Supplemental Figure Legends

Supplemental Figure I. IL-17A deficiency did not affect atherosclerotic plaque formation in ApoE$^{-/-}$ mice under normal chow diet feeding.

Atherosclerotic plaque formation was quantitatively analyzed by staining of aortae with oil red O. ApoE$^{-/-}$ and ApoE$^{-/-}$IL-17A$^{-/-}$ mice: day 0 (n = 5; each group), 8 weeks after normal chow diet feeding (normal diet 8w; n =4 and 3, respectively), 16 weeks after normal chow diet feeding (normal diet16w; n = 6; each group). *$p < 0.05$. **$p < 0.005$. N.S. denotes difference between two groups is not significantly different.

Supplemental Figure II. Atherosclerotic plaque area of abdominal aorta area was decreased in ApoE$^{-/-}$IL-17A$^{-/-}$ mice compared to ApoE$^{-/-}$ mice.

A, Representative microphotographs of abdominal aortic sections stained with H&E in ApoE$^{-/-}$ and ApoE$^{-/-}$IL-17A$^{-/-}$ mice after 8 weeks of HFD feeding. Scale bars indicate 30 μm. B, Quantitative analysis of plaque areas in both ApoE$^{-/-}$ (n= 8) and ApoE$^{-/-}$IL-17A$^{-/-}$ (n= 8) mice. *$p < 0.05$.

Supplemental Figure III. Type I collagen-positive area was decreased in ApoE$^{-/-}$IL-17A$^{-/-}$ mice compared to ApoE$^{-/-}$ mice

A, Representative microphotographs of aortic root sections stained with type I collagen in ApoE$^{-/-}$ and ApoE$^{-/-}$IL-17A$^{-/-}$ mice after 8 weeks of HFD feeding. Scale bars in upper panels indicate 300 μm and in under panels indicate 50 μm. B, Quantitative analysis of the percentage of type I collagen-positive areas in both ApoE$^{-/-}$ (n= 4) and ApoE$^{-/-}$IL-17A$^{-/-}$ (n= 11) mice. *$p < 0.05$.

Supplemental Figure IV. IL-17A deficiency did not significantly affect IL-4, IL-6, IL-10, and IL-17C production in ApoE$^{-/-}$ mice.

Quantitative analysis of IL-4 (A), IL-6 (B), IL-10(C), and IL-17C (D) production in the supernatants of splenic CD4-positive T cells from ApoE$^{-/-}$ and ApoE$^{-/-}$IL-17A$^{-/-}$ mice before (n= 4 and 6, respectively) and after 8 (n= 5 and 8, respectively) or 16 (n= 4 and 7, respectively) weeks of HFD feeding. Splenic CD4-positive T cells were cultured *in vitro* with PMA and ionomycin; culture supernatants were examined by ELISA. Data were obtained from at least three independent experiments. *$p < 0.05$. N.D. denotes not detectable.

Supplemental Figure V. IL-17A deficiency did not significantly affect production of MDA-LDL-specific IgG_{2a}, IgG, and IgM antibodies in $ApoE^{-/-}$ mice after HFD feeding.

Quantitative analysis of titers of MDA-LDL-specific antibodies, IgG2a(A), IgG(B), and IgM(C) in $ApoE^{-/-}$ (n= 21) and $ApoE^{-/-}IL\text{-}17A^{-/-}$ (n= 35) mice before and after 8 or 16 weeks of HFD feeding. Values are indicated by the relative protein levels against MDA-LDL-specific antibody titers of $ApoE^{-/-}$ mice at day 0 and value at day 0 was set as 1. $*p < 0.05$. $***p < 0.0005$. N.S., not significantly different. Note that only IgM class of anti- MDA-LDL antibody was reduced in $ApoE^{-/-}IL\text{-}17A^{-/-}$ compared to $ApoE^{-/-}$ mice at 16 weeks after HFD feeding.

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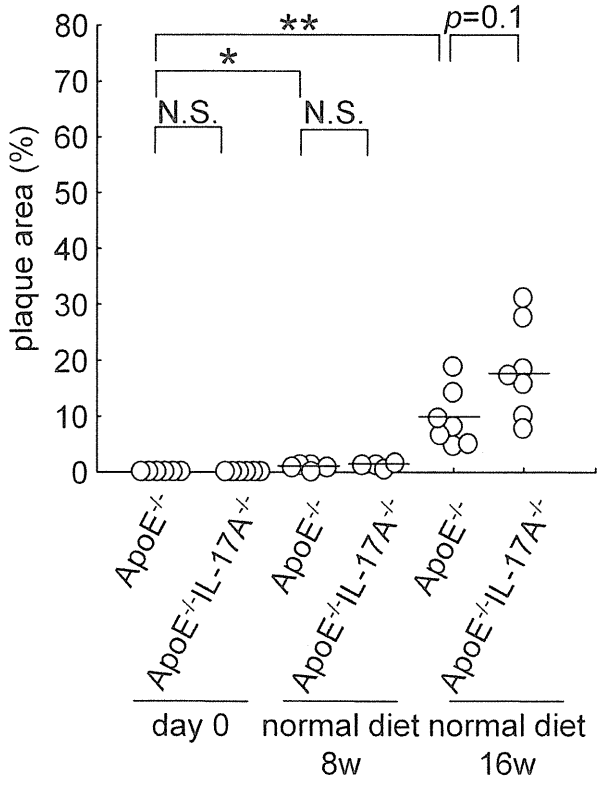
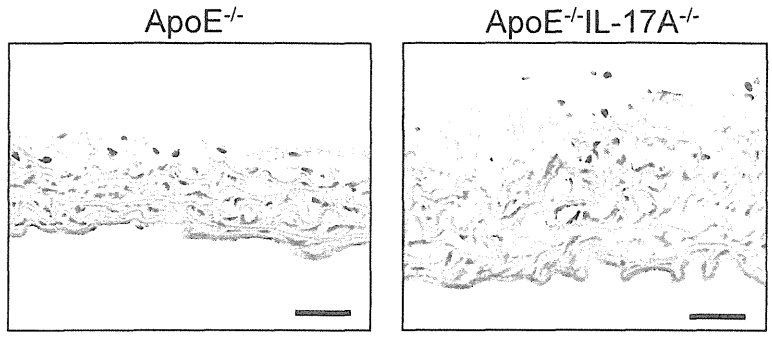


Supplemental Figure I

A



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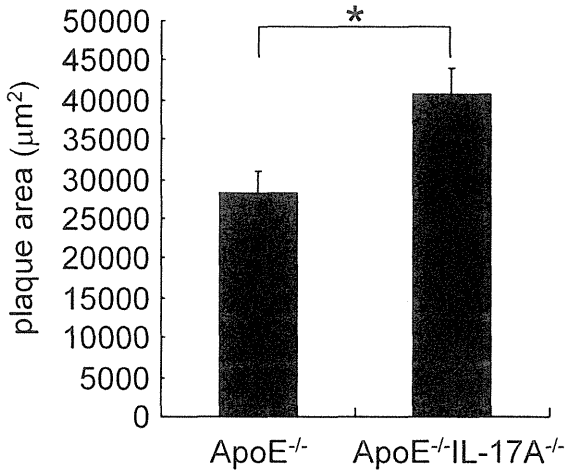
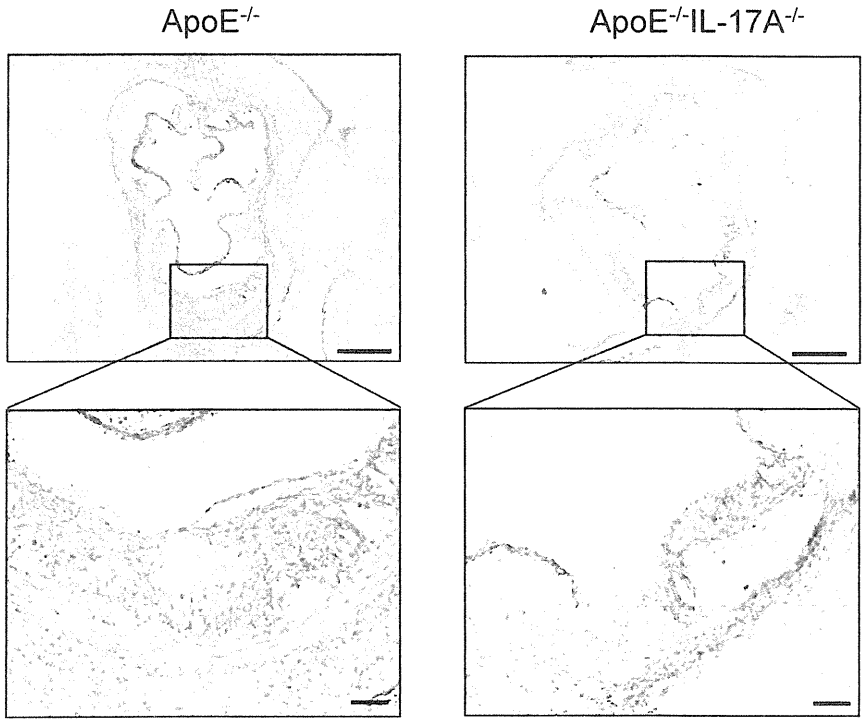


Supplemental Figure II

A



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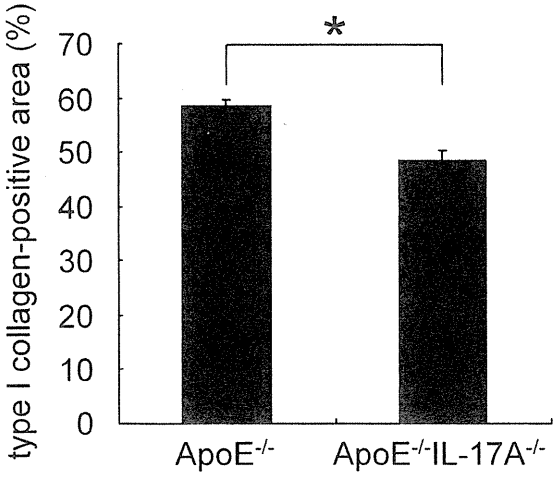
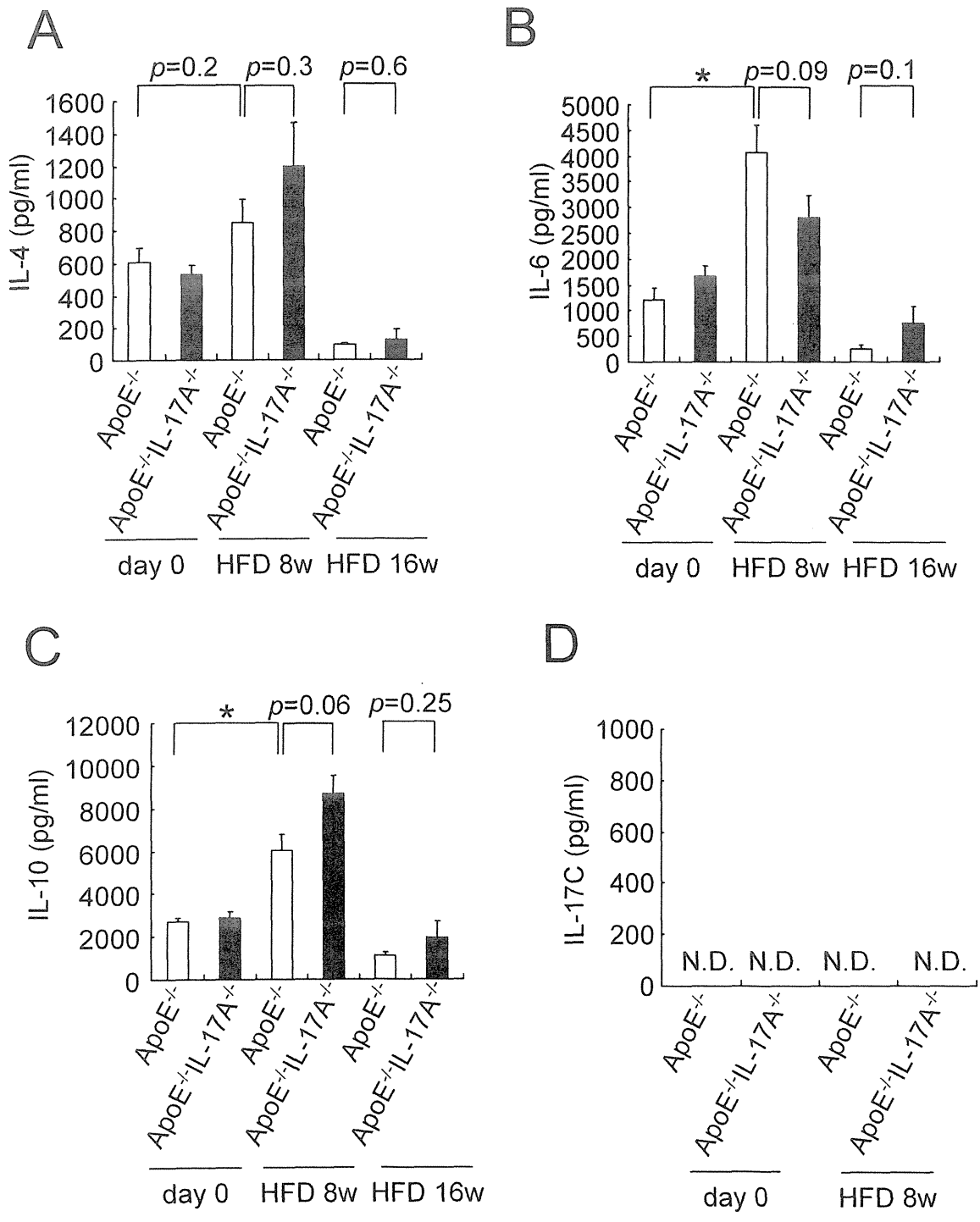
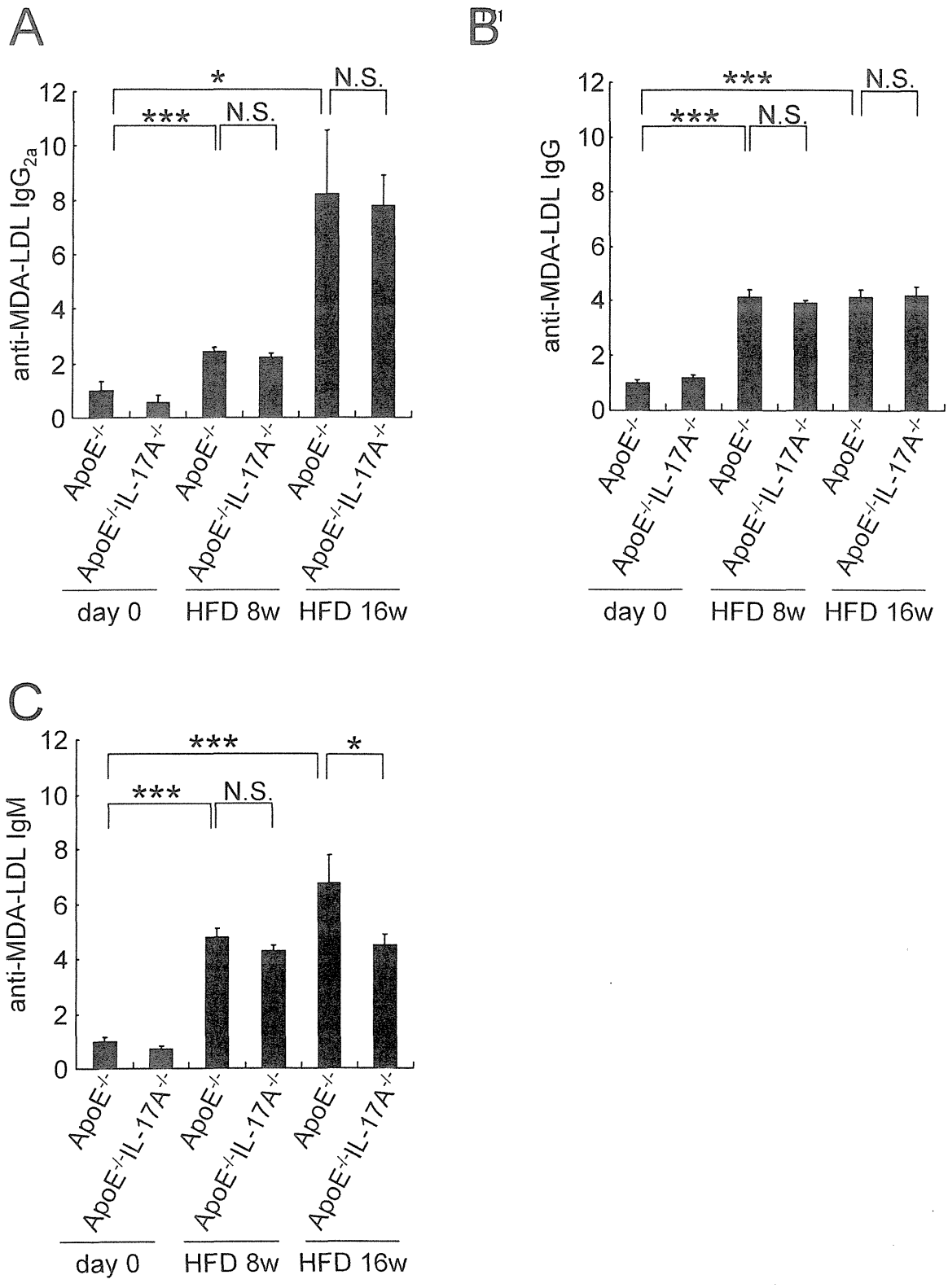


Supplemental Figure III



Supplemental Figure IV



Supplemental Figure V

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Diastolic stiffness as assessed by diastolic wall strain is associated with adverse remodelling and poor outcomes in heart failure with preserved ejection fraction

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Aims The pathophysiology of heart failure with preserved ejection fraction (HFpEF) is complex but increased left ventricular (LV) diastolic stiffness plays a key role. A load-independent, non-invasive, direct measure of diastolic stiffness is lacking. The diastolic wall strain (DWS) index is based on the linear elastic theory, which predicts that impaired diastolic wall thinning reflects resistance to deformation in diastole and thus, increased diastolic myocardial stiffness. The objectives of this community-based study were to determine the distribution of this novel index in consecutive HFpEF patients and healthy controls, define the relationship between DWS and cardiac structure and function and determine whether increased diastolic stiffness as assessed by DWS is predictive of the outcome in HFpEF.

Methods and results Consecutive HFpEF patients ($n = 327$, EF $\geq$ 50%) and controls ($n = 528$) from the same community were studied. Diastolic wall strain was lower in HFpEF (0.33 ± 0.08) than in controls (0.40 ± 0.07, $P < 0.001$). Within HFpEF, those with DWS $\leq$ median (0.33) had higher LV mass index, relative wall thickness, E/e', Doppler-estimated LV end-diastolic pressure to LV end-diastolic volume ratio, left atrial volume index, and brain natriuretic peptide (BNP) levels than those with DWS > median. Heart failure with preserved ejection fraction patients with DWS $\leq$ median had higher rate of death or HF hospitalization than those with DWS > median ($P = 0.003$) even after the adjustment for age, gender, log BNP, LV geometry, or log E/e' ($P < 0.01$).

Conclusion These data suggest that DWS, a simple index, is useful in assessing diastolic stiffness and that more advanced diastolic stiffness is associated with worse outcomes in HFpEF.

Keywords Diastolic function • Heart failure • Preserved ejection fraction • Outcomes

Introduction

Half of the patients with the clinical syndrome of heart failure (HF) in the community have preserved ejection fraction (HFpEF).[1,2] The pathophysiology of HFpEF is complex but increased left ventricular (LV) diastolic stiffness is thought to play a key role in many patients. Diastolic stiffness is inferred from Doppler indices, which reflect filling pressures and myocardial relaxation but do not directly measure diastolic stiffness.[3] Recently, indices based on laws of physics have been shown to represent a more direct measure of myocardial stiffness[4] (Supplementary material online, *Figure S1* and video). Diastolic wall strain (DWS) is based on the linear elastic theory, which predicts that in the presence of preserved EF, impaired diastolic wall thinning reflects resistance to deformation in diastole

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and thus, increased diastolic myocardial stiffness. Diastolic wall strain correlated with the diastolic stiffness constant measured invasively in an animal model.[4] However, the utility of this new index has not been tested in a large cohort of patients with HFpEF.

Several studies have shown the association of echo Doppler indices with poor outcomes in patients with reduced ejection function[5,6] or acute myocardial infarction,[7] but fewer studies have established the association of diastolic function indices with outcomes in HFpEF.[8,9] However, the association between diastolic stiffness and outcomes in a community-based cohort of HFpEF is not well established. The objectives of this community-based study were to determine the distribution of DWS in consecutive HFpEF patients and healthy controls from the same community, define the relationship between DWS and cardiac structure and function, and determine whether increased diastolic stiffness as assessed by DWS is predictive of outcomes in HFpEF.

Methods

Study population

The unique aspects of the Rochester Epidemiology Project for population-based research have been previously described.[1] The study was approved by the Mayo Clinic Institutional Review Board.

Consecutive patients with clinical HF (Framingham criteria), no significant left-sided valvular disease, hypertrophic or infiltrative cardiomyopathy or pericardial disease, and EF $\geq$50% were identified through an Olmsted County, MN, prospective population-based HF surveillance study.[10,11] Between September 2003 and August 2006, 397 HFpEF patients were identified. Control subjects ($n = 617$) without obesity, hypertension, diabetes, or known cardiovascular disease were identified from a random sample ($n = 2042$; age >45 years) of the same community who underwent echocardiography and medical record review as part of a community-based, echocardiographic survey study. Of these, 528 had two-dimensional (2D) measurement of LV wall thickness and chamber dimension.[11]

Outcome data

Mortality data were ascertained from medical records, death certificates for Olmsted County residents, obituaries, and notices of death in the local newspapers, as previously described.[12] Heart failure hospitalization was obtained through the Olmsted County Heathcare Expenditure and Utilization Database with ICD-9 codes, as previously described.[13]

Laboratory data

Plasma brain natriuretic peptide (BNP) was determined by the Biosite Triage® assay.

Echocardiography

Echocardiography was performed by registered diagnostic cardiac sonographers.[11] Ventricular dimensions and wall thickness were determined from 2D echocardiography (parasternal long-axis view) at end diastole based on the recommendation of the American Society of Echocardiography.[14,15] Systolic and diastolic blood pressure and the heart rate were obtained at echocardiography. As previously illustrated,[4] DWS was calculated using the formula: DWS = (PWs − PWd)/PWs, where PWs is the posterior wall thickness at end-systole and PWd is the posterior wall thickness at end-diastole and where end-diastolic and end-systolic measurements were made according to ASE recommendations.[14,15] Mean ± SD of intraobserver and interobserver variability of DWS was 0.003 ± 0.051 and 0.009 ± 0.072 ($n = 50$). Diastolic wall strain calculated with 2D or M-mode measurements ($n = 50$) correlated well ($r = 0.84$, $P < 0.001$), with no systematic error (mean ± SD difference: 0.007 ± 0.055, Bland–Altman analysis). Dichotomous assessments of DWS were performed with continuous values as a semi-qualitative assessment because DWS showed variability due to errors of measuring the wall thickness.

Of the 397 HFpEF patients, 327 (82%) patients had measurable DWS, with the remaining patients excluded due to posterior wall motion abnormalities ($n = 23$), significant pericardial effusion ($n = 4$), or inadequate images ($n = 43$). The LV end-diastolic volume (EDV) and EDV index adjusted by body surface area (EDVI) were calculated by Teichholz methods.[16] The LV mass index and relative wall thickness (RWT) were calculated by standard methods.[17] Based on LV hypertrophy (LVH) defined as LV mass index >95 g/m^2 (woman) or >115 g/m^2 (man) and RWT, LV geometry was classified as normal, concentric remodelling, concentric hypertrophy, or eccentric hypertrophy as previously described.[17] The septal mitral annular early diastolic velocity (e') was determined by spectral tissue Doppler imaging using standard methods. Early transmitral flow velocity (E) was measured by pulsed-wave Doppler, and end-diastolic pressure (EDP) was estimated as follows: (EDP = 11.96 + 0.596 × E/e'), as previously determined from Doppler and invasive EDP measurements at our institution.[18] Pulmonary arterial systolic pressure (PASP) and left atrial volume index (LAVI) were calculated as previously described.[19]

Statistical methods

Categorical variables were compared by the χ^2 test. Continuous variables were compared by a one-way analysis of variance with Bonferroni correction or the Steel–Dwass test for multiple unadjusted comparisons when appropriate after the assessment of normal distribution. Bivariate regressions used the Pearson or Spearman correlation as appropriate. Regression analysis was used to adjust for age, gender, and the presence of other diseases, where the dependent variable was the normally distributed continuous (linear least-squares regression). For variables without normal distribution, log-transformed variables were confirmed to have normal distribution and were used in regression models. The Kaplan–Meier method tested for differences in the survival rate between groups by the log-rank test. Cox proportional-hazards regression was used to adjust for the effect of differences in baseline characteristics or pertinent covariates on outcomes. Statistical comparisons were performed with JMP, version 9. All analyses were two-sided, and significance was judged at $P < 0.05$.

Results

Subject characteristics

In the 397 HFpEF patients, 327 had assessable DWS and constituted the HFpEF group. Of the 617 control subjects, 528 had a measurable DWS and constituted the control group. The distribution of DWS in HFpEF was different from that of control (*Figure 1*). The median (25th to 75th percentiles) DWS was 0.33 (0.29–0.38) in HFpEF and 0.40 (0.36–0.44) in control. *Table 1* shows the characteristics of control and selected elderly control group and of HFpEF patients with lower (≤ median) or higher (> median) DWS. When compared with control, both HFpEF groups were older, more obese, and had higher prevalence of hypertension, diabetes mellitus and coronary artery disease and higher blood pressure, heart rate, and BNP level, consistent with previous reports.[11] As there was an age

difference in the control and HFpEF patients, we selected healthy controls who were over age 70 as elderly control group. Findings were similar when comparing HFpEF patients with elderly controls. The prevalence of coronary artery disease and beta blocker use was higher than that reported in some but not all clinical trials.[20,21] Within HFpEF patients, those with low DWS had similar prevalence of comorbidities and use of angiotensin-converting enzyme-inhibitors or angiotensin receptor blockers, beta-blocker, and diuretics, and higher BNP levels when compared with those with high DWS.

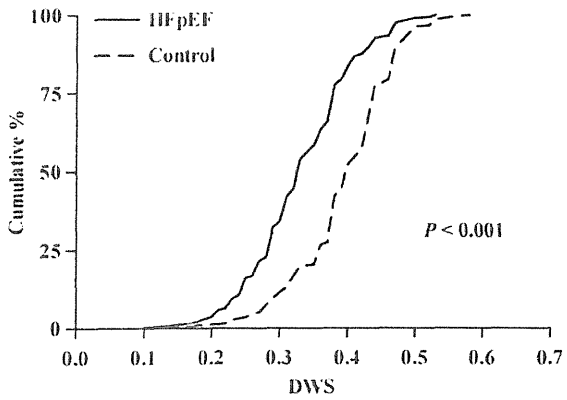

Figure 1 Cumulative frequency distribution of diastolic wall strain (DWS) in patients with heart failure and preserved ejection fraction (HFpEF, black solid) and control subjects (dashed).

Diastolic wall strain and left ventricular structure in heart failure with preserved ejection fraction

When compared with control, LV end-diastolic dimension and EDVI were not different in either HFpEF group, but HFpEF patients had greater wall thickness, LV mass index, and RWT (*Table 2*). Findings were similar when comparing HFpEF patients with elderly controls. Among HFpEF patients, those with high vs. low DWS had similar LV end-diastolic dimension, septal systolic wall thickness, and EDVI, whereas LV end-systolic dimension, PWs, PWd, septal diastolic wall thickness, LV mass index, and RWT differed with HFpEF patients with lower DWS having more abnormal geometry. Within HFpEF patients, DWS was inversely correlated with LV mass index and RWT ($r = -0.28$, $P < 0.001$, $r = -0.26$, $P < 0.001$, respectively). The association of DWS with LV mass index and RWT persisted ($P < 0.001$ for both) after the adjustment for age, gender, and comorbidities (hypertension, diabetes, and CAD).

Diastolic wall strain and left ventricular function in heart failure with preserved ejection fraction

When compared with control, EF was slightly but significantly lower in HFpEF (62.0 ± 6.2 vs. 63.7 ± 4.7%, $P < 0.001$) but among HFpEF patients, EF was similar in those with high or low DWS (*Table 2*). As expected, HFpEF patients had more indices reflective of diastolic dysfunction than control (shorter DT, lower e′, and higher *E*/*e*′, PASP and LAVI). In HFpEF patients with low DWS, mitral *E*/*A* ratio, *E*/*e*′, EDP/EDV ratio, and LAVI were significantly

Table 1 Clinical characteristics of control, elderly control, and heart failure with preserved ejection fraction subjects with high- or low-diastolic wall strain

	Control $n = 528$	Elderly control (age ≥ 70); $n = 46$	HFpEF with high DWS (>median); $n = 151$	HFpEF with low DWS (≤median); $n = 176$
Age (years)	56.7 ± 8.3	74.8 ± 4.8	76.5 ± 13.2*	77.7 ± 11.7*
Male (%)	43	39	40	47
BMI (kg/m^2)	25.3 ± 2.8	25.2 ± 2.9	29.3 ± 8.1***	29.9 ± 7.1***
Hypertension (%)	—	—	82	84
Diabetes (%)	—	—	29	32
Coronary artery disease (%)	—	—	44	51
Beta blocker (%)	—	—	60	59
ACE-I or ARBs (%)	—	—	45	48
Diuretics (%)	—	—	62	65
BNP (pg/mL)	27 ± 30	45 ± 98*	348 ± 397***	542 ± 648*******
Systolic BP (mmHg)	117 ± 12	125 ± 12*	131 ± 23*	132 ± 22*
Diastolic BP (mmHg)	70 ± 8	69 ± 8	68 ± 14	67 ± 14
Heart rate (b.p.m.)	64 ± 9	63 ± 10	70 ± 14***	70 ± 14***

Values are expressed as the mean ± SD unless otherwise noted. BMI, body mass index; ACE-I, angiotensin-converting enzyme-inhibitors; ARBs, angiotensin receptor blockers; BNP, brain natriuretic peptide; BP, blood pressure.
*$P < 0.05$ vs. control group.
**$P < 0.05$ vs. elderly control group.
***$P < 0.05$ vs. HFpEF with the high-DWS (>median) group.

Table 2 Echocardiographic characteristics of control, elderly control, and heart failure with preserved ejection fraction subjects with high- or low-diastolic wall strain

	Control, $n = 528$	Elderly control, $n = 46$	HFpEF with high-DWS (>median), $n = 151$	HFpEF with low-DWS (≤median), $n = 176$
LV structure				
LV end-diastolic dimension (mm)	48.4 ± 4.4	47.4 ± 5.1	47.5 ± 6.0	48.2 ± 6.5
LV end-systolic dimension (mm)	29.2 ± 4.2	27.6 ± 4.4*	29.3 ± 4.9	30.9 ± 5.5******
IVSd (mm)	10.0 ± 1.5	10.9 ± 1.6*	10.7 ± 2.2*	11.7 ± 2.2******
IVSs (mm)	14.8 ± 2.0	15.9 ± 1.8*	15.1 ± 2.7	14.8 ± 2.9**
PWd (mm)	9.1 ± 1.1	9.7 ± 1.2*	9.8 ± 1.6*	11.1 ± 1.7******
PWs (mm)	15.4 ± 1.9	16.2 ± 1.8*	16.4 ± 2.5*	15.4 ± 2.4***
EDVI (mL/m^2)	60.2 ± 10.9	59.5 ± 13.7	57.5 ± 14.4	58.3 ± 15.3
LV mass index (g/m^2)	88.5 ± 16.3	97.2 ± 17.6*	95.2 ± 26.7*	110.3 ± 31.2******
RWT	0.38 ± 0.06	0.41 ± 0.08*	0.44 ± 0.08*	0.48 ± 0.09******
Normal geometry (%)	67	41	31	11******
Concentric remodelling (%)	16	26	32	32
Concentric hypertrophy (%)	4	13	22	41
Eccentric hypertrophy (%)	13	20	15	16
Indices reflective of LV function				
EF (%)	63.7 ± 4.7	65.0 ± 5.1	62.7 ± 6.1	61.4 ± 6.2***
E (m/s)	0.67 ± 0.13	0.62 ± 0.15*	0.94 ± 0.33***	0.96 ± 0.31***
A (m/s)	0.54 ± 0.14	0.70 ± 0.20*	0.87 ± 0.30*	0.79 ± 0.28******
E/A ratio	1.30 ± 0.38	0.93 ± 0.27*	1.14 ± 0.54*	1.38 ± 0.92******
Deceleration time (ms)	219 ± 32	236 ± 46*	207 ± 56***	201 ± 53***
e′ velocity (cm/s)	9.5 ± 3.4	7.2 ± 1.9*	6.4 ± 2.2*	5.6 ± 1.9***
E/e′ ratio	7.6 ± 2.3	9.2 ± 2.8*	16.4 ± 8.2***	19.1 ± 9.5******
Estimated EDP (mmHg)	16.5 ± 1.4	17.4 ± 1.7*	21.7 ± 4.9***	23.3 ± 5.6******
EDP/EDV (mmHg/mL)	0.16 ± 0.04	0.15 ± 0.05	0.21 ± 0.08*	0.24 ± 0.12******
LAVI (mL/m^2)	21.9 ± 5.2	23.8 ± 5.2*	43.7 ± 17.0***	47.8 ± 15.5******
PASP (mmHg)	25.8 ± 3.7	27.7 ± 3.4*	47.3 ± 14.2***	49.6 ± 16.3***
DWS	0.40 ± 0.07	0.40 ± 0.06	0.40 ± 0.04	0.28 ± 0.05

Values are expressed as the mean ± SD. LV, left ventricular; IVSd, interventricular wall thickness at end-diastole; IVSs, interventricular wall thickness at end-systole; PWd, posterior wall thickness at end-diastole; PWs, posterior wall thickness at end-systole; EDVI, end-diastolic LV volume index; RWT, relative wall thickness; EF, ejection fraction; *E*, early transmitral flow velocity; *A*, late transmitral flow velocity; e′, septal mitral annular early diastolic velocity; EDP, end-diastolic LV pressure; LAVI, left atrial volume index; PASP, pulmonary arterial systolic pressure; DWS, diastolic wall strain.
*$P < 0.05$ vs. control group.
**$P < 0.05$ vs. elderly control group.
***$P < 0.05$ vs. HFpEF with the high-DWS (>median) group.

higher than those in HFpEF patients with high DWS, whereas PASP and e′ were similar (*Table 2*). Among HFpEF patients, DWS was modestly correlated with e′ ($r = 0.19$, $P = 0.001$), *E*/e′ ($r = -0.14$, $P = 0.02$), and LAVI ($r = -0.13$, $P = 0.02$). After the adjustment for age and gender, the association of DWS with these indices was still apparent ($P < 0.05$ for all).

Diastolic wall strain and outcomes

The mean follow-up in HFpEF patients who did not die or experience an HF hospitalization during follow-up was 700 ± 312 days. Death ($n = 98$, 30%) or HF hospitalization ($n = 67$, 20%) was common in HFpEF patients. Heart failure with preserved ejection fraction patients with lower DWS had a higher event (death or HF hospitalization) rate (*Figure 2*).

Univariate analysis showed that increased age, lower DWS and higher log BNP, PASP, LAVI, *E*/e′, e′, LV mass index, and RWT were all associated with higher combined event rate, whereas EF was not (*Table 3*). After adjusting for age and gender, DWS, log BNP, PASP, and LV mass index were still associated with a higher combined event rate, whereas LAVI, *E*/e′, and e′ were not (*Table 3*).

DWS was still associated with significant increases in risk of death or HF hospitalization in models adjusting for age, gender, and EF (*Table 4*, Model 1); age, gender, and Doppler-estimated EDP (Model 2); age, gender, and LV mass index (Model 3); and age, gender, EDP, and LV mass index (Model 4). The additive value of DWS to two variables potently associated with outcomes (pulmonary artery systolic blood pressure and BNP) is also shown [*Table 4* (Models 5 and 6) and Supplementary material online, *Figure S2*].

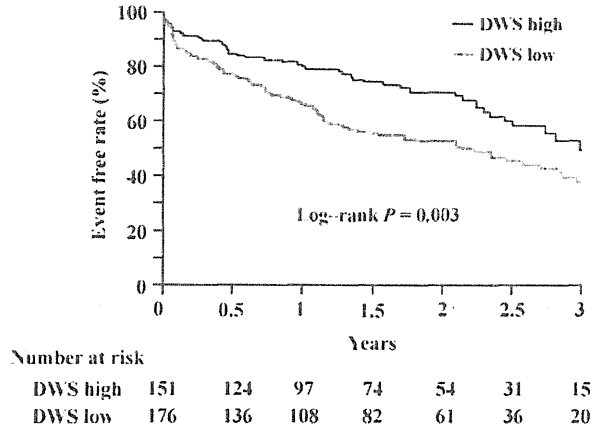

Figure 2 Event-free survival in heart failure with preserved ejection fraction patients according to diastolic wall strain (DWS). Kaplan–Meier plot of event-free (death or heart failure hospitalization) survival in patients with heart failure and preserved ejection fraction patients with diastolic wall strain above (DWS high, black) and below (DWS low, red) the median value of diastolic wall strain in heart failure with preserved ejection fraction patients.

Table 3 Factors related to events (combined outcome of death and heart failure hospitalization) among patients with heart failure and preserved ejection fraction: unadjusted and age-sex adjusted analysis

	HR (95% CI)	*P*-value	Adjusted HR (95% CI)	*P*-value
Age (per 1 year increase)	1.04 (1.02–1.05)	<0.001		
Male		0.13		
DWS (per 0.01 decrease)	1.04 (1.01–1.06)	<0.01	1.03 (1.01–1.06)	<0.01
Log BNP (per 1.0 log unit increase)	1.58 (1.35–1.86)	<0.001	1.50 (1.27–1.78)	<0.001
PASP (per 1 mmHg increase)	1.03 (1.02–1.04)	<0.001	1.02 (1.01–1.03)	<0.001
LAVI (per 1 mL/m^2)	1.01 (1.00–1.02)	0.02		0.31
Log *E*/*e*′ (per 1.0 increase)	1.70 (1.19–2.43)	<0.01		0.24
e′ (per 1 cm/s decrease)	1.11 (1.02–1.22)	0.02		0.54
EF (per 1% decrease)		0.94		
LVMI (per 1 g/m^2 increase)	1.01 (1.00–1.01)	0.02	1.01 (1.00–1.01)	0.01
RWT (per 0.01 increase)	1.02 (1.00–1.04)	0.02		0.06

Abbreviations as in *Tables 1* and *2*.

Findings were consistent when analysing death or HF hospitalizations as independent outcomes. Continuous or dichotomous DWS was associated with death even after the adjustment for age, gender, and other indices (same indices as in *Table 4*). Continuous DWS tended to be associated with HF hospitalization ($P = 0.06$), and dichotomous DWS was associated even after the adjustment for age and gender ($P < 0.05$).

Discussion

In this large, prospectively and consecutively enrolled, community-based cohort of HFpEF patients, we found that, on average, DWS was lower in HFpEF patients than in control subjects, suggesting higher diastolic stiffness in HFpEF patients. However, among HFpEF patients, there was variability in DWS. Heart failure with preserved ejection fraction patients with lower DWS (higher diastolic stiffness) had more abnormal geometry, more impaired relaxation, and higher filling pressures as assessed by conventional Doppler and biochemical (BNP) indices. Further, lower DWS was associated with increased rates of the combined endpoint of death or HF hospitalization, even the after adjustment for age, gender, and other prognostic indices. Based on these data, we conclude that DWS, a simple index easily derived from standard echocardiographic data, may be useful in assessing the severity of diastolic stiffness in HFpEF patients. The association of decreased DWS and worse outcomes supports the concept that increased LV diastolic stiffness contributes to the progression of HFpEF.

Table 4 Hazard ratio of lower diastolic wall strain as continuous and dichotomous (≤ median) values for events (death or heart failure hospitalization) among patients with heart failure and preserved ejection fraction adjusting for pertinent covariates

	Continuous DWS (per 0.01 decrease)		Dichotomous DWS (≤ median)	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Model 1 (age, gender, EF)	1.03 (1.01–1.06)	<0.01	1.73 (1.22–2.48)	<0.01
Model 2 (age, gender, EDP)	1.04 (1.02–1.07)	0.01	1.91 (1.31–2.84)	<0.001
Model 3 (age, gender, LVMI)	1.03 (1.01–1.05)	0.02	1.62 (1.13–2.33)	<0.01
Model 4 (age, gender, EDP, LVMI)	1.03 (1.01–1.06)	0.014	1.75 (1.19–2.62)	0.004
Model 5 (age, gender, log BNP)	1.03 (1.01–1.05)	<0.01	1.76 (1.23–2.54)	<0.01
Model 6 (age, gender, PASP)	1.03 (1.01–1.05)	0.02	1.61 (1.11–2.35)	0.01

HR, hazard ratio; CI, confidence interval. Other abbreviations as in *Tables 1* and *2*.

Diastolic wall strain as a measure of diastolic stiffness

Impaired LV relaxation and increased LV diastolic stiffness are the major components of diastolic dysfunction thought to lead to the elevation of filling pressures in HFpEF. Increased BNP, LAVI, and *E*/*e*′ are correlated with increased filling pressure and infer the presence of increased stiffness[18,22,23] but are load dependent and do not provide specific information on intrinsic passive myocardial stiffness.[24,25] The concept of DWS is based on the linear elastic theory, which considers the physical properties of myocardial tissue in diastole, whereby distending forces exerted on the stiff myocardium in diastole produce less diastolic deformation (wall thinning) and greater translational (epicardial) wall movement than forces applied to compliant or 'compressible' myocardium (see also Supplementary material online).[4] Diastolic wall strain correlated with the diastolic stiffness constant obtained invasively, and moderate changes in filling pressure by acute volume loading did not affect DWS. Decreased DWS is theoretically associated with increased LV stiffness rather than an abnormality of active relaxation because at the heart rates observed in these resting studies, relaxation would be complete before end-diastole even if there were a marked relaxation impairment.[26] However, very severely impaired LV relaxation or atrial systolic function could influence the measurement in select cases.

In the current study, DWS was lower in HFpEF patients, suggesting increased myocardial stiffness. However, among HFpEF patients, DWS varied with significant overlap in values between control and HFpEF patients, suggesting either insensitivity of DWS to increases in diastolic stiffness in HFpEF, poor specificity of DWS with falsely low values in some control subjects, or that increased diastolic stiffness is not an invariant feature in an unselected, community cohort of HFpEF patients. The association of lower DWS values with more abnormal geometry, Doppler evidence of elevation in filling pressures, and adverse clinical outcomes provides evidence that this index reflects intrinsic myocardial properties which are related to disease severity and progression. Importantly, the association between DWS and adverse clinical outcomes persisted after the adjustment for LV geometry, Doppler evidence of filling pressure, or BNP levels. Nonetheless, limitations imposed by the accuracy of measurement of wall thickness in systole and diastole have the potential to limit the accuracy with which DWS reflects myocardial properties.

Association between diastolic stiffness as assessed by diastolic wall strain and outcomes

Diastolic function grade, PASP, BNP, *E*/*e*′, and LAVI were previously reported to be associated with poor outcomes.[8,19,27–29] We showed that DWS as well as BNP or PASP was associated with worse outcomes in HFpEF after the adjustment for age and gender, whereas *E*/*e*′ and LAVI were not. *E*/*e*′ and LAVI were correlated with age, which was strongly associated with worse outcome in this population. BNP and PASP were also strong predictors of worse outcomes, but these indices also are load sensitive and may be altered by concomitant renal dysfunction, obesity, or pulmonary disease. Importantly, DWS remained predictive of outcomes after the adjustment for these potent prognostic factors, suggesting that DWS reflects a fundamental abnormality contributing to the progression of HFpEF. Although DWS was associated with the severity of LVH, the association of DWS with poorer outcomes remained significant after adjusting for LV mass index. This observation suggests that myocardial stiffness is likely influenced by factors beyond the severity of hypertrophy, including fibrosis or alterations in myofibrillar protein expression or post-translational modification as has been described for differences in the isoform distribution and phosphorylation status of titin.[30]

Strengths and limitations

The community-based setting, consecutive and prospective enrolment of HF patients defined by diagnostic criteria validated in numerous epidemiology studies, uniform performance of echocardiography and outcome data inclusive of HF hospitalizations represent strengths of the current study. Heart failure with preserved ejection fraction patients were older than control subjects; however, the differences in LV structure and function

between control subjects and HFpEF patients were generally similar when only control subjects who were ≥70 years old ($n = 46$, mean age 74.8) were considered (*Tables 1* and *2*). Limitations include the lack of cause-specific mortality data, exclusion of the epicardial motion component when applying the linear elastic theory to the quantification of diastolic stiffness, lack of invasive gold standard measurement of diastolic stiffness, the possibility that regional assessment of LV stiffness at the posterior wall may not reflect global LV myocardial stiffness, and inherent variability in the measurement of wall thickness by 2D echocardiography. Failure to measure both diastolic and systolic wall thickness limited the calculation of DWS in some patients, although the feasibility of this index compares favourably with other diastolic indices.[18] Although echocardiographic data were used to calculate DWS, this index could potentially be derived from carefully focused M-mode imaging or magnetic resonance or computed tomographic imaging modalities, enhancing the functional data derived from such imaging techniques.

Conclusions

The severity of diastolic stiffness as assessed by DWS is associated with more severe abnormalities of LV structure and conventional Doppler and biochemical (BNP) indices, which imply underlying diastolic dysfunction and higher filling pressures in HFpEF patients. In this community-based cohort of HFpEF patients, lower DWS was associated with higher rates of death or HF hospitalization as a combined outcome even after adjusting for LV geometry or other potent prognostic factors such as BNP and PASP. DWS is a feasible and simple calculation which does not require complex analysis. The findings of the current study support a potential role for DWS as part of an integrated approach to the assessment of diastolic function and suggest that future studies with focused and optimized assessment of DWS should be performed to further evaluate its clinical utility.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Effects of sitagliptin beyond glycemic control: focus on quality of life

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