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Myocardial steatosis as a possible mechanistic link between diastolic dysfunction and coronary microvascular dysfunction in women

Janet Wei,[1] Michael D. Nelson,[2] Edward W. Szczepaniak,[2] Laura Smith,[2] Puja K. Mehta,[1] Louise E. J. Thomson,[3] Daniel S. Berman,[3] Debiao Li,[2] C. Noel Bairey Merz,[1] and Lidia S. Szczepaniak[2]

[1]*Barbra Streisand Women's Heart Center, Cedars-Sinai Heart Institute, Los Angeles, California;* [2]*Biomedical Imaging Research Institute, Cedars-Sinai Medical Center, Los Angeles, California; and* [3]*S. Mark Taper Foundation Imaging Center, Cedars-Sinai Medical Center, Los Angeles, California*

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Wei J, Nelson MD, Szczepaniak EW, Smith L, Mehta PK, Thomson LE, Berman DS, Li D, Bairey Merz CN, Szczepaniak LS. Myocardial steatosis as a possible mechanistic link between diastolic dysfunction and coronary microvascular dysfunction in women. *Am J Physiol Heart Circ Physiol* 310: H14–H19, 2016. First published October 30, 2015; doi:10.1152/ajpheart.00612.2015.—Women with coronary microvascular dysfunction (CMD) and no obstructive coronary artery disease (CAD) have increased rates of heart failure with preserved ejection fraction (HFpEF). The mechanisms of HFpEF are not well understood. Ectopic fat deposition in the myocardium, termed myocardial steatosis, is frequently associated with diastolic dysfunction in other metabolic diseases. We investigated the prevalence of myocardial steatosis and diastolic dysfunction in women with CMD and subclinical HFpEF. In 13 women, including eight reference controls and five women with CMD and evidence of subclinical HFpEF (left ventricular end-diastolic pressure >12 mmHg), we measured myocardial triglyceride content (TG) and diastolic function, by proton magnetic resonance spectroscopy and magnetic resonance tissue tagging, respectively. When compared with reference controls, women with CMD had higher myocardial TG content (0.83 ± 0.12% vs. 0.43 ± 0.06%; $P = 0.025$) and lower diastolic circumferential strain rate (168 ± 12 vs. 217 ± 15%/s; $P = 0.012$), with myocardial TG content correlating inversely with diastolic circumferential strain rate ($r = -0.779$; $P = 0.002$). This study provides proof-of-concept that myocardial steatosis may play an important mechanistic role in the development of diastolic dysfunction in women with CMD and no obstructive CAD. Detailed longitudinal studies are warranted to explore specific treatment strategies targeting myocardial steatosis and its effect on diastolic function.

myocardial steatosis; magnetic resonance spectroscopy; coronary microvascular dysfunction; diastolic dysfunction; women

NEW & NOTEWORTHY

Heart failure with preserved ejection fraction (HFpEF) is highly prevalent in women but poorly understood. Mechanistic understanding is critical to the development of HFpEF management strategies and guidelines. In this article, we present magnetic resonance spectroscopy data that identify cardiomyocyte fat accumulation as a potential novel mechanistic pathway.

Address for reprint requests and other correspondence: J. Wei, Barbra Streisand Women's Heart Center, Cedars-Sinai Heart Institute, 127 S. San Vicente Blvd A3600, Los Angeles, CA 90048 (e-mail: janet.wei@cshs.org).

ISCHEMIC HEART DISEASE IS a leading cause of death in women, with annual mortality rates 10-fold higher than mortality from breast cancer (13). Unlike their male counterparts, women with persistent chest pain and evidence of myocardial ischemia are more likely to have normal or no obstructive coronary artery disease (CAD) (4, 6, 7). As a result of targeted research initiatives to elucidate this sex difference, including the National Heart, Lung, and Blood Institute-sponsored Women's Ischemia Syndrome Evaluation (WISE) study, it is now recognized that coronary microvascular dysfunction (CMD) plays an important role in the development of ischemic heart disease in women (15, 34). Women with CMD have a 2.5% annual risk of major adverse cardiac events, including death, nonfatal myocardial infarction, nonfatal stroke, or hospitalization for heart failure (20, 37). Five-year WISE follow-up data also demonstrated that the most prevalent event for women with CMD is hospitalization for heart failure (15), predominantly heart failure with preserved ejection fraction (HFpEF), a condition that is associated with a ~25% mortality rate in 3 years (27). HFpEF mechanistic pathways and treatment strategies remain poorly understood.

We recently reported that women with signs and symptoms of ischemia with no obstructive CAD have evidence of left ventricular (LV) diastolic dysfunction (29). The exact mechanism responsible for the dysfunction, however, remains incompletely understood. We hypothesize that CMD triggers a metabolic shift away from free fatty acids, resulting in ectopic fat deposition in cardiomyocytes. Cardiac steatosis has indeed been mechanistically linked with diastolic dysfunction in various rodent models (8, 10–12, 51) and is an independent predictor of diastolic dysfunction in men (31, 47). The purpose of this study was, therefore, twofold: *1*) to determine whether myocardial steatosis is prevalent in women with CMD and *2*) to determine the relationship between cardiac steatosis and diastolic dysfunction.

METHODS

Study Population

Five women with persistent chest pain, evidence of ischemia by stress testing, no obstructive CAD, and evidence of subclinical HFpEF were recruited from the WISE study, and all had been diagnosed with CMD by prior invasive coronary reactivity testing per WISE protocol (49). After coronary angiography demonstration of no obstructive CAD and measurement of resting LV end-diastolic pressure (LVEDP), four coronary reactivity testing measures were assessed as previously published: *1*) abnormal coronary flow reserve, defined as

<2.5 in response to adenosine; *2*) abnormal coronary endothelial function defined as a change in epicardial coronary artery diameter ≤0% in response to ACh; *3*) abnormal microvascular endothelial dysfunction, defined as an increase in coronary blood flow ≤50% in response to ACh; and *4*) abnormal nonendothelial function defined as a change in epicardial coronary artery diameter ≤20% in response to nitroglycerin (44). The WISE group previously demonstrated that myocardial perfusion reserve worsened as the number of abnormal coronary reactivity measures increased (44).

Inclusion criteria for evidence of subclinical HFpEF consisted of resting LVED*P* > 12 mmHg and preserved ejection fraction >50% (9). Exclusion criteria comprised type 2 diabetes, age <18 years, body mass index (BMI) ≥44 kg/m^2, and irregular heartbeat, as well as any contraindication to MRI.

A reference control group consisted of eight women selected by using the following inclusion criteria: absence of any cardiovascular symptoms, absence of cardiac risk factors according to the National Cholesterol Education Program (1), and a normal maximal exercise treadmill stress test (Bruce protocol). Exclusion criteria for the control group were the same as for the group of WISE cases.

All subjects underwent cardiac magnetic resonance (CMR) imaging for evaluation of heart morphology and function, abdominal MRI for evaluation of abdominal visceral and subcutaneous fat mass, proton magnetic resonance spectroscopy (^{1}H MRS) for evaluation of myocardial and hepatic steatosis, and in body-impedance testing for the evaluation of lean and fat body mass. The study was approved by the Institutional Review Board at Cedars-Sinai Medical Center, and all study subjects provided informed consent before any experimental procedure.

Cardiac magnetic resonance imaging and spectroscopy. CMR tissue tagging and cardiac ^{1}H MRS testing were performed at 3.0T whole body Magnetom Verio MR system (Siemens Healthcare, Erlangen, Germany) using a phased array 3.0T body matrix coil, highly standardized protocol (36, 41) and localized spectroscopy with PRESS (PointRESolvedSpectroscopy) sequence for spatial localization and signal acquisition.

CMR and myocardial tissue tagging for LV function. LV morphology and global function were assessed using retrospective ECG-gated, segmented steady state short-axis cine images, across the entire LV. All images were acquired at end-expiration using the following parameters: repetition, time 3 ms; echo time, 1.3 ms; flip angle, 50°; slice thickness, 8 mm with a 2-mm gap between slices; matrix, 224 × 224; field of view, 300 × 225 mm; 33% oversampling. LV volumes, ejection fraction, and mass were measured by tracing the epicardial and endocardial borders of the short-axis cine images at end diastole and end systole using commercial software (cvi42; Circle Cardiovascular Imaging, Calgary, Alberta, Canada).

Two short-axis CMR tissue tagging images were acquired at the mid-LV level (papillary muscles), using a standard gradient echo sequence. Typical imaging parameters included slice thickness, 8 mm; 7-mm grid tags; temporal resolution, 20–30 ms; echo time, 1.8 ms; repetition time, 4.1 ms; matrix, 224 × 100; flip angle, 8°; field of view, 330 × 247 mm^2. For systolic function assessment, the first tissue tagging image was acquired with the trigger at end diastole (R wave of ECG). For diastolic function assessment, because tags fade within 400 to 500 ms (5), myocardial tagging was acquired with the trigger delay applied at end systole, to ensure persistence of tags throughout diastole (29). Time delay was adjusted individually in each subject for optimal tag persistence during diastole. Tagged CMR images were analyzed using commercial software (HARP, Diagnosoft 3.0; Palo Alto, CA) to determine circumferential strain and strain rate in both systole and diastole. Tag analysis was semi-automated, with user input limited to tracing the endo- and epicardium at a single reference cardiac phase for each slice.

^{1}H MRS for myocardial triglyceride measurements and quantification. ^{1}H MRS is a preferred method for non-invasive and in vivo measurement of steatosis. It permits precise and reproducible quantitation of intracellular TG content in cytosol of nonadipose cells. Evaluation of steatosis by ^{1}H MRS is now broadly accepted in clinical studies as fast, safe, and reliable. As previously described (26, 36, 40, 41), a spectroscopic volume of interest (a single voxel, 0.8 × 1.8 × 2.4 cm^3) was positioned over the interventricular septum using end-systolic cardiac cine images in two cardiac planes (short axis and semi 4 chamber axis), collected at end-expiration (Fig. 1). During acquisition of spectroscopic data, patients breathed freely. Data acquisition was triggered simultaneously at end systole (via ECG gating) and end expiration (via a respiratory navigator PACE) (46). The spectra were collected without water suppression and with the following parameters: repetition time was ~4 s depending on heart rate; echo time, 35 ms; 1,024 data points over a 2,000-Hz spectral width; and 32 acquisitions. Spectroscopy data were processed using commercial software (NUTS, Acorn NMR, Fremont, CA). Final calculation of fat and water signal intensities accounted for fat and water signal decay due to spin-spin relaxation (41, 42). Myocardial TG content was expressed as a percentage of tissue water content.

Abdominal MRI and Spectroscopy

MRI for abdominal fat distribution and hepatic ^{1}H MRS were performed following cardiac imaging.

MRI for abdominal fat distribution. Subcutaneous and intra-abdominal fat masses were determined from a single abdominal axial image at the level of lumbar L2-L3. Image analysis via mapping of the subcutaneous and intra-abdominal adipose tissue compartments (2) was performed by an observer blinded to patient clinical history using Slice-O-Matic software (4.3 rev 10; Virtual Magic, Montreal, Canada). Fat area was reported in square centimeters.

^{1}H MRS for hepatic TG measurements and quantification. As described previously (40, 42), a testing volume of 8 cm^3 was selected within the right hepatic lobe, avoiding major blood vessels, intrahepatic bile ducts, and neighboring adipose tissue, was selected from high resolution morphological hepatic images acquired with breath hold at end-exhalation. During acquisition of spectroscopic data patients breathed freely and acquisition was triggered at end-exhalation. Respiratory motion was compensated with a respiratory navigator PACE. Spectra were collected without water suppression and with the following data acquisition parameters: repetition time, 3 s; echo time, 35 ms; 1,024 data points over a 2,000-Hz spectral width; and 16 acquisitions. Final calculation of fat and water signal intensities accounted for fat and water signal

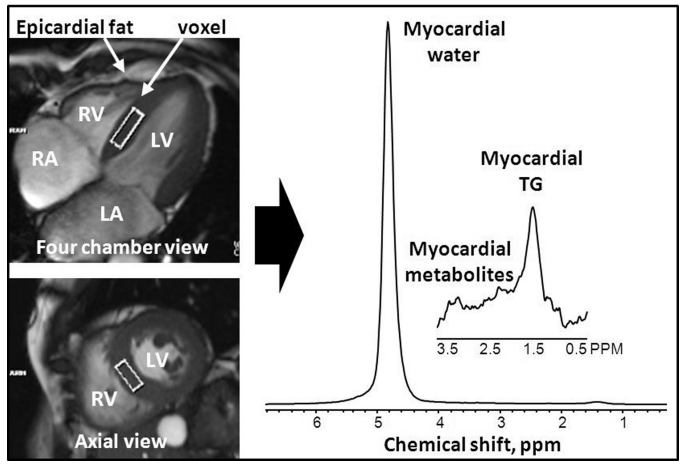

Fig. 1. ^{1}H magnetic resonance spectroscopy (MRS) measurement of myocardial fat accumulation. Volume of interest (single voxel, 3.4 cm^3) was positioned in the interventricular septum, and ^{1}H MRS was acquired at end systole and in end-expiration. Myocardial water (4.8 ppm), myocardial metabolites [e.g., carnitine, creatinine, trimethylamine, 3–3.5 parts per million (ppm)], and methylenes of fatty acids in myocardial TG (1.4 ppm) are shown. LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle; TG, triglyceride.

Table 1. *Characteristics of study population*

Variable	Cases	Controls	*P* Value
Anthropometric data			
Age, yr	52 ± 5	44 ± 6	0.28
Systolic BP, mmHg	110 ± 4	122 ± 6	0.14
Diastolic BP, mmHg	62 ± 3	69 ± 4	0.26
BMI, kg/m^2	29.0 ± 3.6	26.4 ± 1.6	0.48
Lean body mass, kg	45.6 ± 3.4	46.5 ± 1.9	0.82
Body fat mass, kg	34.2 ± 7.9	25.1 ± 3.6	0.28
MRI visceral fat, %	13.8 ± 3.0	22.0 ± 4.6	0.16
MRI subcutaneous fat, %	37.9 ± 6.2	36.3 ± 3.2	0.85
Hepatic TG content, %	4.2 ± 2.6	5.1 ± 1.9	0.80
LV hemodynamics and global function			
LV end-diastolic pressure, mmHg	18.4 ± 2.6	n/a	—
LV end-diastolic volume, ml	129.5 ± 13.1	125.7 ± 8.7	0.80
LV end-systolic volume, ml	39.4 ± 6.3	44.0 ± 4.5	0.56
LV stroke volume, ml	90.1 ± 8.1	81.7 ± 5.5	0.39
LV ejection fraction, %	70 ± 3	65 ± 2	0.17
LV concentricity, g/ml	0.68 ± 30.03	0.59 ± 0.07	0.37

Values are means ± SD; $n = 5$ cases and $n = 8$ controls. BMI, body mass index; BP, blood pressure; LV, left ventricular; n/a, not applicable; TG, triglyceride.

decay due to spin-spin relaxation (40, 42). Hepatic TG content was expressed as a percentage of tissue water content.

Statistical Analysis.

Subject characteristics were presented as means ± SD or 95% confidence interval. Differences between WISE cases and reference controls were compared using Student's two-sided *t*-test. Pearson correlation was performed for evaluation of linear dependence between pairs of variables, i.e., myocardial TG content and circumferential strain rate in WISE cases and reference controls. Statistical significance was defined as P value <0.05.

RESULTS

The cases and reference controls had similar age, BMI, and blood pressure (Table 1). Three of the five WISE cases had hypertension, five had dyslipidemia, one had hypothyroidism, and one had prior history of smoking. None were diabetic/prediabetic. Cardiac medications of the WISE cases included statins (80%), β-blockers (80%), ranolazine (40%), calcium-channel blockers (40%), and diuretics (40%); all subjects held their β-blockers, calcium channel blockers, ranolazine, and/or diuretics for at least 24 h before their procedures. All five cases were postmenopausal. All eight reference controls had no cardiovascular risk factors and did not take any cardiac medications; four were postmenopausal. None received hormone replacement therapy.

All five cases had persistent chest pain, evidence of ischemia by stress testing, a diagnosis of CMD by invasive coronary reactivity testing, and no obstructive CAD. Three cases had abnormal coronary flow reserve to adenosine, one case had abnormal endothelial function to ACh with both epicardial constriction and abnormal coronary blood flow response, and all five cases had abnormal nonendothelial function in response to nitroglycerin. Two cases had evidence of nonobstructive CAD (stenosis, 20–30%) in the left anterior descending artery, while the other three cases had no angiographic evidence of CAD. The mean LVEDP of the cases was elevated at 18 ± 3 mmHg, whereas the mean NT-proBNP was normal at 7 ± 26 pg/ml.

Diastolic dysfunction was present in women with CMD as evidenced by a mean diastolic circumferential strain rate that was lower in cases than in reference controls (168 ± 12%/s vs. 217 ± 15%/s; $P = 0.012$; Fig. 2*A*). Myocardial TG content was higher in cases than in reference controls (0.83 ± 0.12% vs. 0.43 ± 0.06%; $P = 0.025$; Fig. 2*B*), documenting presence of myocardial steatosis in women with CMD. In addition myocardial TG content and diastolic circumferential strain rate correlated inversely ($r = -0.779$; $P = 0.002$; Fig. 2*C*).

There were no group differences in LV mass, LV concentricity (mass/end-diastolic volume), or LV ejection fraction (Table 1). There were also no differences in lean body mass, body fat mass, and percentage body fat measured by impedance scale testing between the cases and reference controls. There was no difference in the percentage of visceral fat and subcutaneous fat measured by MRI between the cases and reference controls. Hepatic TG was within normal range (42) and was not different between cases and reference controls (4.2 ± 2.6% vs. 5.1 ± 1.9%; $P = 0.8$). Moreover, hepatic TG content did not correlate with myocardial TG content ($R^2 = 0.09$; $P = 0.39$).

DISCUSSION

The major novel findings of this investigation are fourfold. First, in women with CMD, myocardial steatosis was 100% higher than in reference controls. Second, diastolic circumferential strain rate, a measure of LV relaxation, was impaired by ~20% in WISE cases than in reference control subjects. Third, myocardial TG content and diastolic circumferential strain rate

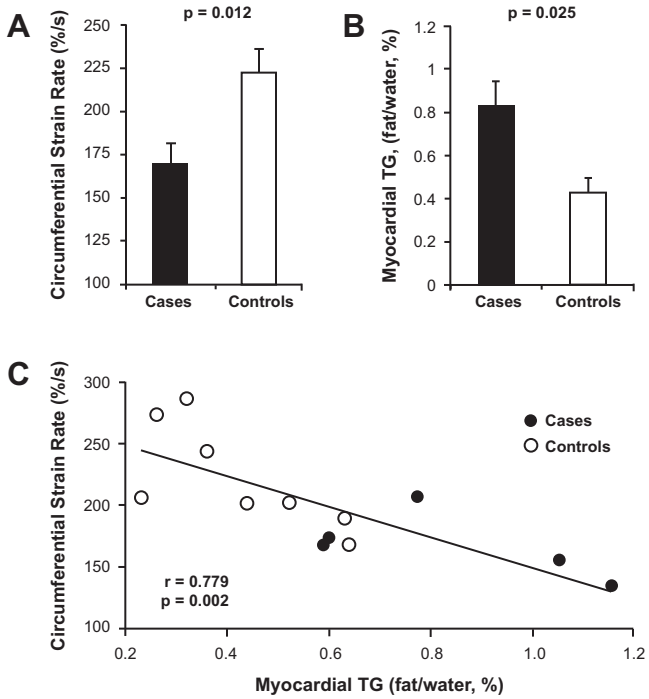

Fig. 2. Diastolic circumferential strain rate and myocardial TG content. *A*: diastolic circumferential strain rate was significantly higher in reference controls than in cases (217 ± 15 vs. 168 ± 12%/s; $P = 0.012$). *B*: myocardial TG content was significantly higher in cases than in reference controls (0.83 ± 0.12% vs. 0.43 ± 0.06%; $P = 0.025$). *C*: myocardial TG content correlated inversely with diastolic circumferential strain rate ($r = -0.779$; $P = 0.002$), demonstrating that diastolic dysfunction is present in women with coronary microvascular dysfunction.

were inversely related, suggesting a relationship between myocardial TG content and LV diastolic function. Fourth, hepatic steatosis was not associated with myocardial steatosis, suggesting that the ectopic myocardial fat deposition measured in our cases may be due to myocardial ischemia rather than systemic metabolic disease. Taken together, these data support the hypothesis that myocardial ischemia-related steatosis may be mechanistically linked with impairments in ventricular relaxation in women with CMD.

Combining MRI with ^{1}H MRS provides detailed pathophysiologic insights into inter-relationships among cardiac structure, function, and metabolism. ^{1}H MRS offers quantitative assessment of myocardial TG content, which cannot otherwise be assessed without using invasive biopsy or external radiolabeled tracers. Our laboratory has previously shown that this approach is highly reproducible in humans with a broad range of obesity and glucose levels, with TG content expressed as a percentage of tissue water content (26, 41). Moreover, we have shown that the amount of fat stored in a healthy nonadipose tissue (i.e., liver and myocardium) is usually minimal and tightly regulated, but increases significantly when the healthy metabolic milieu is disturbed (26, 30, 35, 48). The reliability of ^{1}H MRS to identify and quantify myocardial TG content is important in the identification of myocardial steatosis as a potential treatment target for diastolic dysfunction. Myocardial steatosis and diastolic dysfunction has been shown to be reversible in subjects with severe aortic stenosis undergoing aortic valve replacement (24) and in pharmacologic and dietary reductions of plasma TG levels (16, 17). Traditional and new anti-ischemic therapies may reduce myocardial steatosis and thus may improve myocardial dysfunction in subjects with CMD and HFpEF. In a pilot study, short-term ranolazine was found to decrease LVEDP but did not improve echocardiographic diastolic parameters (E/A, E/E′) (25); it is unknown whether long-term ranolazine may have beneficial effects on LV filling pressure and on sensitive noninvasive diastolic parameters, like those used in this investigation.

Although the presence of myocardial steatosis in HFpEF has not been previously described, myocardial TG accumulation has been described in ischemic cardiomyopathy (28) and end-stage systolic heart failure, as documented by biopsies of post-transplant hearts in men and women (38). Failing hearts had fourfold higher levels of myocardial TG compared with nonfailing hearts. The buildup of TG within the cytosol of cardiomyocytes was associated with dysfunctional expression of genes related to free fatty acid (FFA) metabolism, contractile dysfunction, and inflammation (38). It has been suggested that accumulation of TG in the failing human heart is due to a preferential substrate shift (14); that is, unlike the healthy myocardium, which converts chemical energy primarily from FFA (3), the ischemic myocardium preferentially oxidizes glucose, leaving FFA unoxidized. This surplus of unoxidized FFA is converted to TG droplets in the cytosol of cardiomyocytes (28, 38). Given that each of our WISE cases had established CMD, we speculate that a similar ischemic metabolic shift likely explains the accumulation of myocardial TG found in the present investigation. Hankiewicz et al. (19) demonstrated that in mice with high myocardial TG content, early impairment of diastolic strain occurs primarily in the endocardium, a region that is hypoperfused in patients with CMD (33). However, a prior ^{1}H MRS and stress CMR study of 42 subjects with type 2 diabetes revealed that although myocardial TG content was inversely associated with diastolic strain rate, no association was found between myocardial TG content and myocardial perfusion reserve index (22); the subjects with type 2 diabetes in that study did not have clinical symptoms of angina or heart failure, and we suspect that the pathophysiology of myocardial steatosis in diabetes is different from the pathophysiology of myocardial steatosis in myocardial ischemia.

It remains unclear from this cross-sectional investigation whether myocardial TG accumulation contributes to the development of HFpEF in women with CMD or whether it is simply a marker of disease progression. In the Zucker diabetic fatty rat, cardiac steatosis has been unequivocally linked to myocellular apoptosis and adverse LV remodeling (51). In human volunteers, Hammer et al. (18) showed that acute elevation of myocardial TG content not only reduced diastolic function but that pharmacological inhibition of the myocardial TG accumulation prevented the cardiac impairment. We believe that myocardial TG accumulation not only marks disease progression but that it also independently contributes to the development of diastolic dysfunction in women with CMD.

Myocardial steatosis and hepatic steatosis were not directly associated in our study, suggesting that the development of myocardial steatosis in the setting of myocardial ischemia is different from the development of myocardial steatosis in the setting of systemic metabolic diseases. In systemic metabolic disease like obesity and type 2 diabetes, the development of myocardial steatosis tends to mirror the development of steatosis in other organs (e.g., liver, pancreas) due to elevated circulating FFA (23, 26, 32, 43). We interpret this dichotomy to reflect differences in disease origin. For example, although ischemic heart disease primarily involves reduced myocardial capacity for FFA oxidation, resulting in steatosis exclusively in the heart, metabolic diseases like obesity and type 2 diabetes reflect systemic FFA overload and thus more global organ steatosis (45, 50).

Our proof-of-concept study has several limitations, including a small number of cases and reference controls. Further investigation is warranted to confirm the present results in a larger cohort of women and men with CMD with comparison with controls matched for age and cardiovascular risk factors. Our inclusion criteria for subclinical HFpEF was met primarily by elevated LVEDP, since our cases had normal NT-proBNP; since the completion of this pilot study, we have performed analysis on NT-proBNP and invasive parameters in the WISE cohort and found that NT-proBNP did not correlate with LVEDP (21). We did not measure plasma metabolic profiles or perform glucose tolerance testing to exclude pre-diabetes. However, we do not believe that such limited metabolic information could have influenced our interpretation of the present results, since neither group had elevated hepatic TG content Or grossly abnormal fasting metabolic parameters or visceral fat percentages.

Another potential limitation is limited number of diastolic end-points used in this investigation. Conventional (Doppler derived) diastolic measurements include mitral inflow velocities, annular tissue velocities, and pulmonary venous flow velocities, which were not assessed in this investigation. Instead we focused on circumferential diastolic strain rate, using gold-standard MR tissue tagging. Indeed, we have previously shown this metric differentiates patients with ischemic syndrome, from healthy age-, sex-, and BMI-matched controls (29). We have also shown that this metric is more sensitive than other traditional MR derived metrics of diastolic function, such as peak volumetric filling rate

(29). Like other measures of diastolic function, however, circumferential diastolic strain rate is likely load dependent and thus would be influenced by pseudo-normalization. We do not believe that this influenced our interpretation of the present results, however, since circumferential diastolic strain rate was lower than our healthy controls, despite all of our patients having significantly elevated LV filling pressures.

Myocardial TG content was measured in the septum. As such, one cannot exclude the possibility of regional differences. Although CMD is considered to be a diffuse phenomenon, it may have regional heterogeneity (39); nevertheless, none of the subjects had asymmetric septal hypertrophic cardiomyopathy, and we do not suspect regional differences in CMD or myocardial steatosis in our subject population.

Finally, many of our patients were on β-blockers and/or calcium-channel blockers. We do not believe these medications influences our present results, however, since these medications were held for at least 24 h before imaging.

The data herein suggest that myocardial steatosis may play an important mechanistic role in the development of diastolic dysfunction in women with CMD and no obstructive CAD. Detailed longitudinal studies are warranted to explore specific treatment strategies targeting myocardial steatosis.

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Present address for E. Szczepaniak and L. Szczepaniak: Biomedical Research Consulting in magnetic resonance spectroscopy, Albuquerque, New Mexico.

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AUTHOR CONTRIBUTIONS

Author contributions: J.W., E.W.S., C.N.B.M., and L.S.S. conception and design of research; J.W., E.W.S., L.S., and L.S.S. performed experiments; J.W., M.D.N., E.W.S., L.S., and L.S.S. analyzed data; J.W., M.D.N., E.W.S., P.K.M., C.N.B.M., and L.S.S. interpreted results of experiments; J.W., M.D.N., E.W.S., and L.S.S. prepared figures; J.W. and M.D.N. drafted manuscript; J.W., M.D.N., L.S., P.K.M., L.E.T., D.S.B., D.L., C.N.B.M., and L.S.S. edited and revised manuscript; J.W., M.D.N., E.W.S., L.S., P.K.M., L.E.T., D.S.B., D.L., C.N.B.M., and L.S.S. approved final version of manuscript.

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