized $\beta = -0.754$; $P < 0.001$).

Conclusions

High-frequency speckle-tracking stress echocardiography is a feasible and sensitive approach for detecting myocardial contractile reserve dysfunction in KD mice during recovery phase.

Introduction

Kawasaki disease (KD) is a systemic vasculitis and a leading cause of acquired heart disease in children [1, 2]. Although long-term surveillance focuses primarily on coronary artery involvement, myocardial injury is also an important component of KD. Acute myocarditis may precede coronary arteritis and occur without clinically apparent coronary disease [3, 4], while persistent cardiomyocyte injury, interstitial

fibrosis, and coronary microvascular abnormalities have been reported beyond the acute inflammatory phase [4–7]. Thus, resolution of the acute illness and preserved conventional systolic function may not indicate complete myocardial recovery.

Resting left ventricular ejection fraction (LVEF) and fractional shortening (LVFS) may remain normal despite impaired myocardial mechanics. Speckle-tracking echocardiography studies have identified abnormalities in left ventricular deformation during acute and mid-term KD [8, 9], although longer-term findings have been inconsistent [10]. We previously reported reduced global longitudinal strain (GLS) and global circumferential strain (GCS), despite preserved conventional systolic indices, together with an inverse association between GLS and circulating markers of collagen turnover [11]. Because LVEF is influenced by loading conditions and ventricular geometry [12], assessment of contractile reserve may reveal dysfunction not apparent at rest; in other cardiac diseases, stress-derived systolic reserve has provided prognostic information and predicted subsequent left ventricular remodeling [13, 14]. We subsequently identified attenuated GLS and GCS reserve during clinical convalescence from KD, including in patients without coronary artery lesions [15]. However, the myocardial substrate underlying this stress-induced functional abnormality remains unknown.

The *Lactobacillus casei* cell wall extract (LCWE) murine model reproduces coronary arteritis, aortitis, myocarditis, and subsequent cardiovascular remodeling [16–18]. We therefore combined high-frequency speckle-tracking stress echocardiography with myocardial histology to determine whether longitudinal deformation reserve remains impaired during late recovery from LCWE-induced experimental KD despite preserved conventional left ventricular systolic function. GLS reserve was prespecified as the primary functional endpoint. We hypothesized that LCWE exposure would selectively impair GLS reserve and that lower reserve would be associated with greater myocardial collagen burden.

Methods

Study design

Forty mice were assigned to independent rest and stress cohorts (n = 20 each). Within each cohort, mice were randomized 1:1 to vehicle control or LCWE treatment. The rest cohort underwent resting echocardiography followed immediately by tissue collection, providing a drug-unexposed assessment of myocardial fibrosis. The stress cohort underwent paired baseline and dobutamine-stress echocardiography followed immediately by tissue collection, permitting animal-level reserve–histology analyses. Absolute GLS reserve was the primary functional endpoint; LVEF, LVFS, GCS, and strain-rate reserves were secondary endpoints. The overall study design, including cohort allocation, echocardiographic assessment, dobutamine stress testing, and tissue collection, is summarized in Fig. 1. Reporting followed the ARRIVE 2.0 guidelines [19].

Animals

Five-week-old male C57BL/6 wild-type mice were obtained from the Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences. Mice were housed under specific pathogen-free conditions in temperature- and humidity-controlled rooms with a 12-hour light–dark cycle and free access to standard chow and water. After at least 7 days of acclimatization, mice were randomized using a computer-generated sequence. Investigators performing echocardiographic and histological analyses were blinded to group allocation. The Institutional Animal Care and Use Committee of Gansu Provincial Hospital approved all procedures (approval no. 2025 – 135), which complied with institutional and national animal-care regulations.

LCWE preparation and induction of experimental KD

LCWE was prepared using an established protocol [20]. *Lactobacillus casei* was cultured in MRS broth (Oxoid, UK) for 48 hours, harvested, washed in phosphate-buffered saline (PBS), and disrupted with 4% sodium dodecyl sulfate. Sequential digestion with RNase, DNase I, and trypsin (250 µg/mL each) removed adherent material. Cell-wall fragments were washed, sonicated in a dry ice–ethanol bath for 2 hours, centrifuged at $20,000 \times g$ for 20 minutes, and the supernatant was centrifuged at $95,000 \times g$ for 1 hour at 4°C. The pellet was discarded, and total rhamnose content was measured using the phenol–sulfuric acid assay.

KD-group mice received a single intraperitoneal injection of 0.5 mL LCWE (1 mg/mL); control mice received the same volume of sterile PBS. Animals were monitored for body weight, activity, survival, and injection-related complications and were maintained without further intervention for 8 weeks before the prespecified imaging protocol.

High-frequency echocardiography

Imaging was performed with a Vevo 3100 high-resolution small-animal ultrasound system and a 22–55-MHz transducer (FUJIFILM VisualSonics, Toronto, Canada). Anesthesia was induced with 2.5% isoflurane in 100% oxygen at 0.5 L/min. After chest depilation, mice were positioned supine on a platform maintained at 37°C, and anesthesia was maintained with 1.0% isoflurane. Electrocardiographic signal, respiratory rate, and rectal temperature were monitored continuously.

Parasternal long- and short-axis cine loops containing at least three consecutive cardiac cycles were acquired at frame rates up to 233 frames/s. Imaging depth, sector width, gain, and frame rate were optimized at baseline and kept constant within each paired examination. M-mode recordings at the papillary-muscle level were used to measure LV end-diastolic dimension (LVIDd), interventricular septal and posterior-wall thickness at end-diastole, LVEF, and LVFS. Values were averaged over three cycles. Acquisition followed published recommendations for murine cardiac physiology and preclinical echocardiography [21–23]. A trained investigator blinded to group allocation performed all conventional measurements.

Dobutamine stress echocardiography

After baseline imaging in the stress cohort, dobutamine (Sigma-Aldrich; product D0676) was administered as a single intraperitoneal bolus (0.7 $\mu\text{g/g}$). This dose was selected in preliminary experiments comparing 0.35, 0.7, and 1.4 $\mu\text{g/g}$ in a separate cohort of male C57BL/6 mice. Imaging was repeated immediately and serially for 10 minutes until heart rate reached its maximum and began to decline. The same views, acquisition settings, and transducer position were reproduced whenever technically feasible, and heart rate was recorded with each cine loop. The acquisition at the peak heart-rate response was used for stress analysis. Technical adequacy required a $\geq 15\%$ increase in heart rate or a reproducible increase in at least one conventional systolic index, without severe arrhythmia or persistent hemodynamic instability.

Speckle-tracking analysis

Cine loops were analyzed offline with dedicated VisualSonics small-animal speckle-tracking software by an investigator blinded to treatment and histology. GLS and global longitudinal systolic strain rate (GLSR) were derived from parasternal long-axis loops; GCS and global circumferential systolic strain rate (GCSR) were derived from midventricular short-axis loops. Endocardial and epicardial contours encompassed the full myocardial wall. Each view was divided into six segments; contours were adjusted when tracking was suboptimal, and segments were excluded if acceptable tracking could not be obtained. Three consecutive cycles with stable heart rate and image quality were averaged. Terminology followed consensus recommendations where applicable [24, 25].

Group analyses report strain and strain rate as absolute magnitudes; the representative software curves retain the conventional negative sign for systolic longitudinal strain. Contractile reserve was defined as peak stress minus baseline (ΔLVEF , ΔLVFS , ΔGLS , ΔGLSR , ΔGCS , and ΔGCSR), so a larger positive Δ indicates greater recruitment.

Histological assessment

After the assigned protocol, hearts were excised, rinsed in ice-cold PBS, weighed, embedded in optimal cutting temperature compound, and sectioned at 5 μm through the midventricular level. Hematoxylin and eosin staining assessed residual inflammation, and Masson trichrome staining assessed collagen. Collagen-positive myocardial area was quantified using a prespecified color threshold in Fiji (ImageJ2 version 2.14.0/1.54f; National Institutes of Health) [26, 27]. CVF was calculated as collagen-positive myocardial area divided by total analyzed myocardial area $\times 100$. The rest cohort provided the drug-unexposed histological comparison; CVF from the stress cohort was used for matched reserve–histology analyses. A blinded investigator performed all measurements.

Reproducibility

Twelve studies were selected at random. The primary observer repeated measurements after at least 4 weeks, and a second blinded observer independently analyzed the same cine loops. Intraobserver and interobserver agreement were quantified using two-way random-effects, absolute-agreement intraclass correlation coefficients (ICCs) and Bland–Altman mean bias with 95% limits of agreement [28, 29].

Statistical analysis

Analyses were performed with GraphPad Prism 10.0 (GraphPad Software, San Diego, California) and SPSS 22.0 (IBM, Armonk, New York). Distributional assumptions were assessed using Shapiro–Wilk tests and Q–Q plots. Continuous variables are presented as mean $\pm$ SD or median (interquartile range), as appropriate. Independent-samples t tests or Mann–Whitney U tests compared groups. Paired baseline-to-stress responses were analyzed using two-way repeated-measures analysis of variance, with the group-by-condition interaction as the principal test and Šídák-adjusted comparisons where appropriate; change scores were also compared between groups.

Pearson or Spearman correlation assessed associations with CVF in animals with matched imaging and histology. An exploratory multiple linear regression modeled CVF using Δ GLS and resting GLS. A sensitivity analysis modeled peak-stress GLS using treatment group and resting GLS. All tests were two-sided, and $P < 0.05$ indicated statistical significance. Effect estimates are reported with 95% confidence intervals where available.

Results

Animal allocation

All 40 mice completed the assigned protocol: 20 in the rest cohort and 20 in the stress cohort (10 control and 10 LCWE-treated mice per cohort). No prespecified exclusions were applied, and all planned observations were available. No pre-injection parameter differed significantly among the four groups (all $P > 0.05$; Table 1).

Table 1
Baseline characteristics after group allocation and before LCWE or vehicle administration

Characteristic	Rest cohort		Stress cohort		P
	Control (n = 10)	LCWE (n = 10)	Control (n = 10)	LCWE (n = 10)	
Body weight, g	19.9 ± 1.8	21.1 ± 2.0	19.8 ± 1.7	20.9 ± 1.9	0.724
Heart rate, beats/min	462 ± 28	455 ± 31	468 ± 30	460 ± 32	0.862
LVFS, %	35.6 ± 2.0	35.3 ± 2.1	35.7 ± 2.1	35.4 ± 2.0	0.713
LVEF, %	65.5 ± 2.9	65.1 ± 3.0	65.7 ± 3.1	65.3 ± 3.2	0.653
IVSd, mm	0.74 ± 0.05	0.73 ± 0.05	0.74 ± 0.06	0.73 ± 0.05	0.821
LVPWd, mm	0.72 ± 0.05	0.73 ± 0.05	0.73 ± 0.05	0.73 ± 0.06	0.690
LVIDd, mm	3.82 ± 0.19	3.80 ± 0.20	3.84 ± 0.18	3.81 ± 0.21	0.733
GLS, %	18.8 ± 0.36	18.6 ± 0.35	18.9 ± 0.36	18.7 ± 0.37	0.742
GLSR, s ⁻¹	8.6 ± 0.44	8.5 ± 0.42	8.6 ± 0.39	8.5 ± 0.43	0.713
GCS, %	28.6 ± 0.89	28.4 ± 0.90	28.7 ± 0.95	28.5 ± 0.96	0.651
GCSR, s ⁻¹	12.2 ± 1.02	12.0 ± 1.11	12.3 ± 1.12	12.1 ± 1.02	0.724
Note. Data are presented as mean ± SD. Strain and strain-rate measures are reported as absolute magnitudes. GCS, global circumferential strain; GCSR, global circumferential systolic strain rate; GLS, global longitudinal strain; GLSR, global longitudinal systolic strain rate; HR, heart rate; IVSd, interventricular septal thickness at end diastole; LCWE, Lactobacillus casei cell-wall extract; LVEF, left ventricular ejection fraction; LVIDd, left ventricular internal diameter at end diastole; LVPWd, left ventricular posterior wall thickness at end diastole; LVFS, left ventricular shortening fraction. P values were calculated using one-way analysis of variance across the four groups.					

Resting LV function

At 8 weeks in the rest cohort, LVEF and LVFS did not differ between groups ($P = 0.486$ and $P = 0.529$, respectively; Table 2; Fig. 2A,B). In contrast, GLS ($17.5 \pm 1.3\%$ vs $18.8 \pm 1.2\%$; $P = 0.032$) and GCS ($26.3 \pm 2.2\%$ vs $28.5 \pm 2.0\%$; $P = 0.031$) were lower after LCWE exposure (Table 2; Fig. 2C,D).

Table 2
Resting echocardiographic parameters and myocardial collagen volume fraction at 8 weeks

Variable	Control (n = 10)	LCWE (n = 10)	<i>P</i>
Body weight, g	30.3 ± 1.3	29.8 ± 1.4	0.517
Heart rate, bpm	451 ± 21	455 ± 24	0.696
IVSd, mm	0.75 ± 0.05	0.77 ± 0.06	0.432
LVPWd, mm	0.74 ± 0.05	0.76 ± 0.05	0.350
LVIDd, mm	4.43 ± 0.19	4.52 ± 0.21	0.740
LVEF, %	65.6 ± 3.0	63.9 ± 3.2	0.486
LVFS, %	35.7 ± 1.9	34.6 ± 1.8	0.529
GLS, %	18.8 ± 1.2	17.5 ± 1.3	0.032
GLSR, s ⁻¹	8.45 ± 0.70	7.82 ± 0.75	0.065
GCS, %	28.5 ± 2.0	26.3 ± 2.2	0.031
GCSR, s ⁻¹	12.20 ± 1.00	11.45 ± 1.05	0.219
CVF, %	1.8 ± 0.4	5.6 ± 1.2	< 0.001
Data are presented as mean ± SD. Strain and strain-rate measures are reported as absolute magnitudes. CVF, collagen volume fraction; GCS, global circumferential strain; GCSR, global circumferential systolic strain rate; GLS, global longitudinal strain; GLSR, global longitudinal systolic strain rate; HR, heart rate; IVSd, interventricular septal thickness at end diastole; LCWE, Lactobacillus casei cell-wall extract; LVEF, left ventricular ejection fraction; LVIDd, left ventricular internal diameter at end diastole; LVPWd, left ventricular posterior wall thickness at end diastole; LVFS, left ventricular shortening fraction.			

The independent stress cohort showed the same baseline pattern: LVEF and LVFS were comparable between LCWE-treated and control mice, whereas GLS ($17.4 \pm 1.4\%$ vs $18.7 \pm 1.3\%$; $P = 0.041$) and GCS ($26.3 \pm 2.3\%$ vs $28.4 \pm 2.1\%$; $P = 0.043$) were lower after LCWE exposure (Table 3; Fig. 3C,E,G,I).

Table 3
Paired baseline-to-dobutamine responses in the stress cohort

Variable	Baseline			Peak stress			Contractile reserve (Δ)		
	Control	LCWE	P	Control	LCWE	P	Control	LCWE	Interaction P
HR, beats/min	452 $\pm$ 22	456 $\pm$ 25	0.413	610 $\pm$ 34*	606 $\pm$ 19*	0.504	158 $\pm$ 24	150 $\pm$ 26	0.484
LVFS, %	35.2 $\pm$ 2.1	34.3 $\pm$ 1.7	0.303	48.8 $\pm$ 2.9*	47.6 $\pm$ 3.1*	0.381	13.6 $\pm$ 1.7	13.3 $\pm$ 1.5	0.695
LVEF, %	65.4 $\pm$ 3.1	63.8 $\pm$ 3.3	0.278	79.9 $\pm$ 4.5*	77.9 $\pm$ 5.6*	0.399	14.5 $\pm$ 2.8	14.1 $\pm$ 2.8	0.764
GLS, %	18.7 $\pm$ 1.3	17.4 $\pm$ 1.4	0.041	26.6 $\pm$ 2.0*	21.9 $\pm$ 1.1*	< 0.001	7.9 $\pm$ 1.6	4.5 $\pm$ 1.3	< 0.001
GLSR, s ⁻¹	8.5 $\pm$ 0.7	8.2 $\pm$ 0.8	0.263	11.7 $\pm$ 0.9*	11.3 $\pm$ 0.7*	0.395	3.2 $\pm$ 0.6	3.1 $\pm$ 0.7	0.741
GCS, %	28.4 $\pm$ 2.1	26.3 $\pm$ 2.3	0.043	36.9 $\pm$ 2.8*	32.6 $\pm$ 2.8*	0.024	8.5 $\pm$ 1.8	8.4 $\pm$ 1.0	0.824
GCSR, s ⁻¹	12.2 $\pm$ 1.0	11.5 $\pm$ 1.0	0.302	15.9 $\pm$ 1.4*	15.0 $\pm$ 1.4*	0.260	3.7 $\pm$ 0.7	3.6 $\pm$ 0.8	0.652

Data are presented as mean $\pm$ SD. Strain and strain-rate measures are expressed as absolute magnitudes; contractile reserve (Δ) was calculated as peak stress minus baseline. *P < 0.001 vs baseline within the same group. GCS, global circumferential strain; GCSR, global circumferential systolic strain rate; GLS, global longitudinal strain; GLSR, global longitudinal systolic strain rate; HR, heart rate; LCWE, *Lactobacillus casei* cell-wall extract; LVEF, left ventricular ejection fraction; LVFS, left ventricular shortening fraction. Post-stress CVF was 1.8 $\pm$ 0.3% in control mice and 5.5 $\pm$ 1.2% in LCWE-exposed mice (P < 0.001).

Dobutamine response and contractile reserve

Dobutamine increased heart rate, LVEF, and LVFS in both groups (all within-group P < 0.001; Table 3; Fig. 3A,C,E). Δ HR (158 $\pm$ 24 vs 150 $\pm$ 26; P = 0.484), Δ LVEF (14.5 $\pm$ 2.8% vs 14.1 $\pm$ 2.8%; P = 0.764), and Δ LVFS (13.6 $\pm$ 1.7% vs 13.3 $\pm$ 1.5%; P = 0.695) were comparable in control and LCWE-treated mice (Fig. 3B,D,F).

GLS increased with dobutamine in both groups, but peak-stress GLS was lower after LCWE exposure (21.9 $\pm$ 1.1% vs 26.6 $\pm$ 2.0%; P < 0.001), as was Δ GLS (4.5 $\pm$ 1.3% vs 7.9 $\pm$ 1.6%; P < 0.001; Table 3; Fig. 3G,H). Peak-stress GCS was also lower after LCWE exposure (32.6 $\pm$ 2.8% vs 36.9 $\pm$ 2.8%; P = 0.024), but Δ GCS did not differ (8.4 $\pm$ 1.0% vs 8.5 $\pm$ 1.8%; P = 0.824; Fig. 3I,J). Δ GLSR and Δ GCSR were likewise comparable between groups (Table 3). Representative M-mode and longitudinal-strain recordings are shown in Fig. 4.

Myocardial collagen and association with GLS reserve

Eight weeks following LCWE treatment, at which point there was no observable remnant myocardial inflammation, as determined by H&E staining (Fig. 5A,B). In the rest cohort, CVF was higher in LCWE-treated mice than in controls ($5.6 \pm 1.2\%$ vs $1.8 \pm 0.4\%$; $P < 0.001$; Table 2; Fig. 5C–E). The stress cohort showed a concordant difference ($5.5 \pm 1.2\%$ vs $1.8 \pm 0.3\%$; $P < 0.001$; Table 3; Fig. 5E).

Within LCWE-exposed animals, Δ GLS showed a strong inverse association with CVF ($r = -0.876$; $P = 0.001$; Fig. 5F). In the full stress cohort, an exploratory two-predictor model explained 65.6% of CVF variance (adjusted $R^2 = 0.616$; $F[2, 17] = 16.22$; $P < 0.001$). Δ GLS remained associated with CVF after adjustment for resting GLS ($B = -0.717$ per percentage point; 95% CI, -1.005 to -0.428 ; standardized $\beta = -0.754$; $P < 0.001$), whereas resting GLS did not (Table 4). In the baseline-adjusted sensitivity analysis, LCWE exposure remained associated with lower peak-stress GLS (adjusted coefficient, -3.89 percentage points; 95% CI, -5.38 to -2.40 ; $P < 0.001$; model $R^2 = 0.786$; Table 4).

Table 4 Multivariable models of collagen volume fraction and peak-stress GLS					
Predictor	B	SE	Standardized β	95% CI for B	P
Δ GLS, %	-0.717	0.137	-0.754	-1.005 to -0.428	< 0.001
Resting GLS, %	-0.290	0.208	-0.200	-0.729 to 0.150	0.182
B, unstandardized regression coefficient; β , standardized regression coefficient; CI, confidence interval; GLS, global longitudinal strain; SE, standard error. Δ GLS was calculated as peak stress minus baseline. Tabulated coefficients are from the CVF model. The CVF model had $R^2 = 0.656$, adjusted $R^2 = 0.616$, $F(2,17) = 16.22$, and $P < 0.001$. In the baseline-adjusted sensitivity analysis with peak-stress GLS as the outcome, LCWE exposure had an adjusted coefficient of -3.89 percentage points (95% CI, -5.38 to -2.40 ; $P < 0.001$); model $R^2 = 0.786$.					

Reproducibility

Intraobserver ICCs were 0.96 for resting GLS, 0.95 for stress GLS, and 0.92 for Δ GLS; corresponding interobserver ICCs were 0.93, 0.91, and 0.88. Circumferential-strain agreement was similarly good to excellent, and Bland–Altman biases were small (Table 5).

Table 5
Intraobserver and interobserver reproducibility of myocardial strain measures

Variable	Intraobserver ICC (95% CI)	Intraobserver bias (95% LoA)	Interobserver ICC (95% CI)	Interobserver bias (95% LoA)
Resting GLS	0.96 (0.87–0.99)	0.10 (– 0.80 to 1.00)	0.93 (0.78–0.98)	–0.20 (– 1.30 to 0.90)
Stress GLS	0.95 (0.84–0.99)	0.12 (– 0.91 to 1.15)	0.91 (0.72–0.97)	–0.24 (– 1.52 to 1.04)
ΔGLS	0.92 (0.75–0.98)	0.10 (– 1.00 to 1.20)	0.88 (0.64–0.96)	–0.20 (– 1.60 to 1.20)
Resting GCS	0.95 (0.84–0.99)	0.16 (– 1.25 to 1.57)	0.91 (0.72–0.97)	–0.28 (– 1.82 to 1.26)
Stress GCS	0.94 (0.81–0.98)	0.20 (– 1.39 to 1.79)	0.90 (0.69–0.97)	–0.31 (– 2.02 to 1.40)
ΔGCS	0.90 (0.69–0.97)	0.18 (– 1.42 to 1.78)	0.86 (0.59–0.95)	–0.30 (– 2.00 to 1.40)
Values are presented as ICC (95% CI) or mean bias (lower to upper 95% limits of agreement). Bias represents the mean paired difference, and the 95% LoA were calculated as bias $\pm$ 1.96 SD of the paired differences. Δ was calculated as peak stress minus baseline. CI, confidence interval; GCS, global circumferential strain; GLS, global longitudinal strain; ICC, intraclass correlation coefficient; LoA, limits of agreement.				

Discussion

The principal finding of this study is that preserved chamber-level left ventricular systolic function masks a selective impairment of longitudinal deformation reserve during late recovery from experimental KD. Eight weeks after LCWE exposure, LVEF and LVFS were preserved at rest and augmented comparably during dobutamine stress, whereas modest reductions in resting GLS and GCS were accompanied by marked attenuation of GLS reserve. This discordance indicates that conventional systolic indices may remain compensated despite persistent impairment of longitudinal myocardial recruitment, which becomes evident only under adrenergic challenge. Importantly, GLS reserve but not resting GLS was strongly associated with myocardial collagen burden, supporting a structure–function relationship between residual extracellular matrix remodeling and impaired contractile reserve. These findings support stress-derived GLS reserve as a candidate marker of incomplete myocardial recovery beyond conventional chamber-level measures.

This functional dissociation is physiologically plausible. Both LVEF and LVFS are chamber-level indices influenced by loading conditions and ventricular geometry assumption [12, 30]. Modeling and human imaging data showed that circumferential shortening contributes more to LVEF than longitudinal shortening, allowing chamber ejection to remain preserved despite impaired longitudinal mechanics [30]. Relatively preserved circumferential and radial mechanics may therefore compensate for impaired longitudinal recruitment and maintain chamber-level systolic performance. Dobutamine increases

myocardial workload and the requirement for coordinated fiber recruitment, thereby exposing limited longitudinal reserve that remains inconspicuous at rest. In other cardiac diseases, stress-derived LVEF reserve has provided incremental prognostic information [13], whereas dobutamine CMR-derived contractile reserve has predicted subsequent LV remodeling [14]. Accordingly, preserved LVEF augmentation during stress should not be equated with complete myocardial recovery.

Cardiovascular magnetic resonance studies provide complementary evidence that myocardial abnormalities may persist after KD. Muthusami et al. showed that extracellular volume expansion extended beyond regions of late gadolinium enhancement and that impaired myocardial perfusion was associated with reduced circumferential mechanics, linking tissue remodeling and perfusion abnormalities to regional contractile dysfunction [6]. In another small cohort, late gadolinium enhancement was detected in 5 of 13 patients (38%). The cohort was heavily enriched for coronary disease, with coronary aneurysms present in 12 patients, yet extracellular volume did not differ from that of controls at the group level [7]. These findings suggest that late myocardial involvement after KD is spatially heterogeneous and may manifest as focal fibrosis or regional perfusion and mechanical abnormalities without diffuse extracellular volume expansion. However, the small, aneurysm-enriched cohorts limit generalizability to patients without major coronary sequelae and do not establish whether residual fibrosis contributes to impaired myocardial reserve when resting function is preserved. Prior LCWE studies similarly showed that recovered mice remained susceptible to myocardial fibrosis and dysfunction during adrenergic stress [18]. Our findings extend these observations by demonstrating consistently elevated CVF across two independent LCWE-exposed cohorts and, within LCWE-exposed animals, a strong inverse association between collagen volume fraction and GLS reserve. Collectively, these findings support a structure–function link in which residual extracellular matrix remodeling limits longitudinal recruitment under stress. Interstitial collagen accumulation may increase myocardial stiffness and disrupt force transmission, thereby constraining deformation during adrenergic stimulation [31].

Subclinical myocardial dysfunction despite preserved conventional systolic indices has been reported during acute and intermediate-term KD [8, 9, 11], although a longer-term study found no group-level strain abnormality [10], underscoring heterogeneity across disease stages and study populations. Building on these observations, our previous clinical study identified impaired deformation reserve during convalescence [15], and our serial LCWE study showed that LVEF normalized by day 28 while longitudinal strain remained impaired through day 56 [23]. The present study advances this work by demonstrating a selective impairment of longitudinal deformation reserve and linking this functional deficit to histologically quantified myocardial collagen burden. Together, these findings support a staged recovery phenotype in which chamber-level systolic function normalizes before myocardial mechanics, leaving a compensated abnormality that becomes evident under adrenergic stress. This framework provides a mechanistic basis for evaluating stress deformation imaging in patients with a history of KD and preserved resting LVEF.

Several methodological features strengthen the interpretation of these findings. Blinded analysis and paired rest–stress imaging reduced measurement variability, while comparable chronotropic responses limited heart rate response as an explanation for the between-group differences. Concordant increases in CVF across drug-unexposed and post-stress cohorts further indicate that the histological findings were

not attributable to acute dobutamine exposure. Moreover, baseline-adjusted analysis confirmed that the lower peak-stress GLS in LCWE-exposed animals was not explained solely by the modest resting difference. Resting and stress-derived strain measurements demonstrated good-to-excellent reproducibility.

Limitations

Several limitations merit consideration. The modest sample size limited statistical precision and rendered the multivariable analyses exploratory. Nevertheless, concordant histological findings across independent cohorts, paired rest–stress imaging, baseline-adjusted analyses, and good-to-excellent reproducibility support the internal consistency of the results. As a mechanistic proof-of-concept study, the cross-sectional association between CVF and GLS reserve establishes neither causality nor mechanistic specificity. CVF quantifies overall collagen burden but not its spatial distribution, architecture, or cross-linking, and contributions from microvascular dysfunction, impaired perfusion, residual inflammation, or altered adrenergic signaling cannot be excluded. Murine β -adrenergic responses are protocol and loading dependent [32, 33]; therefore, the absence of blood-pressure measurements leaves residual hemodynamic confounding. Moreover, M-mode indices are geometry dependent, and deformation analysis during stress remains technically challenging at murine heart rates [33]. Finally, the LCWE model reproduces selected, but not all, features of human KD [34]. Larger longitudinal studies integrating direct volumetry, comprehensive hemodynamic assessment, spatial tissue characterization, and clinical validation are required to define the mechanistic and translational significance of GLS reserve.

Conclusions

During late recovery from LCWE-induced experimental KD, preserved LVEF and LVFS responses to dobutamine masked a selective impairment of longitudinal deformation reserve. The strong inverse association between GLS reserve and myocardial collagen burden within LCWE-exposed animals supports stress-derived GLS reserve as a candidate marker of incomplete myocardial recovery beyond conventional systolic indices.

Declarations

Statement of Ethics

This study were approved by the Institutional Animal Care and Use Committee of Gansu Provincial Hospital, approval number (GSSYY-2025-135).

Clinical trial number

Not applicable.

Conflict of Interest Statement

The authors have no potential conflicts of interest to disclose.

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Author Contribution

Haiyong Wang: formal analysis, investigation, writing-original draft, review and editing. Yunshan Zhou: formal analysis, review, editing, project administration and resources. Dong Wang: project administration and resources. Nan Wang: project administration, resources, and supervision.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

References

1. Jone PN, Tremoulet AH, Choueiter NF, et al. Update on diagnosis and management of Kawasaki disease: a scientific statement from the American Heart Association. *Circulation*. 2024;150:e481–500. 10.1161/CIR.0000000000001295.
2. McCrindle BW, Rowley AH, Newburger JW, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a scientific statement for health professionals from the American Heart Association. *Circulation*. 2017;135:e927–99. 10.1161/CIR.0000000000000484.
3. Harada M, Yokouchi Y, Oharaseki T, et al. Histopathological characteristics of myocarditis in acute-phase Kawasaki disease. *Histopathology*. 2012;61:1156–67.
4. Yutani C, Go S, Kamiya T, et al. Cardiac biopsy of Kawasaki disease. *Arch Pathol Lab Med*. 1981;105:470–3.
5. Liu AM, Ghazizadeh M, Onouchi Z, Asano G. Ultrastructural characteristics of myocardial and coronary microvascular lesions in Kawasaki disease. *Microvasc Res*. 1999;58:10–27.
6. Muthusami P, Luining W, McCrindle B, et al. Myocardial perfusion, fibrosis, and contractility in children with Kawasaki disease. *JACC Cardiovasc Imaging*. 2018;11:1922–4. 10.1016/j.jcmg.2018.06.009.
7. Dusenbery SM, Newburger JW, Alexander ME, et al. Myocardial fibrosis in patients with a history of Kawasaki disease. *Int J Cardiol Heart Vasc*. 2021;32:100713. 10.1016/j.ijcha.2021.100713.
8. McCandless RT, Minich LL, Wilkinson SE, et al. Myocardial strain and strain rate in Kawasaki disease. *Eur Heart J Cardiovasc Imaging*. 2013;14:1061–8. 10.1093/ehjci/jet041.

9. Dedeoglu R, Barut K, Oztunc F, et al. Evaluation of myocardial deformation in patients with Kawasaki disease using speckle-tracking echocardiography during mid-term follow-up. *Cardiol Young*. 2017;27:1377–85. 10.1017/S1047951117000580.
10. Kayabey Ö, Tuncer T, Deveci M, et al. Is there myocardial involvement in children with long-term follow-up for Kawasaki disease? *Turk Arch Pediatr*. 2021;56:44–50. 10.5152/TurkArchPediatr.2020.20193.
11. Wang H, Tong M, Mu J, Wu T, Ruan L. Assessment of myocardial function by two-dimensional speckle tracking echocardiography in patients with Kawasaki disease: a mid-term follow-up study. *Coron Artery Dis*. 2021;32:500–8. 10.1097/MCA.0000000000000981.
12. Katsi V, Georgiopoulos G, Laina A, et al. Left ventricular ejection fraction as therapeutic target: is it the ideal marker? *Heart Fail Rev*. 2017;22:641–55. 10.1007/s10741-017-9624-5.
13. Tamarappoo BK, Ling LF, Cerqueira M, Hachamovitch R. Independent prognostic value of left ventricular contractile reserve and chronotropic response in patients with reduced left ventricular ejection fraction undergoing vasodilator stress myocardial perfusion imaging with Rb-82 positron emission tomography. *Eur Heart J Cardiovasc Imaging*. 2018;19:442–9. 10.1093/ehjci/jex157.
14. Tayal U, Wage R, Newsome S, et al. Predictors of left ventricular remodelling in dilated cardiomyopathy: a cardiovascular magnetic resonance study. *Eur J Heart Fail*. 2020;22:1160–70. 10.1002/ejhf.1734.
15. Wang H, Long X, Han Z, Dong X, Yan R. Myocardial contractile reserve impairment in patients with Kawasaki disease during convalescent phase: a study based on two-dimensional speckle-tracking stress echocardiography. *Pediatr Cardiol*. 2026;47:229–39. 10.1007/s00246-024-03751-y.
16. Rosenkranz ME, Schulte DJ, Agle LMA, et al. TLR2 and MyD88 contribute to *Lactobacillus casei* extract-induced focal coronary arteritis. *Circulation*. 2005;112:2966–73. 10.1161/CIRCULATIONAHA.105.537530.
17. Soni PR, Noval Rivas M, Arditi M. A comprehensive update on Kawasaki disease vasculitis and myocarditis. *Curr Rheumatol Rep*. 2020;22:6. 10.1007/s11926-020-0882-1.
18. Matundan HH, Sin J, Noval Rivas M, et al. Myocardial fibrosis after adrenergic stimulation as a long-term sequela in a mouse model of Kawasaki disease vasculitis. *JCI Insight*. 2019;4:e126279. 10.1172/jci.insight.126279.
19. Percie du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0. *PLoS Biol*. 2020;18:e3000410. 10.1371/journal.pbio.3000410.
20. Lee Y, Schulte DJ, Shimada K, et al. Interleukin-1 β is crucial for coronary artery inflammation in a mouse model of Kawasaki disease. *Circulation*. 2012;125:1542–50. 10.1161/CIRCULATIONAHA.111.072769.
21. Lindsey ML, Kassiri Z, Virag JAI, et al. Guidelines for measuring cardiac physiology in mice. *Am J Physiol Heart Circ Physiol*. 2018;314:H733–52. 10.1152/ajpheart.00339.2017.
22. Donner DG, Kiriazis H, Du XJ, et al. Improving the quality of preclinical research echocardiography. *Am J Physiol Heart Circ Physiol*. 2018;315:H58–70. 10.1152/ajpheart.00157.2018.

23. Wang H, Han Z, Zhou Y, Dong X, Wang N. Dynamic assessment of myocardial contractile dysfunction and its recovery after IVIG treatment in a murine model of Kawasaki disease using high-resolution speckle-tracking echocardiography. *Front Cardiovasc Med.* 2026;13:1695337. 10.3389/fcvm.2026.1695337.
24. Li Z, Li Y, Zhang L, et al. Reduced myocardial reserve in young X-linked muscular dystrophy mice diagnosed by two-dimensional strain analysis combined with stress echocardiography. *J Am Soc Echocardiogr.* 2017;30:815–e8279. 10.1016/j.echo.2017.03.009.
25. Voigt JU, Pedrizzetti G, Lysyansky P, et al. Definitions for a common standard for 2D speckle tracking echocardiography. *Eur Heart J Cardiovasc Imaging.* 2015;16:1–11. 10.1093/ehjci/jeu184.
26. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods.* 2012;9:671–5. 10.1038/nmeth.2089.
27. Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open-source platform for biological-image analysis. *Nat Methods.* 2012;9:676–82. 10.1038/nmeth.2019.
28. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients. *J Chiropr Med.* 2016;15:155–63. 10.1016/j.jcm.2016.02.012.
29. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet.* 1986;1:307–10.
30. Stokke TM, Hasselberg NE, Smedsrud MK, et al. Geometry as a confounder when assessing ventricular systolic function: comparison between ejection fraction and strain. *J Am Coll Cardiol.* 2017;70:942–54. 10.1016/j.jacc.2017.06.046.
31. Frangogiannis NG. Cardiac fibrosis. *Cardiovasc Res.* 2021;117:1450–88. 10.1093/cvr/cvaa324.
32. Porritt RA, Markman JL, Maruyama D, et al. Interleukin-1 beta-mediated sex differences in Kawasaki disease vasculitis development and response to treatment. *Arterioscler Thromb Vasc Biol.* 2020;40:802–18. 10.1161/ATVBAHA.119.313863.
33. Wang X, Wei Z, Wang P, et al. Echocardiographic evaluation of cardiac reserve to detect subtle cardiac dysfunction in mice. *Life Sci.* 2023;331:122079. 10.1016/j.lfs.2023.122079.
34. Zacchigna S, Paldino A, Falcão-Pires I, et al. Towards standardization of echocardiography for the evaluation of left ventricular function in adult rodents: a position paper of the ESC Working Group on Myocardial Function. *Cardiovasc Res.* 2021;117:43–59. 10.1093/cvr/cvaa110.
35. Noval Rivas M, Arditi M. Kawasaki disease: pathophysiology and insights from mouse models. *Nat Rev Rheumatol.* 2020;16:391–405. 10.1038/s41584-020-0426-0.

Figures

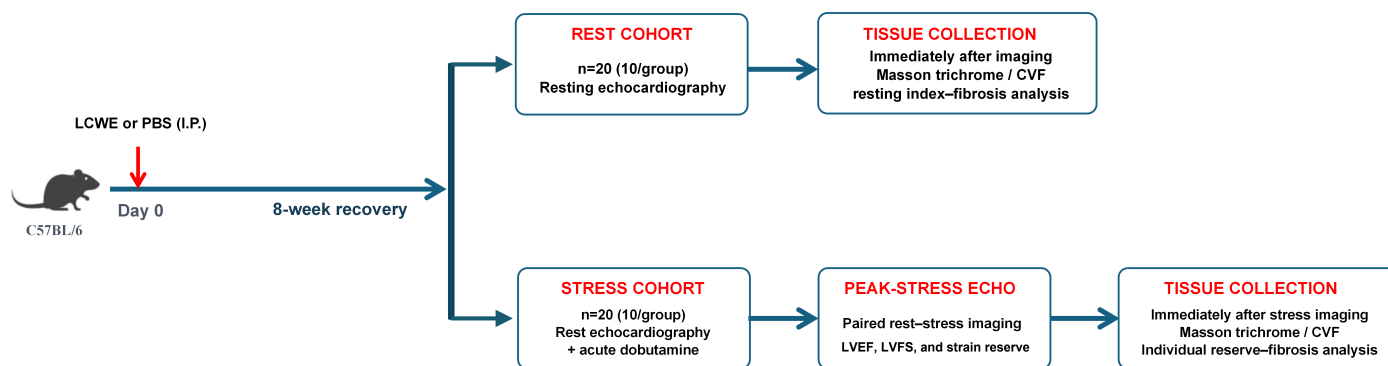


Figure 1

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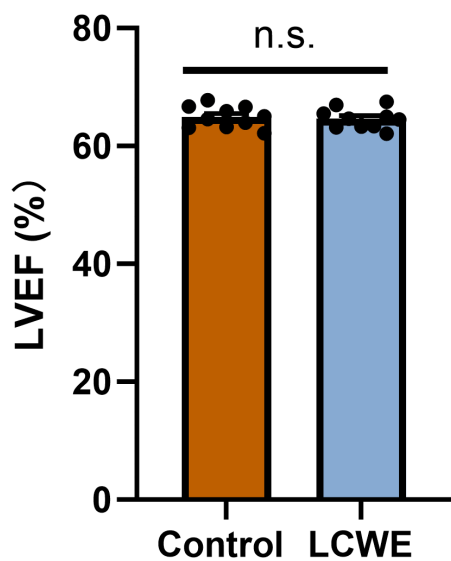
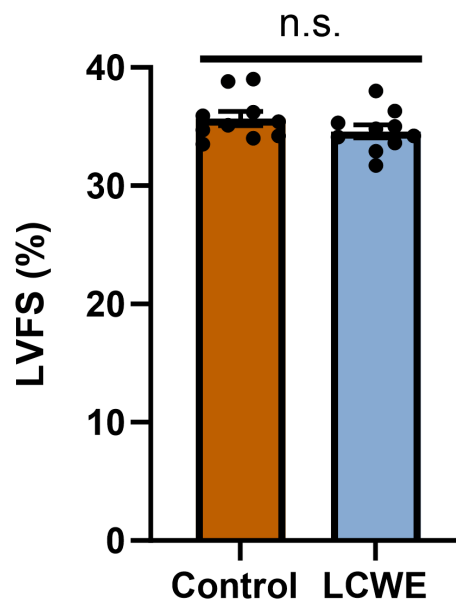
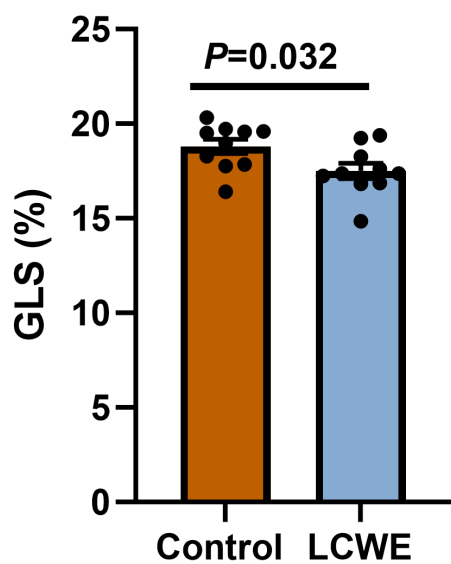
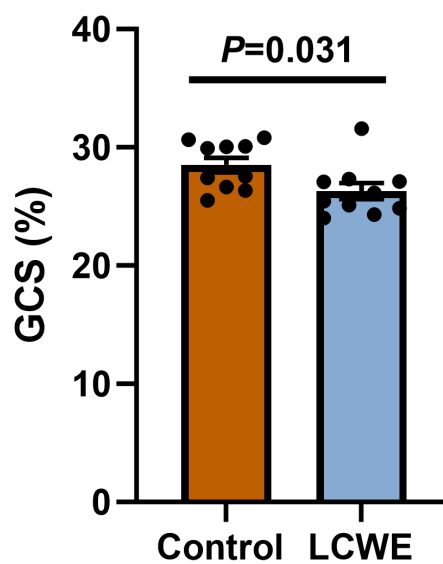
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Figure 2

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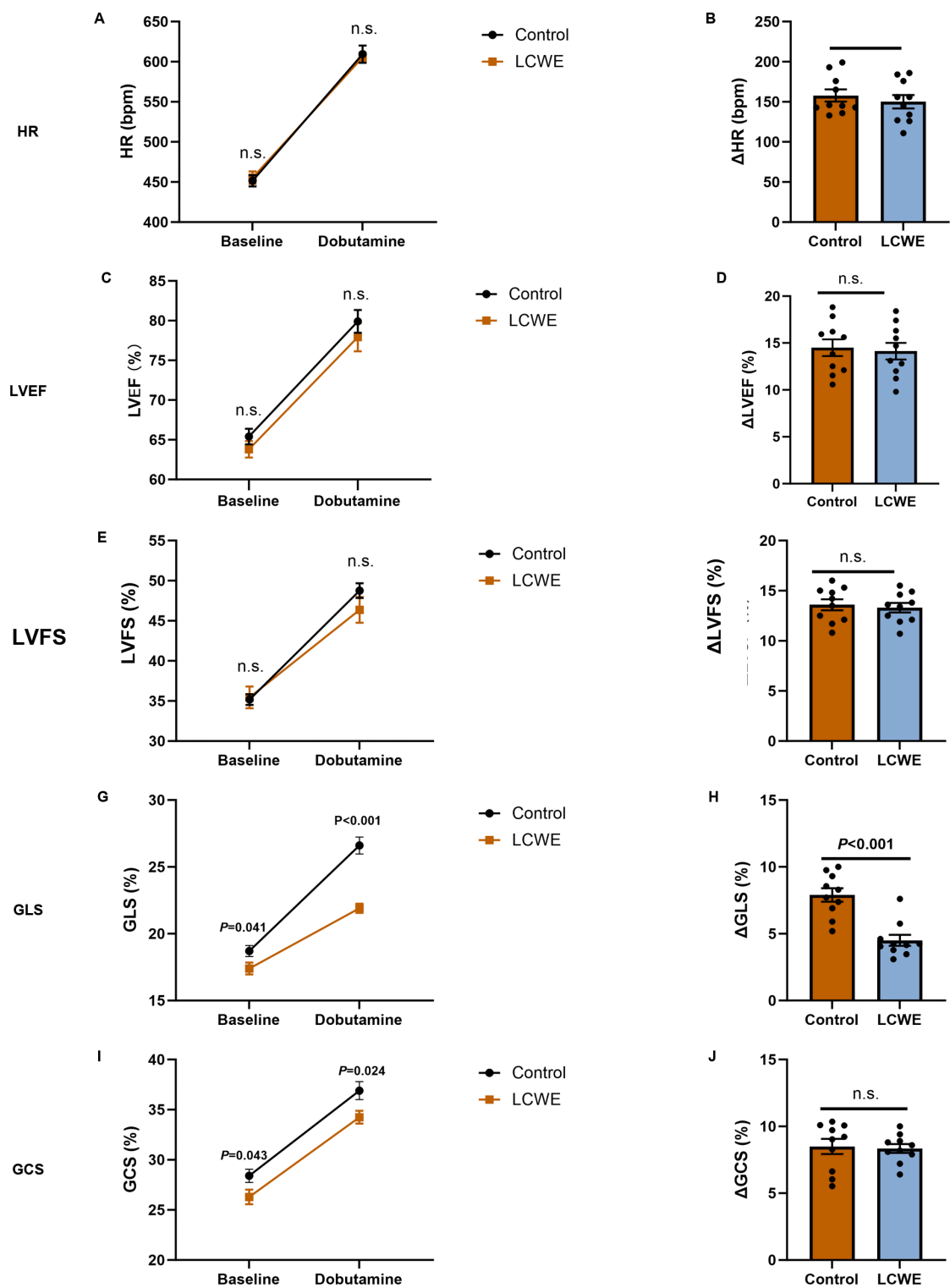


Figure 3

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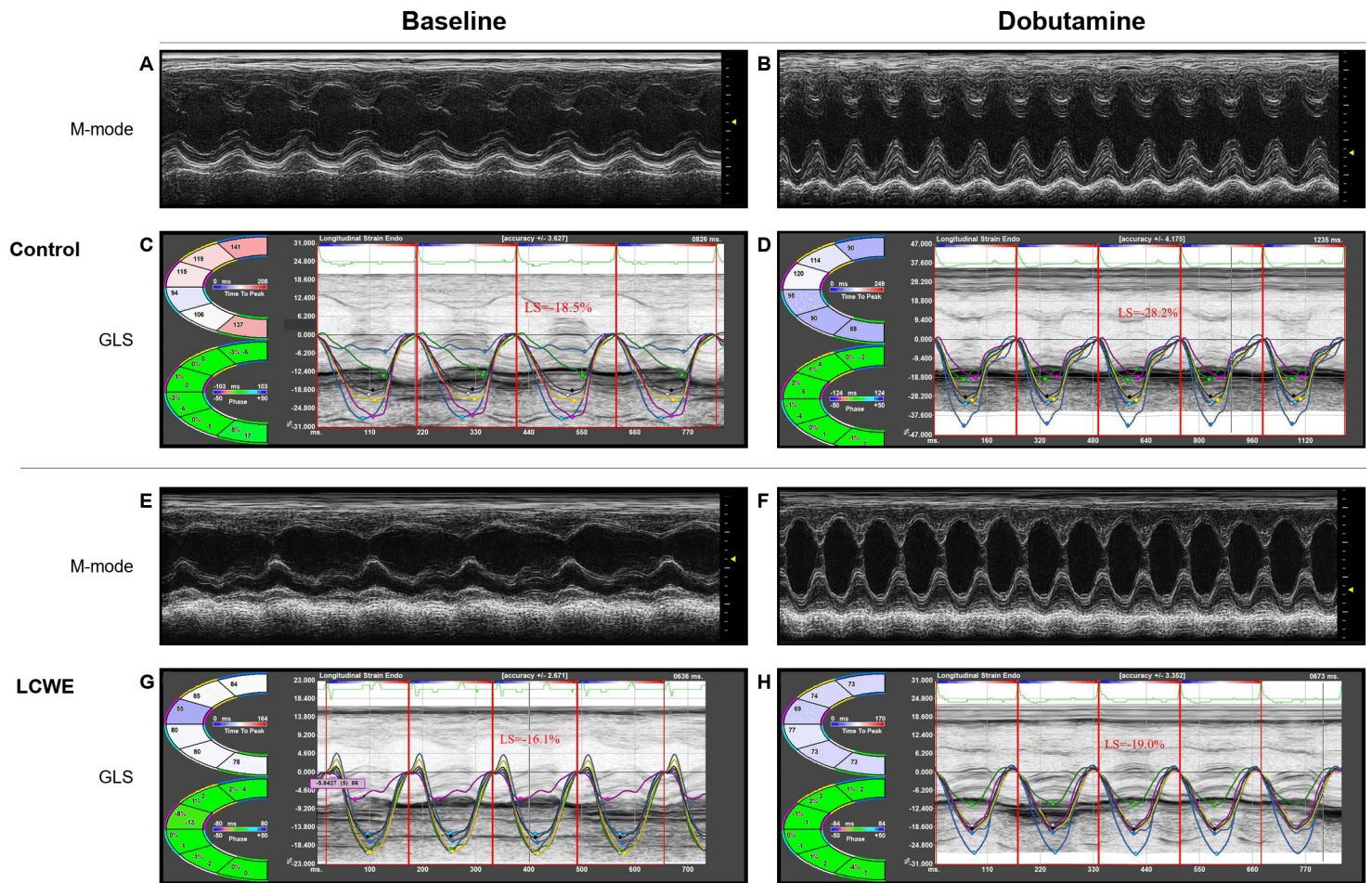


Figure 4

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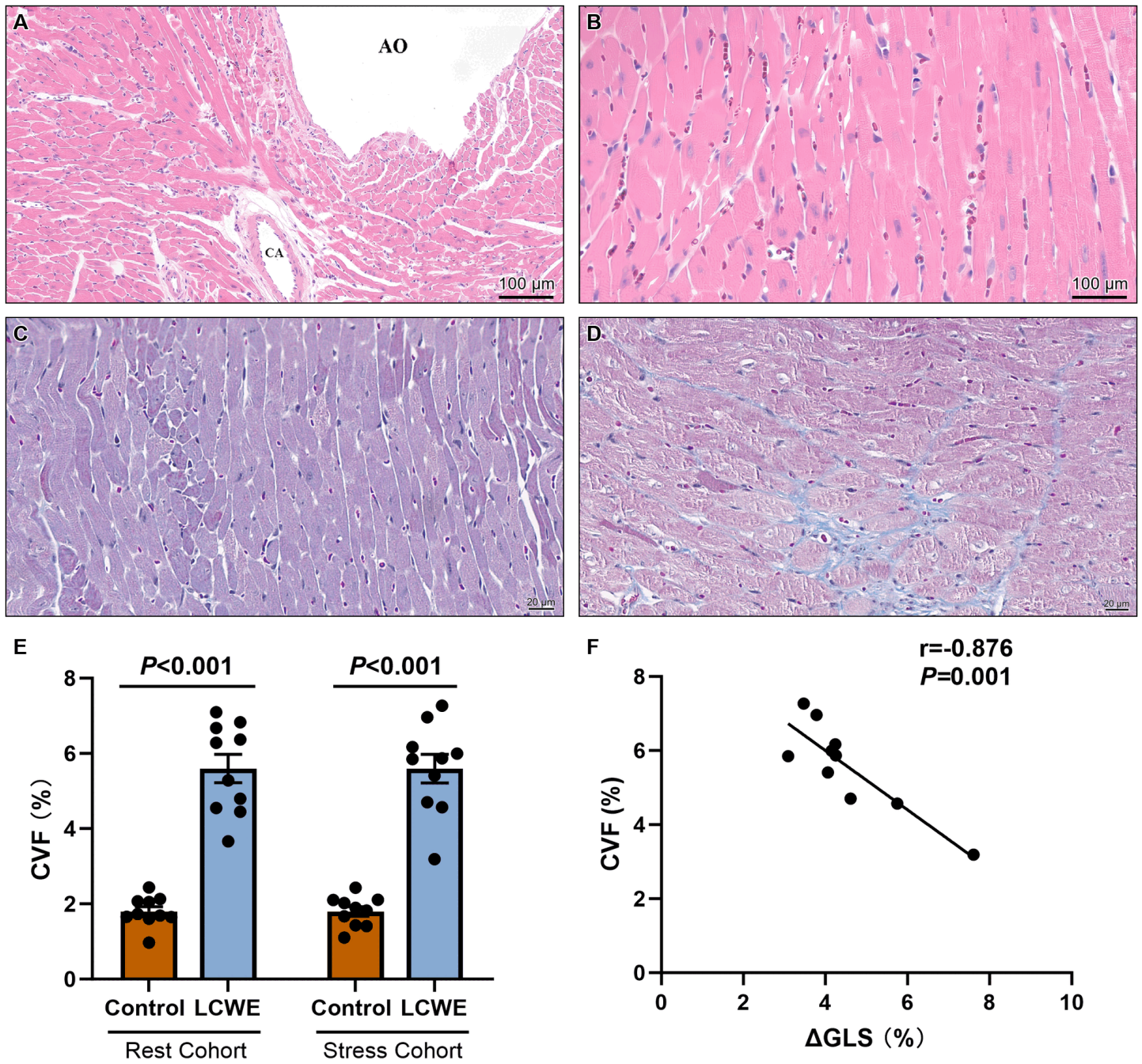


Figure 5

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