Diastolic Tissue Doppler Indexes Correlate With the Degree of Collagen Expression and Cross-Linking in Heart Failure and Normal Ejection Fraction

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Objectives
We attempted to correlate echocardiographic analysis of diastolic function with changes of myocardial collagen in middle-aged patients with heart failure (HF) despite normal ejection fraction (EF).

Background
Increased collagen deposition may contribute to the deterioration of the left ventricular compliance and diastolic dysfunction in HF.

Methods
We investigated 41 patients (median age 50 years [interquartile range: 41 to 57 years]) with normal EF (median 62% [interquartile range: 56% to 70%]) whose endomyocardial biopsies were taken previously. Assessment of diastolic function was performed by mitral-flow and tissue Doppler measurements. Sirius red and immunohisto logic staining was performed to determine collagen volume fraction (CVF) and cross-linking, collagen types I and III expression, and lysyl-oxidase (LOX) expression. Expression of collagen messenger ribonucleic acid was determined by real-time polymerase chain reaction.

Results
Twenty-six patients with HFNEF with diastolic dysfunction showed a significant increase in total collagen and collagen I expression compared with that of 15 controls. This was accompanied with enhanced collagen cross-linking and LOX overexpression in HFNEF. Among all flow Doppler, only deceleration time of E was associated with CVF (R² = 0.43), whereas tissue Doppler parameters correlated with CVF, collagen I at the protein and mRNA levels (E' [R² = −0.58, −0.60, −0.45]; E'/A' [R² = −0.32, −0.36, −0.31]), and left ventricular filling index (E/E' [R² = 0.72, 0.68, 0.63]), respectively. No correlation with collagen III was found. The degree of collagen cross-linking and LOX expression was related to E' (R² = 0.55 and 0.60) and E/E' (R² = 0.72 and 0.71), respectively, but not to flow Doppler. Collagen overexpression correlated with reduced exercise capacity.

Conclusions
Patients with HFNEF showed increased content of myocardial collagen type I, enhanced collagen cross-linking, and LOX expression, which were associated with impaired diastolic tissue Doppler parameters. (J Am Coll Cardiol 2011;57:977–85) © 2011 by the American College of Cardiology Foundation

Differential diagnosis of exertional dyspnea and reduced exercise capacity provide a challenge in the clinical routine. Particularly, patients with mild symptoms are misdiagnosed, an event followed by either inadequate treatment or no treatment at all. Their symptomatology could be of noncardiac (1–3) or cardiac (4–6) origin. One-half of the patients presented with a preserved left ventricular (LV) ejection fraction (EF) (7). This clinical syndrome, called heart failure with normal ejection fraction (HFNEF), is found predominantly in the elderly, especially with hypertension and hypertensive heart disease (HHD). However, underlying pathophysiologic mechanisms in HFNEF are only partially understood. Predominantly diastolic dysfunction (7–11) with impaired LV compliance and diastolic filling (12–16) are thought to be responsible for the symptoms. In clinical practice, echocardiography is the first-line diagnostic method (17,18). However, patients with a lower grade of comorbidity and/or an early stage of disease are difficult to diagnose (19). Because mitral-flow Doppler revealed lower accuracy (13,20), tissue Doppler (TDI) is suggested as the ultimate diagnostic approach (17). Nevertheless, because of...
known limitations (11,13,21), its prognostic role is still under investigation.

In post-mortem studies in patients with HHD (22,23), an enhanced accumulation of collagen I and III fibers within the myocardial interstitium and perivascular extracellular matrix was found (24). In hypertensive animal models with LV hypertrophy and preserved systolic function, increased amounts of collagen were accompanied by abnormal diastolic stiffness (25,26) but not ultimately in hypertrophied myocardium without fibrosis (27,28). Because increased myocardial fibrosis may contribute to the deterioration of the LV compliance and diastolic dysfunction in heart failure (29,30), we investigated the reliability of echocardiography to recognize the changes in amounts and quality of myocardial collagen as a sign of early cardiac remodeling in HFNEF. Therefore, we included middle-aged patients who were symptomatic under exercise conditions, thus aiming to find an optimal noninvasive diagnostic approach for early diagnosis and prevention of disease progression.

Methods

Study population. Patients presenting with heart failure (HF) symptoms and reduced exercise capacity despite preserved LVEF (>50%) who showed diastolic dysfunction according to echocardiographic analysis and European Society of Cardiology recommendations (17) were enrolled in the study. All patients had at least 1 HF-related hospitalization and were screened for noncardiac causes of symptoms, including lung function tests. Ventilatory system-related exertional dyspnea was excluded by performing spiroergometry. Exercise capacity and exertional dyspnea were evaluated by a 6-min walk test, bicycle ergometry, and NT-proBNP plasma levels (31,32). Patients with significant coronary artery diseases verified by angiography; atrial fibrillation; and significant heart valve, lung, or renal diseases were excluded. Patients without symptoms and signs of congestive heart failure but with atypical angina or intermittent arrhythmias who underwent right ventricular (RV) biopsy for evaluation of cardiomyopathy (33) and showed regular systolic and diastolic LV function were enrolled as controls. Endomyocardial biopsies (EMBs) were obtained following international recommendations (33). All patients investigated provided their written informed consent for invasive diagnostic procedures. The research protocol was approved by the local institutional review committee (approval 225-07).

Echocardiography and Doppler measurements. Transthoracic echocardiography was performed using a GE Vingmed ultrasound system 5 or VIVID 7 (2.5 MHz) (Horten, Norway) by an independent investigator blinded for invasive data analysis. Cardiac cycles were stored in a cineloop format. Chamber dimensions were determined by standard procedures, including left ventricular mass index (LVMI) and left atrial volume index (LAVI). End-diastolic wall stress was calculated as previously described (34). Mitral and pulmonary venous flow Doppler and TDI were recorded in the apical 4-chamber view according to guidelines (17). Mitral in-flow measurements included peak early (E) and late (A) flow velocities, the E/A ratio, deceleration time of E (DT), and isovolumic relaxation time (IVRT). Pulmonary venous flow measurements included peak S, D, and atrial reversal velocities. The S’ and early (E’) and late (A’) diastolic peak velocities were assessed at septal and lateral mitral origin and expressed as mean. The mean values of the E’/A’ ratio and LV filling index (E/E’) were determined.

EMBs. EMBs were obtained from the RV septum using a flexible biopsy (Westmed, St. Ingbert, Germany) via the femoral vein approach. Endomyocardial biopsies were directly embedded in optimal cutting temperature compound (Miles Laboratories, Inc., Elkhart, Indiana) and frozen at −70°C. With a cryostat, the biopsies were cut into 4-mm thin sections, mounted on a slide, and fixed in acetone for 10 min. The tissue pieces were frozen in liquid nitrogen and stored at −70°C for subsequent histologic analysis (35).

Histology and immunohistology. Formalin-fixed tissue was processed for paraffin embedding, sectioned in standard thickness, and stained with collagen-specific Picrosirius red (F3BA in aqueous picric acid) (6). The fraction of myocardial volume occupied by fibrillar collagen (collagen volume fraction [CVF]) was determined by quantitative morphometry with an automated image analysis system (AnalySYS, Soft Imaging System GmbH, Hamme, Germany) (6). To distinguish between cross-linked (insoluble) and non–cross-linked (soluble) collagen, a colorimetric procedure was used (36). First, a fast green/Sirius red assay was performed to identify and quantify total collagen. In a second step, a sircol-based assay was performed to obtain and quantify soluble collagen. The amount of insoluble collagen was calculated by subtracting the amount of soluble collagen from the amount of total collagen. The amount of cross-linking was calculated as the ratio between the insoluble and soluble forms of collagen. Cross-linking was determined retrospectively in only 23 patients (14 with HFNEF and 9 controls) for whom EMB samples were available. To determine expression of collagen I and III, immunohistologic staining was performed using standard techniques with...
collagen antibodies and polarized light for recognition of stained collagen. The exact procedure is described in the Online Appendix. The expression of lysyl-oxidase (LOX), the enzyme responsible for collagen cross-linking, was determined retrospectively in 15 patients for whom EMB samples were available (10 with HFNEF and 5 controls).

The results are expressed as semiquantitative values (absent, mild, moderate, and intense expression). The detailed procedure is available in the Online Appendix.

The histomorphologic study was performed by a pathologist blinded to patient data. To determine myocyte dimensions, the maximal myocyte diameter was measured through the nucleus for 10 cells per sample. Using a standard equation for sample size, 10 measurements reduced the sampling error to <3%.

RNA isolation, reverse transcription, pre-amplification, and real-time polymerase chain reaction. Total RNA was extracted from the EMB by the Trizol method. Additional purification was performed using the ChargeSwitch total RNA cell kit (Invitrogen GmbH, Karlsruhe, Germany). TaqMan gene expression assays (Applied Biosystems Deutschland GmbH, Darmstadt, Germany) were used for preamplification and real-time polymerase chain reaction. The exact procedure is described in the Online Appendix.

Statistical analysis. SPSS (version 13.0, SPSS Inc., Chicago, Illinois) was used for statistical analyses. Continuous variables are expressed as median with interquartile range. Two-sample comparisons were performed using an unpaired t test for normally distributed variables, Mann-Whitney U test for non-normally distributed variables, and the Fisher exact or Chi-square test for categoric data. The whiskers in the box plots are defined as highest/lowest values under the outlier cutoff (1.5 × interquartile range). The Pearson coefficients for linear and Spearman coefficients for nonlinear correlations were used. Regression analyses and curve fitting were performed to determine exact relations. A value of p < 0.05 was considered to be statistically significant in all analyses.

Results

Patient characteristics. Patient characteristics are summarized in Table 1. The study population was relatively young (median age 50 years) and included 26 patients with HFNEF and 15 controls. Patients with HFNEF showed a significantly higher prevalence of hypertension, diabetes, and obesity. They had higher New York Heart Association functional class and NT-proBNP levels and lower 6-min walk test and bicycle exercise-test levels than the controls. There was no difference between groups in age, sex, and body mass index.

Echocardiography. Heart dimensions and flow and tissue Doppler measurements are listed in Table 2. In patients with HFNEF and controls, LV was not dilated. Patients with HFNEF showed a trend toward increased LVMi and had increased wall stress and LAVi. They had normal systolic function but showed borderline flow Doppler and significantly impaired TDI diastolic parameters: decreased E’ and E'/A and increased E/E'.
Histologic staining: total collagen and collagen I and III in patients with HFNEF. Figure 1 illustrates representative histologic staining from EMBs in patients with HFNEF showing increased Sirius red collagen content and collagen I and LOX expression compared with controls. A larger proportion of collagen I–stained area fraction was detected in patients with HFNEF compared with controls (median 11.5% [interquartile range (IQR): 6.7% to 14.7%] vs. 2.7% [IQR: 1.8% to 4.2%]; p = 0.005), but expression of collagen III did not differ (Fig. 2), which resulted in an increased collagen I/III ratio (1.11 [IQR: 0.72 to 1.52] vs. 0.58 [IQR: 0.20 to 0.96]; p = 0.020). The Picrosirius red staining showed higher values of CVF in patients with HFNEF (13.1% [IQR: 10.08% to 18.40%] vs. 4.1% [IQR: 3.25% to 7.04%]; p < 0.001) (Fig. 2E) that are also within the expected range for patients with chronic fibrosing cardiac diseases. Myocyte diameters were not significantly increased in comparison with those of the controls (20.0 μm [IQR: 17.0 to 23.0 μm] vs. 18.0 μm [IQR: 17.0 to 20.2 μm]; p = 0.258).

Collagen cross-linking and expression of myocardial LOX in patients with HFNEF. The degree of collagen cross-linking was significantly increased in the myocardium of patients with HFNEF compared with controls. The amount of insoluble collagen was higher and amount of soluble collagen was lower in patients with HFNEF than in controls (Table 3). Insoluble collagen and cross-linking correlated strongly with CVF (r = 0.802; p = 0.002 and r = 0.880; p < 0.001, respectively). Considering LOX expression as a semiquantitative variable and taking into account the distribution within groups (3 intense, 6 moderate, and 1 mild in patients with HFNEF vs. 1 absent and 4 mild in controls), a Mann-Whitney U test showed significant difference (Mann-Whitney U = 2.000; p = 0.003). A regression analysis provided a significant correlation of LOX
expression to CVF ($r = 0.63$; $p = 0.013$), cross-linking ($r = 0.63$; $p = 0.012$), and amount of insoluble collagen ($r = 0.68$; $p = 0.005$).

Expression level of mRNA for collagen I and III in patients with HFNEF. The results of increased collagen I expression in patients with HFNEF with diastolic dysfunction were confirmed on the mRNA level. The expression of collagen I mRNA was 2.1-fold increased in patients with HFNEF ($p = 0.001$), and a trend was shown toward increased expression of collagen III mRNA by 1.6-fold (Fig. 2).

**Diastolic parameters versus myocardial collagen content in HFNEF.** Approximated wall stress showed moderate correlations with CVF and collagen I expression ($r = 0.40$; $p = 0.031$ and $r = 0.45$; $p = 0.021$, respectively), whereas LVMi and LAVi did not. Flow Doppler showed that DT and A correlated weakly with CVF ($r = 0.43$; $p = 0.017$ and $r = 0.52$; $p = 0.031$, respectively), but E, E'/A, and IVRT were not related either to total collagen or collagen I or III expression at the protein or mRNA level. Pulmonary venous flow was available in 42% of patients; S/D ratio correlated with collagen I expression, and S, D, and atrial reversal did not show any correlation. The TDI data showed significant correlations with CVF and collagen I expression but not collagen III expression; E' showed an inverse correlation with CVF ($r = -0.60$; $p = 0.001$) and collagen I expression at the mRNA ($r = -0.58$; $p = 0.045$) and protein levels ($r = -0.36$; $p = 0.030$) (Fig. 3). A' was not associated with myocardial collagen. E'/A' correlated with CVF ($r = -0.32$; $p = 0.048$) and collagen I expression at the protein ($r = -0.36$; $p = 0.026$) and mRNA levels ($r = -0.31$; $p = 0.035$). The correlation with collagen III expression was not significant ($r = -0.18$; $p = 0.267$ and $r = -0.20$; $p = 0.356$, respectively). E/E' was related significantly to CVF ($r = 0.72$; $p < 0.001$) and collagen I expression at the protein ($r = 0.68$; $p < 0.001$) (Fig. 3) and mRNA levels ($r = 0.63$; $p < 0.001$). E/E' was weakly related to collagen III expression at the protein ($r = 0.33$; $p = 0.029$) and mRNA levels ($r = 0.44$; $p = 0.030$). There were no associations between myocyte diameter and diastolic TDI parameters.

**Diastolic parameters versus collagen cross-linking and LOX expression.** From flow Doppler, only DT correlated with collagen cross-linking, whereas TDI parameters E', E'/A', and E/E' showed significant correlations with soluble and insoluble collagen and cross-linking (Table 4). The LOX expression was associated with TDI but not with conventional flow Doppler parameters. Elevated E/E' provided the best correlation, with an increasing degree of collagen cross-linking, suggesting an impaired LV compliance in patients with HFNEF with myocardial collagen overexpression.

**Table 3** Collagen Cross-Linking in Patients With HFNEF Versus Controls

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 9)</th>
<th>HFNEF (n = 14)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble collagen (µg/mg)</td>
<td>0.58 (0.52–0.68)</td>
<td>0.41 (0.35–0.52)</td>
<td>0.005</td>
</tr>
<tr>
<td>Insoluble collagen (µg/mg)</td>
<td>0.45 (0.41–0.59)</td>
<td>0.66 (0.57–0.73)</td>
<td>0.008</td>
</tr>
<tr>
<td>Cross-linking (%)</td>
<td>0.77 (0.63–1.01)</td>
<td>1.69 (1.04–2.09)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Variables are expressed as median (25% to 75% percentiles), values of collagen are expressed as µg of collagen/mg of total protein.

Abbreviation as in Table 1.
Binary logistic and stepwise multiple regression analyses showed that insoluble collagen (likelihood ratio [LR] 16.8; p < 0.001), collagen I (LR 5.1; p = 0.024), and collagen cross-linking (LR 10.3; p = 0.001) correlated independently, with evidence of LV diastolic dysfunction and LV filling index E/E', on top of CVF.

**Figure 3** Correlations of Diastolic TDI With Collagen Expression in Patients With HFNEF

Tissue Doppler indexes (TDI) E' and E/E' correlate significantly with (A) CVF (89.8/E' = 0.43 and 10.9 × E/E' + 0.60), (B) collagen type I (0.53/E' + 0.012 and 0.009 × E/E' − 0.028), and (C) collagen cross-linking (8.8/E' + 0.14 and 0.11 × E/E' + 0.12). Dotted lines represent correlation curve fits for the HFNEF group separately: (A) R = 0.50 and R = 0.46. (B) R = 0.47 and R = 0.51. (C) R = 0.74 and R = 0.76; p < 0.05. Open circles represent controls. Abbreviations as in Figure 2.
Correlation of Diastolic Indexes From Mitral-Flow and Tissue Doppler With CVF, Insoluble Collagen, Collagen Cross-Linking, and LOX Expression

<table>
<thead>
<tr>
<th></th>
<th>CVF (n = 41)</th>
<th>Insoluble Collagen (n = 23)</th>
<th>Cross-Linking (n = 23)</th>
<th>LOX (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>0.03 (0.431)</td>
<td>0.22 (0.336)</td>
<td>0.33 (0.139)</td>
<td>0.11 (0.705)</td>
</tr>
<tr>
<td>E/A</td>
<td>-0.25 (0.130)</td>
<td>-0.04 (0.867)</td>
<td>-0.02 (0.938)</td>
<td>-0.40 (0.135)</td>
</tr>
<tr>
<td>IVRT</td>
<td>-0.12 (0.526)</td>
<td>-0.21 (0.448)</td>
<td>-0.21 (0.449)</td>
<td>-0.09 (0.790)</td>
</tr>
<tr>
<td>DT</td>
<td>0.43 (0.017)</td>
<td>0.51 (0.050)</td>
<td>0.41 (0.135)</td>
<td>0.14 (0.687)</td>
</tr>
<tr>
<td>E_mean</td>
<td>-0.58 (&lt;0.001)</td>
<td>-0.47 (0.031)</td>
<td>-0.55 (0.010)</td>
<td>-0.60 (0.018)</td>
</tr>
<tr>
<td>E'/A_mean</td>
<td>-0.32 (0.048)</td>
<td>-0.30 (0.186)</td>
<td>-0.28 (0.230)</td>
<td>-0.35 (0.198)</td>
</tr>
<tr>
<td>E/E_mean</td>
<td>0.72 (&lt;0.001)</td>
<td>0.55 (0.007)</td>
<td>0.72 (&lt;0.001)</td>
<td>0.71 (0.003)</td>
</tr>
</tbody>
</table>

Data are expressed as Pearson correlation coefficient (p value). CVF = collagen volume fraction; LOX = lysyl-oxidase; other abbreviations as in Table 2.

Discussion

The key finding of our study was evidence of a relationship between structural changes of the extracellular matrix and echocardiographic TDI in middle-aged patients already exhibiting HFNEF largely owing to HHD. Increased myocardial collagen content and cross-linking in EMBs indicated an early remodeling process that is able to increase ventricular stiffness, compromising LV filling. Opposite to conventional flow Doppler, TDI of diastolic function and LV filling index as a noninvasive measure of impaired LV compliance revealed significant correlations with myocardial collagen content and collagen cross-linking, which confirmed their superior diagnostic accuracy in patients with HFNEF showing mild diastolic dysfunction.

The pathophysiology of HFNEF is poorly understood, and the correct diagnosis in everyday clinical settings still remains a challenge. Mitral-flow Doppler is no longer recommended as the method of choice because of its low accuracy (17,18). Tissue Doppler provides the most reliable diagnostic approach; however, its key parameter, LV filling index (E/E'), also reveals disadvantages relating to unclear cut-off values and its prognostic role in HFNEF. To further evaluate the role of different echocardiographic techniques in HFNEF, we correlated their diastolic parameters with changes of the collagen amount/quality in EMBs, known to be critical for LV compliance. Besides increased myocyte tension (37) and endothelial dysfunction (35,38), there are changes in the extracellular matrix, and particularly those in collagen composition that influence the passive mechanical properties of the myocardium (27,30,39). Because myocardial fibrosis is uniform for all sites in the heart in HHD, these changes in extracellular matrix and collagen accumulation can be observed by biopsy of the RV interventricular septum as performed in our study. We were able to find an increased total amount of cardiac collagen in patients with HFNEF, which is in agreement with findings from patients with HHD (22,23), including preclinical data (25,40,41).

However, the measurement of collagen content is a less accurate expression of fibrosis in hypertrophied hearts because it is influenced by increments of ventricular mass. Formation and distribution of new collagen fibers, as characterized by collagen cross-linking or collagen I/III ratio, contribute to the functional consequence of myocardial fibrosis and therefore are found to be related to myocardial diastolic stiffness (24,41). Therefore, LV chamber stiffness will be affected by the amount and quality of cardiac collagen, as previously shown (6,23,30,36,42,43), indicating that changes in plasma levels of matrix metalloproteinase/tissue inhibitor of metalloproteinase and collagen propeptides reflect an increase in systemic collagen turnover.

The association between TDI and myocardial fibrosis in our study was independent from the LV hypertrophy parameter obtained by LVMI, indicating the importance of fibrosis for the compliance properties of the LV (27), which support the findings from animal models (25,26,28). Data from patients with hypertension have led to the suggestion that the myocyte cross-sectional area is almost double its normal value but does not change with progression to cardiac failure, when cell lengthening is the predominant cellular change (44). Similarly, we found no significant changes in myocyte diameters in our HFNEF population with a short history of disease and borderline increased LVMI. However, there exists variability in muscle fiber diameter because fibers surrounded by collagen may become atrophic, whereas those at a distance may be hypertrophied (Fig. 1). Therefore, although the association of TDI and myocardial collagen appears not to be influenced by hypertrophy and myocyte shape changes, the variability of fiber diameters calls for caution in interpretation of these results.

Correlation of mitral-flow Doppler with myocardial collagen in patients with HFNEF. Among measured-flow Doppler parameters, only DT was associated with myocardial collagen content. This result is in line with findings showing that DT reflects LV chamber stiffness (45). We did not find any significant relationship with early in-flow velocity or IVRT. They represent mainly early diastolic changes reflecting an active process of diastolic LV relaxation, which also might explain its unmodifiable correlation with cardiac fibrosis. Pulmonary vein flow Doppler was also not found to be sensitive enough to detect early changes of cardiac remodeling in patients with mild HFNEF. In contrast to other studies involving diastolic HF (11,15,16,19), our population was younger and showed only a mild increase in LVMI and borderline LA volume, without any apparent severe concomitant diseases such as severe hypertension, coronary
artery disease, and hypertrophic and diabetic cardiomyopathy which per se can influence mitral-flow properties.

In conclusion, flow Doppler measurements correlate with hemodynamic changes in patients with HFNEF only indirectly and therefore cannot provide unequivocally any of the changes in intrinsic LV characteristics, at least during an early form of the disease. According to our findings, this becomes clinically relevant especially in patients with only mild disturbances, relatively low filling pressure, and an early stage of remodeling when transmural flow alone cannot serve as a reliable diagnostic method.

**Correlation of TDI with myocardial collagen content in patients with HFNEF.** Because it is believed that intrinsic changes of LV tissue precede the impairment of transmural flow, TDI might already recognize early changes in the tensile characteristics of LV, not yet manifested as being part of a mitral-flow pattern. In support of this belief, we showed a significant association of TDI parameters with not only the extent of myocardial collagen accumulation but also with the quality alteration of collagen, as characterized by the degree of cross-linking and expression of the crucial regulatory enzyme LOX, even in a mild form of diastolic dysfunction. Early diastolic mitral movement E', which is also partially related to the compliance disturbance of the LV (13), correlated with CVF and cross-linking. Opposite of this correlation, atrial contraction movement A' was not related to myocardial collagen content. In our patients with borderline LAVI changes, A' seemed not to be affected.

The strongest association was found between LV filling index E/E' and the degree of myocardial collagen amount, collagen cross-linking, and expression of LOX. These findings confirm the hypothesis that cardiac fibrosis and the remodeling process are associated with the passive LV compliance characteristics (13,30). This relationship was supported in particular by a connection to collagen I expression, which is known to possess extreme tensile properties. Consequently, our patients with HFNEF with elevated E/E' suffered from exercise intolerance, as obtained by the 6-min walk test. This indicates that LV compliance disturbances—associated as well with cardiac fibrosis—can indeed contribute to the lower cardiac performance despite normal contractile LV function as confirmed by rest echocardiography.

**Conclusions**

Patients with HFNEF showed increased myocardial collagen content, particularly collagen type I, but also enhanced collagen cross-linking and LOX expression, which were associated with impaired diastolic function and reduced exercise tolerance. This association suggested that quantity and quality of myocardial collagen influence the compliance characteristics and performance of the LV in HFNEF. Correlation of diastolic TDI parameters with increased cardiac collagen expression and cross-linking implicates that TDI reflects the hemodynamic properties of the LV already present among mild forms of HFNEF. Therefore, in middle-aged patients with symptoms manifested solely during exercise conditions, TDI should substantiate a method of choice for the evaluation of diastolic function. Additionally, our findings suggest that therapeutic strategies directed not just at reducing the amount of collagen but also at reducing collagen cross-linking should be implemented in the prevention of the adverse impact of myocardial fibrosis on cardiac function in patients with HFNEF. In this regard, it was reported that treatment with the loop diuretic torasemide was associated with correction of LOX overexpression, enhanced collagen cross-linking, and normalization of LV chamber stiffness in patients with HF (36).

**REFERENCES**


Key Words: cardiac collagen • cross-linking • diastolic function • heart failure with normal EF • tissue Doppler imaging.