Dobutamine Stress and Speckle-Tracking Echocardiography Unmask Impaired Diastolic Reserve in a Two-Hit Experimental HFpEF Model

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Background

Heart failure with preserved ejection fraction (HFpEF) accounts for approximately half of heart failure cases and remains difficult to diagnose early. Diastolic dysfunction is a hallmark of HFpEF, but early abnormalities may be subtle or absent under resting conditions. Assessment of cardiac reserve may reveal functional abnormalities before overt HFpEF develops. We tested whether dobutamine stress echocardiography combined with speckle-tracking echocardiography (STE) could detect early diastolic reserve impairment in a two-hit experimental HFpEF model.

Objective

To determine whether 12 weeks of high-fat diet plus L-NAME (HFD+L) exposure induces early HFpEF-like remodeling and impaired diastolic reserve detectable by dobutamine stress echocardiography combined with STE.

Key Take-Home Message

Despite preserved ejection fraction and largely preserved systolic reserve, HFD+L mice exhibited impaired diastolic deformation reserve that was more clearly revealed during dobutamine stress. Stress STE identified abnormalities not fully captured by conventional resting echocardiography.

Methods

A total of 24 male AKR/J mice (Jackson Laboratories, 7−8 weeks old, body weight range at study initiation: 20.9 g–29.0 g) were assigned to either the low-fat diet (LFD) control group (n=14) or HFD+L treatment group (n=10) for 12 weeks. Conventional echocardiography and STE were performed under isoflurane anesthesia (2−3% induction; 1−2% maintenance) at rest and during dobutamine stress to assess cardiac structure, systolic reserve and diastolic deformation reserve. Body temperature and heart rate (HR) were monitored throughout the procedure.

Group	Diet and Water
Low-Fat Diet (LFD)	10% kcal from fat (Teklad, TD.08806) + tap water *ad libitum*
High-Fat Diet + L-NAME (HFD+L)	60% kcal from fat (Teklad, TD.06414) + L-NAME (0.5 g/L) *ad libitum*

Echocardiography:
Cardiac structure and function were assessed by conventional echocardiography using Vevo 2100 (FUJIFILM VisualSonics) equipped with a 40-MHz solid state transducer (MS550). STE analysis at rest and during dobutamine stress was performed using the Vevo Strain Software (Vevo Strain 1.0).

All measurements and calculations were performed according to the American Society of Echocardiography guidelines. Briefly, left ventricular (LV) M-mode echocardiography was performed in a parasternal short axis view at the mid papillary level to measure LV wall and chamber dimensions. Left atrial (LA) M-mode echocardiography was performed in a parasternal long axis view by placing the sample volume at the region of the aortic root and left atria to measure LA dimension. Pulsed-wave Doppler was acquired from the apical 4-chamber view to assess LV diastolic function.

High-quality PLAX B-mode images were collected for STE analysis. The time at maximum relaxation (end-diastole) was used to determine the starting phase of the strain trace. Global Longitudinal Strain (GLS) was used for systolic function evaluation. To measure longitudinal and radial strain and strain rate during early LV filling, the "reverse peak" algorithm of the Vevo Strain Software was utilized, these parameters were reported as diastolic radial/longitudinal strain and strain rate. After completing the echocardiography assessment as described above and while the animal was still anesthetized, the positive inotropic agent dobutamine (1 mg/kg, IP) was given. Once the HR increased and remained stable for approximately 30 seconds, PLAX B-mode images were collected for strain analysis. LV M-mode imaging in the parasternal short-axis view and pulsed-wave Doppler from the apical 4-chamber view were repeated.

Data Analysis:
Statistical analyses were performed using GraphPad Prism 11.0.0. Between-group comparisons, including reserve responses calculated from rest to dobutamine, were performed using an unpaired two-tailed t-test with Welch's correction. A p-value of < .05 was considered statistically significant.

Results

Animal In-Life

- All animals gained weight between Study Day 1 and Study Day 81. Mice fed LFD gained 16 ± 2 g over the 12-week study (+63%); mice fed HFD+L gained 19 ±1 g (+77%) over the 12-week study.
- A total of 4 animals (n=2/group) died prior to terminal procedures during the conduct of study-related procedures.
- No relevant clinical or necropsy observations were noted in the mice that survived to the terminal procedures..

Ejection Fraction Was Preserved in Both Groups at 12 Weeks

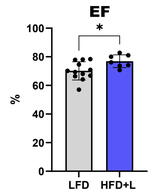

Figure 1. During resting echocardiography at 12 weeks, ejection fraction (EF) was preserved in both groups and was slightly higher in mice fed HFD+L than mice fed LFD (+77% vs +70%, respectively, *p < .05).

Left Ventricular Concentric Remodeling Occurred in Mice Fed High-Fat Diet + L-NAME as Compared to Low-Fat Diet

- Mice fed HFD+L diet had a statistically significant increase in relative wall thickness (RWT) and a reduction in LV internal diameter in diastole (LVIDd).
- Both end-diastolic and end-systolic volumes (LVEDV, LVESV) were significantly smaller in the HFD+L group compared with the LFD group.
- LA size in HFD+L mice was slightly increased compared with LFD mice but did not reach statistical significance.

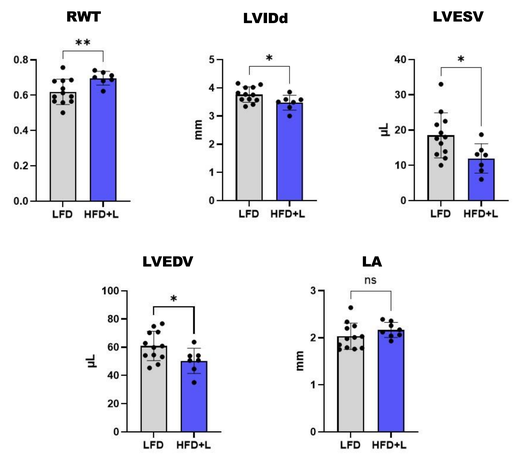

Figure 2. Statistically significant differences were noted in LV morphology in the mice fed HFD+L vs LFD (*p < .05; **p < .01; ns, not significant).

At 12 Weeks, Resting Conventional Echocardiography Detected Only Mild Impairment in Relaxation in Mice Fed High-Fat Diet + L-NAME as Compared to Low-Fat Diet

- E (velocity of blood flow across the mitral valve during early diastole) and A (velocity of blood flow across the mitral valve during atrial contraction) were similar in mice fed HFD+L and LFD.
- Isovolumic relaxation time (IVRT) was slightly increased in mice fed HFD+L mice as compared with LFD though this difference did not reach statistical significance.

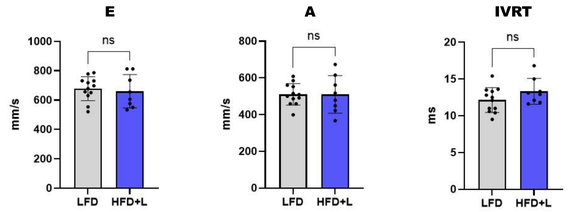

Figure 3. Resting echocardiography showed similar transmitral E and A velocities between groups, while IVRT was slightly increased in HFD+L mice but did not reach statistical significance (ns, not significant).

Dobutamine Stress Echocardiography Was Performed to Evaluate Cardiac Function Beyond Resting Conditions, which Showed Largely Preserved Cardiac Systolic Reserve, with Modest Attenuation of EF Reserve in Mice Fed a High-Fat Diet + L-NAME

- Dobutamine stress did not reveal significant differences in HR reserve between mice fed LFD (+11%) and HFD+L (+20%).
- Despite LV remodeling, mice fed HFD+L demonstrated a reduction in LVESV similar to that observed in mice fed LFD.
- GLS did not differ significantly between groups during dobutamine stress, consistent with largely preserved systolic deformation.
- During dobutamine stress, EF increased by 21% in HFD+L fed mice versus 30% in LFD fed mice, suggesting largely preserved systolic reserve with modest attenuation of EF reserve in HFD+L mice.

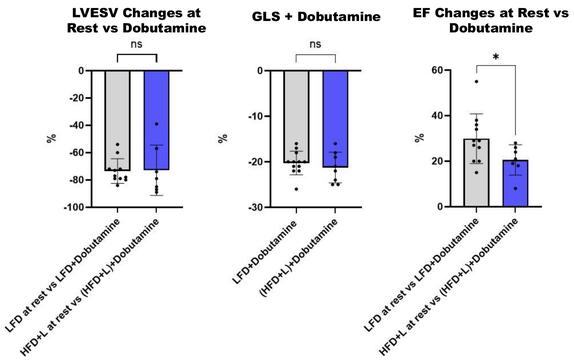

Figure 4. Dobutamine stress echocardiography showed a preserved systolic reserve with a slight, yet statistically significant, attenuation of EF reserve in HFD+L mice (*p < .05; ns, not significant).

Dobutamine Stress STE Revealed Impaired Diastolic Deformation Reserve in HFD+L mice

- Early diastolic radial and longitudinal strain were reduced in HFD+L fed mice at rest as compared with LFD fed mice, with blunted augmentation during dobutamine stress, consistent with impaired myocardial relaxation.

Parameter	LFD at Rest	LFD + Dob	LFD Reserve	HFD+L at Rest	HFD+L + Dob	HFD+L Reserve
Radial Diastolic Strain Average (%)	-0.37 ± 0.40 (n=12)	-1.25 ± 1.50 (n=12)	+241%	-0.16 ± 0.15 (n=8)	-0.45 ± 0.44 (n=8)	+173%
Longitudinal Diastolic Strain Average (%)	0.41 ± 0.42 (n=12)	1.28 ± 1.64 (n=12)	+209%	0.26 ± 0.17 (n=8)	0.51 ± 0.51 (n=8)	+100%

Dob, dobutamine; HFD+L, high-fat diet + L-NAME; LFD, low-fat diet.

Table 1. Early diastolic deformation reserve assessed by STE. Values are mean ± SD. Reserve was calculated as percent change from rest to following dobutamine dose using absolute mean strain magnitude.

- Despite reduced diastolic strain magnitude, early diastolic strain rate increased with dobutamine in HFD+L mice, revealing a dissociation between diastolic strain magnitude and strain rate, suggesting altered myocardial relaxation mechanics.

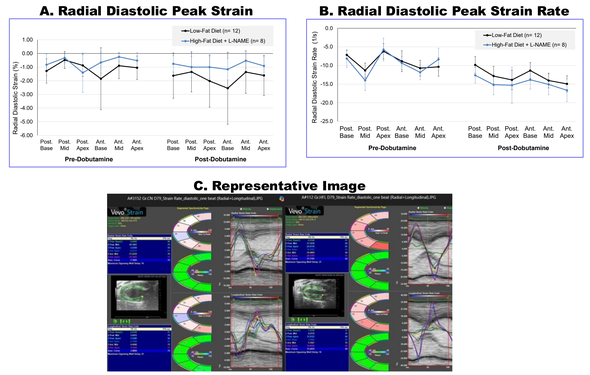

Figure 5. A) Regional early diastolic radial strain at rest and during dobutamine stress.; B) Regional early diastolic radial strain rate at rest and during dobutamine stress; C) Representative diastolic strain rates curves [LFD vs (HFD+L) + Dobutamine].

Conclusions

- 12 weeks of HFD+L induced concentric LV remodeling while preserving resting EF.
- Conventional resting echocardiography detected structural remodeling and mild relaxation impairment but did not fully reveal abnormalities in cardiac functional reserve.
- Dobutamine stress echocardiography combined with STE uncovered impaired early diastolic deformation reserve despite largely preserved systolic reserve.
- These findings support stress STE as a sensitive approach for detecting early impairment of diastolic reserve in experimental HFpEF-like model.

Disclosures

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