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Skeletal Muscle Mitochondrial Content, Oxidative Capacity, and Mfn2 Expression are Reduced in Older Patients with Heart Failure and Preserved Ejection Fraction and are Related to Exercise Intolerance

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Abstract

Objectives—The aim of this study was to examine skeletal muscle mitochondria content, oxidative capacity, and the expression of key mitochondrial dynamics proteins in patients with heart failure with preserved ejection fraction (HFpEF) and to determine potential relationships with measures of exercise performance.

Background—Multiple lines of evidence indicate that severely reduced peak exercise oxygen uptake (peak VO_2) in older patients with HFpEF, is related to abnormal skeletal muscle oxygen utilization. Mitochondria are key regulators of skeletal muscle metabolism, however little is known about how these organelles are affected in HFpEF.

Methods— *Vastus lateralis* skeletal muscle citrate synthase activity, and the expression of porin and regulators of mitochondrial fusion were examined in older patients with HFpEF (n=20) and healthy, age-matched controls (n=17).

Results—In HFpEF patients compared to age-matched healthy controls, mitochondrial content assessed by Porin expression was 46% lower (p-value= 0.01), citrate synthase activity was 29% lower (p= 0.01), and Mfn2 expression was 54% lower (p= <0.001). Expression of Porin was

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CONFLICTS OF INTEREST

Dr. Kitzman serves as a consultant for Relypsa, Corvia Medical, Abbvie, Merck, Bayer, GSK, and Regeneron, and owns stock in Gilead Sciences and Relypsa. No other authors have conflicts of interest to declare.

significantly positively correlated with both peak VO_2 and 6 minute walk distance (r=0.48, p=0.003 and r=0.33, p=0.05, respectively). Expression of Mfn2 was also significantly positively correlated with both peak VO_2 and 6 minute walk distance (r=0.40, p=0.02 and r=0.37, p=0.03 respectively).

Conclusion—These findings suggest that skeletal muscle oxidative capacity, mitochondrial content, and mitochondrial fusion are abnormal in older patients with HFpEF and may contribute to their severe exercise intolerance.

Keywords

heart failure; preserved ejection fraction; exercise; aging; mitochondria; skeletal muscle

INTRODUCTION

Heart failure with preserved left ventricular ejection fraction (HFpEF) is the most prevalent form of heart failure (HF) and is nearly unique to older adults, particularly older women (1, 2). The primary manifestation of chronic stable HFpEF is severe exercise intolerance measured objectively as reduced peak exercise oxygen uptake (peak VO_2) (3). Multiple recent reports indicate that, in addition to underlying cardiac dysfunction, 'non-cardiac' factors contribute to severe exercise intolerance in HFpEF. Previously, our group has reported that reduced cardiac output accounts for only 50% of the markedly reduced peak oxygen uptake (peak VO_2) in HFpEF patients (4), suggesting a significant role for peripheral factors. Endurance exercise training significantly improves peak VO_2 in clinically stable older individuals with HFpEF; however, a majority of the improvement is mediated by non-cardiac factors, presumably skeletal muscle function (5, 6). Although we and others have found significant abnormalities in central arterial stiffness and their relation to exercise intolerance, we reported that arterial stiffness and conduit arterial endothelial dysfunction do not improve with exercise training in HFpEF (7). Altogether, these studies suggest that in HFpEF, skeletal muscle alterations contribute to exercise intolerance and that improvements in skeletal muscle function contribute to the beneficial effects of exercise training for these patients.

Several lines of evidence indicate that older adults with HFpEF have altered skeletal muscle metabolism. We have reported that older HFpEF patients have abnormal skeletal muscle oxygen utilization and that this is related to their severely reduced peak VO_2 (8). Using magnetic resonance spectroscopy in HFpEF patients, Bhella et al found reduced skeletal muscle oxidative metabolism (9). More recently, Dhakal et al, using invasive hemodynamic monitoring during exercise, showed that oxygen extraction was significantly reduced in HFpEF and was a major contributor to their reduced peak VO_2 (10). While HFpEF patients have a lower percent lean mass, the increase in peak exercise VO_2 relative to lean mass is lower in HFpEF compared to healthy controls (8). Using skeletal muscle biopsies, we recently showed that HFpEF patients have a decreased number of type I oxidative fibers compared to healthy controls (11). Taken together, these data suggest the hypothesis that skeletal muscle mitochondrial dysfunction contributes to impaired skeletal muscle aerobic metabolism in patients with HFpEF.

To examine this hypothesis, we measured the expression of key mitochondrial proteins in skeletal muscle biopsy specimens from patients with HFpEF and healthy, age-matched, controls (HC). We measured the expression of Mitofusins 1 and 2 (Mfn1 and Mfn2), proteins localized to the mitochondrial outer membrane that play an essential role in the fusion of these organelles. Mitofusins, in particular Mfn2, play an important role in mitochondrial quality control by mediating complementation of organelles and the elimination of dysfunctional mitochondria by autophagy (12, 13). A potential difference in skeletal muscle mitochondrial content was determined by analysis of Porin expression. Further validation was provided by the measurement of citrate synthase activity, a biomarker widely recognized as the most reliable indicator of skeletal muscle oxidative capacity and mitochondrial content (14). Finally, we examined the relationships of these mitochondrial parameters with measures of exercise intolerance, peak VO_2 and 6-minute walk distance.

METHODS

Participants

As previously described in studies from our laboratory (3, 4, 6, 15–17), and in accord with the 2013 ACC/AHA recommendations (18), HFpEF was defined as symptoms and signs of HF according to the National Health and Nutrition Examination Survey HF clinical score of ≥3 and the criteria of Rich et al (19, 20), preserved resting left ventricular systolic function (ejection fraction ≥50%, and no segmental wall motion abnormalities), and no significant ischemic or valvular heart disease, pulmonary disease, anemia, or other disorder that could explain the patients' symptoms (3, 4, 16). HFpEF subjects were ≥ 60yrs of age at study entry. Age-matched, sedentary HC subjects were recruited and screened and excluded if they had any chronic medical illness, were on any chronic medication, had current complaints or an abnormal physical examination (including blood pressure ≥ 140/90 mmHg), had abnormal results on the screening tests (including electrocardiogram, exercise echocardiogram, and spirometry), or regularly undertook vigorous exercise (8, 21). The protocol was approved by the Wake Forest School of Medicine institutional review board, and all participants provided written, informed consent.

Exercise Testing

As previously described (3, 22), exercise testing was performed on a treadmill using the modified Naughton protocol for the HFpEF subjects and using the modified Bruce protocol for the HC subjects. Expired gas analysis was conducted using a commercially available system (CPX-2000 and Ultima; MedGraphics; Minneapolis, MN) that was calibrated before each test with a standard gas of known concentration and volume. Breath-by-breath gas exchange data were measured continuously during exercise and averaged every 15 seconds, and peak values were averaged from the last two 15-second intervals during peak exercise. A six-minute walk test was performed using the method of Guyatt (23).

Skeletal muscle biopsy

As previously described, skeletal muscle biopsies were performed in the early morning after an overnight fast (24–26). Subjects were asked to refrain from taking aspirin, non-steroidal anti-inflammatory drugs, and other compounds that may affect bleeding, platelets, or

bruising for the week prior to the biopsy, and to refrain from any strenuous activity for at least 36 hours prior to the biopsy. Muscle was obtained from the vastus lateralis using the percutaneous needle biopsy technique with a University College Hospital needle under local anesthesia with 1% lidocaine (27). There were no medical complications or other reported adverse events from the procedure.

Visible blood and connective tissue were removed from muscle specimens, and portions for Western blot and enzyme analyses were partitioned for freezing. Muscle portions used for mitochondrial analyses were stored at −80°C prior to homogenization and analysis.

Protein Expression

Expression of mitochondrial proteins was determined by Western blotting. Frozen skeletal muscle biopsy samples (10–15 mg) were homogenized with stainless steel beads using a BBX24 Bullet Blender (Next Advance, Averill Park, NY) and lysed with radioimmunoprecipitation assay (RIPA). Equal amounts of total protein, determined by BCA protein assay (Thermo Scientific, Rockford, IL), were separated by electrophoresis in Laemmli buffer on 12% polyacrylamide-SDS gels (Invitrogen, Carlsbad, CA). The samples were electrophoretically transferred to nylon polyvinyl difluoride (PVDF) membrane and the blots were incubated with commercially-available primary antibodies to Mfn1 (1:1000), Mfn2 (1:1000), porin (1:1000), and GAPDH (1:2000) (Abcam, Cambridge, MA). Appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies were added and immunoreactive products were visualized using the Supersignal West-Pico chemiluminescent reagent (Thermo Scientific, Rockford, IL). Kaleidoscope markers (BioRad, Hercules, CA) were used to monitor protein transfer and Magic Markers (Invitrogen, Carlsbad, CA) were used for molecular weight approximations. The density of each immunoreactive product was quantified using a Kodak imaging system (Kodak, Rochester, NY). Densitometry values for Mfn1 and Mfn2 were normalized to Porin in order to account for differences in mitochondrial content. Measurement of Porin was normalized to GAPDH.

Citrate Synthase Activity

The activity of a mitochondrial enzyme, citrate synthase, in skeletal muscle homogenates was measured using the Citrate Synthase Assay Kit (Sigma, CS0720). Measurements of citrate synthase activity were carried out at room temperature in 0.2 ml of assay medium containing 20μg of muscle homogenate in the presence of saturating concentrations of acetyl-coenzyme A (0.3 mM), dithionitrobenzoic acid (0.1 mM) and oxaloacetate (0.5 mM). The reaction was initiated by adding 0.5 mM oxaloacetate and monitored by measuring the increase in absorbance at 412 nm due to thionitrobenzoic acid (TNB) formation using a SpectraMax Microplate Reader (UV/Vis) and SoftMax Pro software (Molecular Devices). Citrate synthase activities were expressed as nmol/min/mg protein using molar extinction coefficient of TNB, ε412=13.6/mM/cm.

Statistical Methods

Intergroup (HFpEF vs. HC) comparisons of participant characteristics were made by independent samples t-tests for continuous variables, by Fisher’s exact tests for binomial

variables, and by Chi-square tests for general categorical variables. To ensure confidence in our findings, comparisons of exercise capacity variables between groups were made by analysis of covariance, adjusting for gender. Comparisons of mitochondrial protein expression between groups were made initially by independent samples t-test and then by analysis of covariance, adjusting for gender, BMI, and race. Sex, BMI, and race were selected because of prior data suggesting their influence on mitochondrial function, in addition to the intergroup imbalances observed. The tests for group differences were made by the Type III sum of squares test for removal. The final analysis was conducted as a single test with subsequent adjustments. Logarithmic transformation was performed for Porin which was highly skewed. The relationships between mitochondrial protein expression variables and exercise capacity (peak VO2, six-minute walk distance) were assessed by Spearman correlations. For all analyses, a two-tailed p-value of <0.05 was required for significance. All statistical tests were conducted using SAS version 9.1.3 .

RESULTS

Subject Characteristics

The patients had characteristics typical of chronic, stable HFpEF with NYHA class II-III symptoms, and abnormal Doppler LV diastolic function compared to HC (Table 1). A history of chronic systemic hypertension was present in 95% of HFpEF patients. HFpEF and HC were well matched for age; however, there was a trend for a greater number of women in the HFpEF group. No participants in the HFpEF or HC groups had a history, signs, or symptoms of thyroid dysfunction. Although body weight, fat mass, percent body fat, and body mass index were higher in HFpEF compared to HC, lean mass was similar. Multiple population-based studies and clinical trials have reported significantly higher BMI in HFpEF patients compared to the general population (28–32). To account for the differences in gender, BMI, and race, adjustments for these variables are included in the comparisons of mitochondrial parameters.

Exercise performance

In accord with prior reports, HFpEF patients had severely reduced peak VO_2 compared to HC (Table 2) (33, 34). This was evident despite similar peak exercise respiratory exchange ratio in HFpEF versus HC, which was ≥1.12 in both groups, indicating exhaustive exercise effort. There was a trend for lower peak HR in HFpEF versus HC. The 6-minute walk distance was also significantly less in HFpEF compared to HC.

Expression of Mitochondrial Proteins

Protein expression of Porin and Mfn2 (normalized to Porin) were significantly lower (p=0.01 and p=<0.001 respectively) in skeletal muscle tissue of patients with HFpEF compared to HC (Table 3). Normalization of mitofusins to Porin, rather than gapdh, was appropriate because these protein reside on the mitochondrial outer membrane. Normalization to Porin accounts for the difference in mitochondrial content, which would exacerbate the observed difference on Mfn2 expression. The lower Mfn2 expression in HFpEF patients remained statistically significant after adjustments for gender (p=<0.001), BMI (p=0.03), race (p=0.002), or gender, BMI, and race (p=0.03). Decreased Porin

expression in HFpEF skeletal muscle remained significant when adjusting for gender alone (p=0.004), race alone (p=0.003) and a strong trend for significance when adjusted for gender, BMI and race (p=0.07). Expression of Mfn1 was not significantly different in HFpEF skeletal muscle compared to HC. No significant differences were found with subgroup analysis comparing the effects of gender. Representative western blot images from three participants from each group are presented in figure 1.

Citrate Synthase activity

Citrate synthase activity was significantly lower (p=0.01) in patients with HFpEF compared to HC (Table 3). The difference remained significant with individual adjustments for gender and BMI, and a strong trend for significance when adjusted for race (p=0.06) and for gender, BMI, and race (p=0.053).

Relationships between skeletal muscle mitochondria and exercise performance

Expression of mitochondrial proteins and citrate synthase activity were compared with aerobic power (i.e. peak VO_2) and endurance (six-minute walk distance, 6MWD) (Table 4). With all subjects combined, Porin expression was significantly positively associated with both peak VO_2 and 6MWD (Figure 2). The expression of Mfn2 was positively associated with both measures of exercise performance (Figure 3). Mfn1 was significantly associated with 6MWD with a trend (p=0.053) for a positive association with peak VO_2. There was a trend toward a relationship (p=0.07) between citrate synthase activity and peak VO_2 (Figure 4).

When analyses were performed only within the HFpEF group, the relationships became non-significant, except for Porin and Mfn1, where there remained a trend with 6 minute walk distance (r=0.33; p=0.16, and r=0.37, p=0.11, respectively); there also remained a trend for Mfn1 with peak VO_2 (r=0.38; p=0.10).

Effects of statin use

By definition, no participants in the HC group were taking statin medications. Among the participants with HFpEF, 11 were on statins and 9 were not. Analyses comparing the effects of statin use on mitochondrial outcomes indicate that there was no effect on Mfn2 expression (p=0.67) and no effect of citrate synthase activity (p=0.94).

DISCUSSION

To our knowledge, this study provides the first report of skeletal muscle mitochondrial alterations in patients with HFpEF, the most common form of HF in older persons. The major new finding of this study is that compared to healthy controls, patients with HFpEF exhibit lower *vastus lateralis* mitochondrial content and oxidative capacity as reported by citrate synthase activity and porin expression. The relationships of these mitochondrial parameters with measures of exercise capacity indicate that these deficits may contribute to severely reduced exercise tolerance. Additionally, the mitochondrial fusion regulator, Mfn2, was significantly decreased in HFpEF and may also contribute to exercise intolerance.

The findings presented in this report are supported by multiple lines of evidence, including a recent study reporting that mitochondrial density, measured in the soleus muscle of a rat model of HFpEF, was reduced compared to controls (35). Further support is provided by our previous report that, compared to age-matched healthy subjects, older HFpEF patients have reduced peak exercise arteriovenous-oxygen difference (A-VO_2diff), which is an important contributor to their severely reduced peak VO_2 (33). In addition, non-cardiac peripheral factors were also a major contributor to improved peak VO_2 after endurance training (6). Using ^{31}phosphate magnetic resonance spectroscopy, Bhella et al reported that during static leg exercise, HFpEF patients had impaired skeletal muscle oxidative metabolism versus healthy controls(9). Recently, we showed that in older HFpEF patients compared to healthy age-matched controls, the slope of the relationship of peak VO_2 with percent leg lean mass was markedly reduced in HFpEF versus age-matched healthy controls, suggesting that skeletal muscle hypoperfusion or impaired oxygen utilization may play an important role in limiting exercise performance in HFpEF(8). Further, using skeletal muscle biopsies, we showed that HFpEF patients had fewer type I oxidative fibers compared to healthy controls (11).

Studies performed in healthy older adults provide further evidence linking mitochondrial bioenergetics with physical function. Coen et. al, has reported that the respiratory capacity of muscle fibers and maximal phosphorylation capacity are associated with peak VO_2 and walk speed in older adults (36). Similarly, we have reported that walk speed in older adults is also positively associated with the function of isolated skeletal muscle mitochondria, assessed as respiratory control (25).

Our data are also supported by previous reports of multiple skeletal muscle mitochondrial abnormalities, including reduced citrate synthase activity, in patients with HF with reduced EF (HFrEF) and their relation to exercise intolerance in those patients (37, 38). A study by Drexler and colleagues reported over 20 years ago that in patients with HFrEF, skeletal muscle mitochondrial morphology is disrupted and characterized by a reduction in mitochondrial volume and surface density (39). Due to its key role in mitochondrial fusion (40), reduced Mfn2 expression may mediate changes in mitochondrial morphology associated with HF. The Drexler study found that the alterations in mitochondrial morphology in HFrEF were significantly correlated with peak VO_2. Similarly, we find that in HFpEF, Mfn2 expression is correlated to both peak exercise peak VO_2 and 6MWD, indicating a potential role for impaired mitochondrial fusion in associated impaired exercise tolerance during sub-maximal (as occurs during activities of daily living) as well as maximal exercise.

The mean BMI of our HFpEF patients and HC reflects that of population-based studies and clinical trials which indicate that approximately 80% of older HFpEF patients are overweight or obese, twice the general older population (41). Excess regional adipose tissue may impair skeletal muscle function and reduce mitochondrial density (42, 43); hence, the potential effects of obesity on skeletal muscle mitochondria are important to consider in the interpretation of the present results. While an earlier study has reported that Mfn2 mRNA is decreased in the skeletal muscle of obese individuals (44), a more recent study has shown that protein content of Mfn2 is unaffected (45). In our study, controlling for BMI did not

alter the statistical difference between Mfn2 expression in patients with HFpEF and HC. The difference in Porin in HFpEF patients compared to HC was weaker statistically (p=0.21) after controlling for BMI, suggesting a role for body mass / adioposity in mitochondrial content and biogenesis in HFpEF.

Interestingly, skeletal muscle Mfn2 has been associated with a number of metabolic abnormalities. In mice, genetic ablation of Mfn2 leads to the development of impaired glucose tolerance, hyperinsulinemia, and insulin resistance (46). In humans, mRNA levels of Mfn2 have been positively correlated with glucose disposal rate (44). Mfn2 depletion in HFpEF may play a role in impaired skeletal muscle metabolism as well as insulin resistance, and glucose intolerance associated with this disease (47). Importantly, Mfn2 plays an important role in mitochondrial quality control by mediating complementation of organelles and the elimination of dysfunctional mitochondria by autophagy (40, 48, 49). Low expression of Mfn2 may lead to the accumulation of dysfunctional organelles within the mitochondrial network, leading to reduced overall oxidative phosphorylation capacity.

Limitations

This study was limited to mitochondrial measures that could be performed on 10–15mg samples of frozen skeletal muscle tissue that were available. Future studies, with access to larger, fresh samples at the time of biopsy, will enable the examination of mitochondrial function by muscle fiber respirometry, and the preparation of tissues for imaging of mitochondria using electron microscopy and for gene expression. Such studies will be able to determine the bioenergetic and morphological consequences of the low citrate synthase activity and low Mfn2 expression we observed patients with HFpEF. This study specifically focused on citrate synthase because this enzyme has been shown to be decreased in HFrEF (37). A recent review of manuscripts published between 1983 and 2013, reported that changes in peak VO_2 are highly correlated with measures of skeletal muscle citrate synthase activity (50). Furthermore, the authors report that changes in whole body oxidative capacity also matched changes in muscle citrate synthase activity.

We selected healthy control subjects who were sedentary since habitual level of physical activity can influence skeletal muscle function. Since we did not use formal survey assessments of habitual physical activity, we cannot ensure that there weren’t subtle intergroup differences that influenced our results. However, similar mitochondrial abnormalities were present in animal models of HF and HFpEF where physical activity was able to be controlled (35). Furthermore, it is known that the mitochondrial abnormalities in HFrEF, which are similar to those we observed in our HFpEF patients, are independent of level of habitual phyical activity and cardiac output (38, 51).

Although relationships of mitochondrial function variables with exercise capacity became less significant when examined within the HFpEF group only, the most appropriate analyses to address the question of mechanisms accounting for differences in exercise capacity between HFpEF and controls is with the groups combined; this also allows a larger range for correlations and a larger sample size.

Since we did not test a control group with hypertension but not heart failure, we cannot determine whether a portion of the intergroup differences in mitochondrial content and biogenesis that we observed was mediated partly by this common comorbidity.

Future directions

The results of this study warrant multiple lines of future investigation. We have previously reported that the capillary-to-fiber ratio is decreased in patients with HFpEF and that these alterations were associated with their decreased peak VO_2 (26). This study highlights the need to better understand the relative contributions of oxygen supply versus utilization in the impairment of skeletal muscle metabolism in patients with HFpEF. Future studies can also examine the effects of interventions, such as exercise training, on skeletal muscle mitochondrial bioenergetics in patients with HFpEF. Alterations in mitochondrial oxidative phosphorylation may underlie our previous observation that the increase in peak VO_2 after exercise training is primarily due to improved A-VO_2diff (6). In a rat model of HFpEF, exercise training prevented the reduction of skeletal muscle citrate synthase activity (35). In healthy humans, exercise training has also been shown to increase the expression of Mfn2 (52, 53). Mitochondria, and Mfn2 in particular, represent potentially important targets for intervention since they can underlie skeletal muscle defects associated with a number of the primary symptoms of HFpEF. Interventions that rescue Mfn2 expression, prevent Mfn2 decline, or promote Mfn2 function may have important implications in the treatment of HFpEF. Understanding the role of mitochondria in HFpEF-associated exercise intolerance and its improvement with intervention could lead to the development of novel treatments for exercise intolerance in HFpEF with strategies specifically designed to improve skeletal muscle metabolism.

CONCLUSION

These findings suggest that skeletal muscle oxidative capacity, mitochondrial content, and mitochondrial fusion are abnormal in older patients with HFpEF and may contribute to their severe exercise intolerance.

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Abbreviations

ACC	American College of Cardiology
AHA	American Heart Association
A-VO_2diff	arterio-venous oxygen difference
BMI	body mass index

DEXA	dual energy x-ray absorptiometry
EF	ejection fraction
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
HC	healthy age-matched control
HF	heart failure
HFpEF	heart failure with preserved ejection fraction
HFrEF	heart failure with reduced ejection fraction
HRP	horseradish peroxidase
LV	left ventricle
Mfn1	mitofusin 1
Mfn2	mitofusin 2
mRNA	messenger ribonucleic acid
NYHA	New York Heart Association
PVDF	polyvinyl difluoride
RIPA	radioimmunoprecipitation assay
TNB	thionitrobenzoic acid

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Clinical Perspectives

Recent studies have reported that alterations in skeletal muscle metabolism are major contributors to exercise intolerance in patients with HFpEF. This study provides the first evidence that skeletal muscle mitochondrial defects are present in patients with HFpEF and are related to key measures of exercise intolerance. Understanding the role of mitochondria in HFpEF may uncover potential new targets for the development of therapeutic interventions.

Translational Outlook

The relationship of low citrate synthase activity and low Mfn2 expression with mitochondrial function and skeletal muscle metabolism in patients with HFpEF remains to be determined. In addition, future studies should examine the role of mitochondria in interventions that have been shown to improve exercise capacity in patients with HFpEF (e.g. exercise training). The results of these studies will inform on whether targeting mitochondria can effectively improve the exercise capacity of patients with HFpEF.

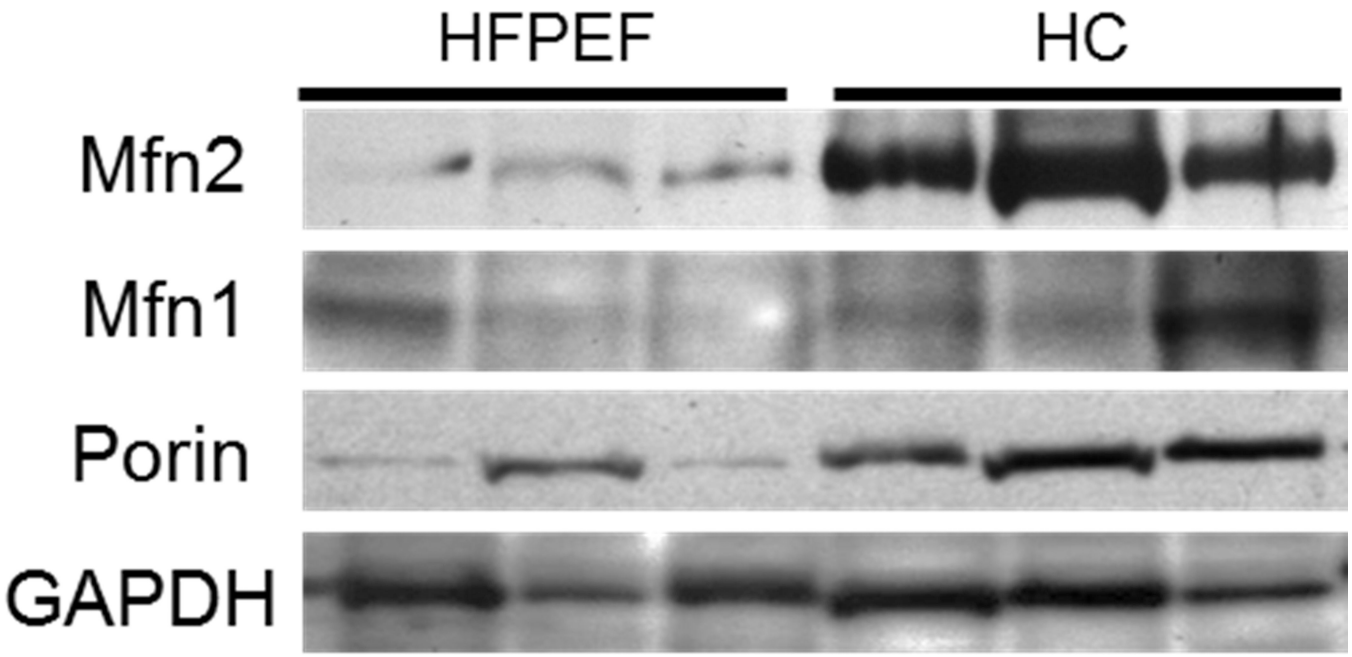

Figure 1.
Representative western blot bands from 3 patients with HFPEF and 3 healthy controls (HC). For each protein, images were obtained from the same blot and exposure.

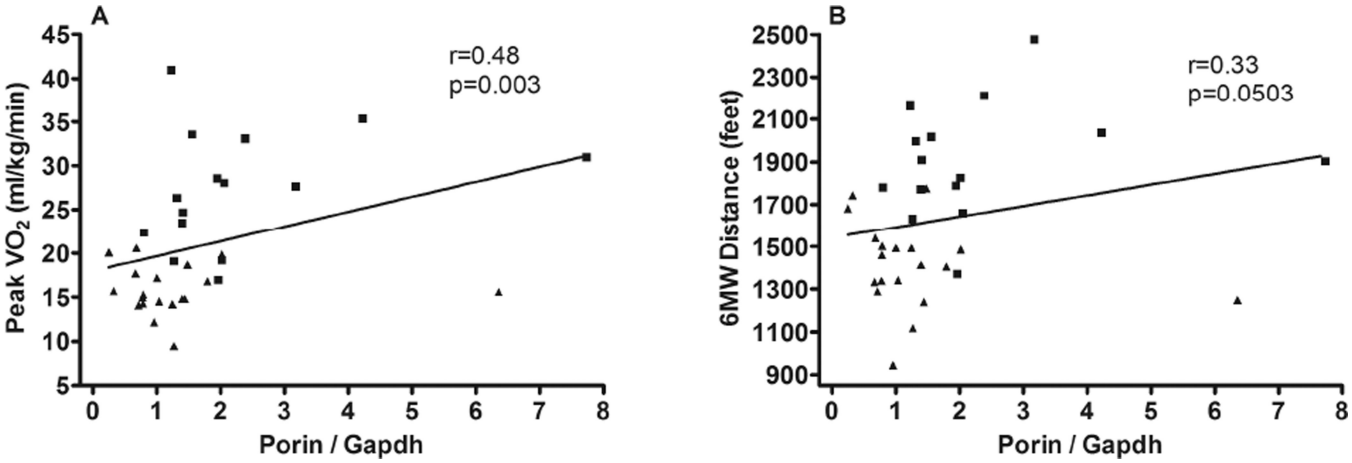

Figure 2.

Regression analysis (Spearman) comparing *Vastus lateralis* Porin expression with peak VO2 and 6 minute walk distance. Both mitochondrial markers were normalized to the housekeeping protein, GAPDH. HFpEF participants are designated by triangles and HC participants are designated by squares.

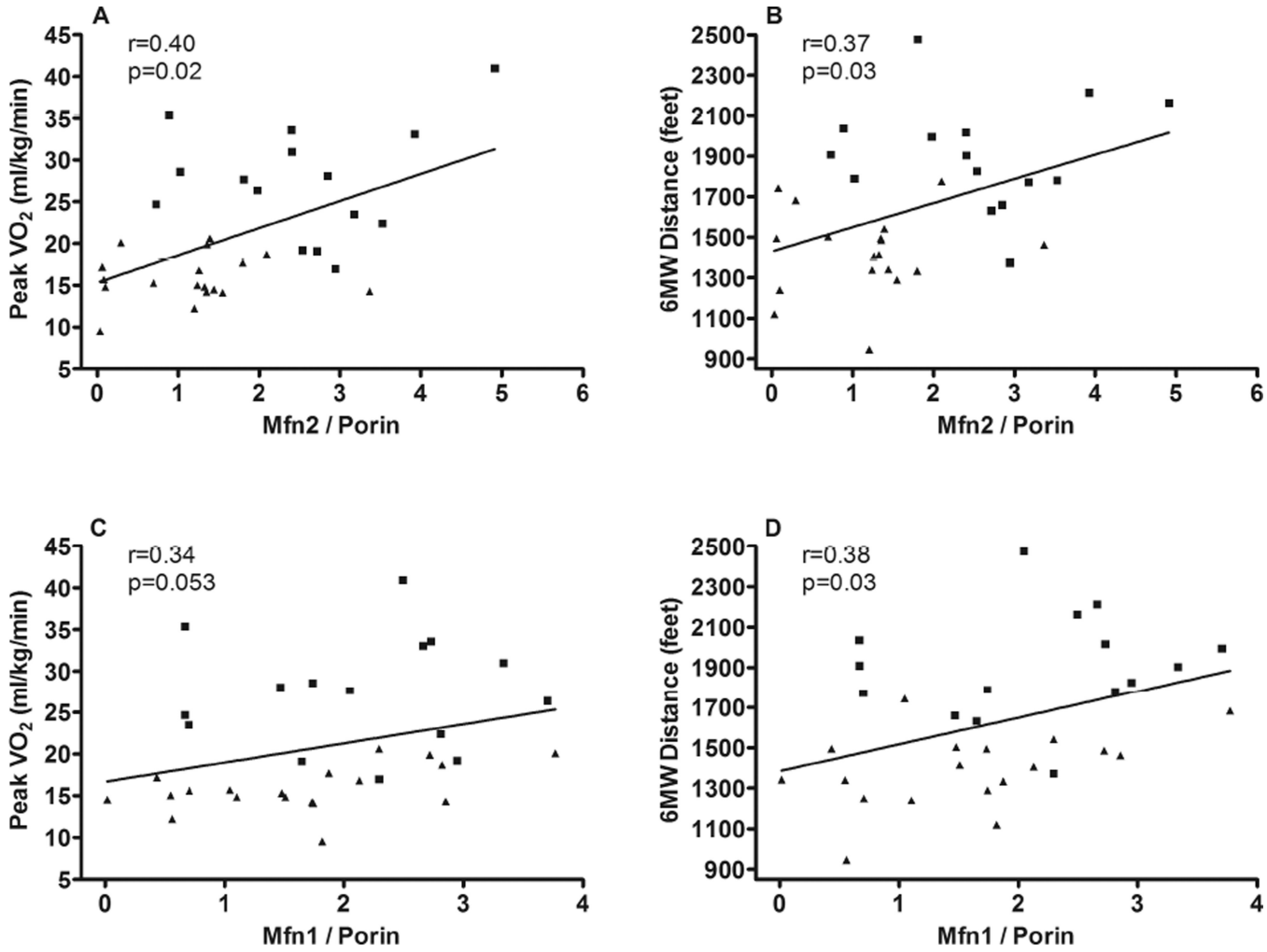

Figure 3.

Regression analysis (Spearman) comparing *Vastus lateralis* Mfn1 and Mfn2 expression with peak VO2 and 6 minute walk distance. Both mitochondrial markers were normalized to the expression of Porin to account for differences in mitochondrial content. HFpEF participants are designated by triangles and HC participants are designated by squares.

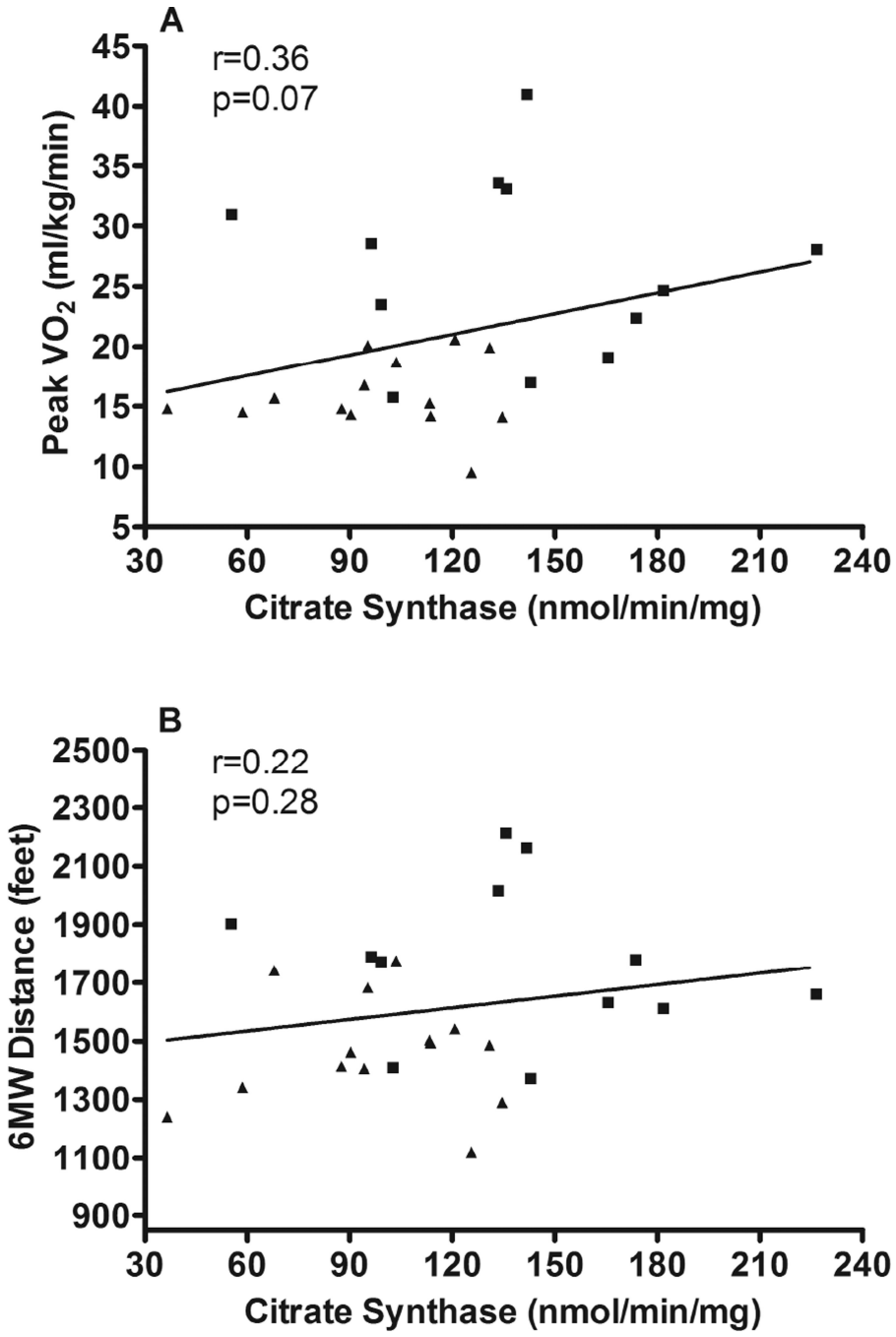

Figure 4.

Regression analysis (Spearman) comparing *Vastus lateralis* citrate synthase activity with peak VO2 and 6 minute walk distance. HFpEF participants are designated by triangles and HC participants are designated by squares.

Table 1

Participant Characteristics

	HFpEF (N=20)	HC (N=17)	p-value
Age (years)	68.2 ± 6.0	71.2 ± 7.7	0.18
Women	12 (60%)	5 (29%)	0.10
White	11 (55%)	17 (100%)	0.002
Height (cm)	167 ± 8	173 ± 8	0.02
Weight (kg)	101 ± 17	83 ± 20	0.005
Body mass index (kg/m^2)	36.2 ± 4.9	27.4 ± 5.3	<0.001
Total Fat Mass (kg, by DEXA)	39.7 ± 9.6	24.2 ± 10.6	<0.001
Total Lean Mass (kg, by DEXA)	56.0 ± 11.5	56.8 ± 11.2	0.84
Percent Body Fat, %	40.5 ± 7.9	28.3 ± 7.5	<0.001
Systolic blood pressure, mmHg	132 ± 14	125 ± 9	0.07
Diastolic blood pressure, mmHg	79 ± 9	75 ± 6	0.16
Ejection Fraction, %	61 ± 5	58 ± 5	0.07
Lateral mitral annulus velocity (e', cm/s)	7.3 ± 1.8	9.1 ± 1.8	0.004
Early mitral flow velocity / e'	10.0 ± 2.5	7.5 ± 1.4	<0.001
Diastolic Filling Pattern			
Normal	0 (0%)	13 (76%)	<0.001
Impaired relaxation	19 (95%)	4 (24%)	
Pseudonormal	1 (5%)	0 (0%)	
Restrictive	0 (0%)	0 (0%)	
History of hypertension	19 (95%)	--	--
Diabetes mellitus	5 (25%)	--	--
New York Heart Association class			
II	14 (70%)	--	--
III	6 (30%)	--	--
Medications			
Diuretics	15 (75%)	--	--
Angiotensin converting enzyme inhibitors	9 (45%)	--	--
Beta blockers	8 (40%)	--	--
Calcium channel blockers	5 (25%)	--	--

HFpEF, Heart failure and preserved ejection fraction; HC, Healthy age-matched control; DEXA, Dual-Energy X-ray absorptiometry; Values are Mean ± SD, or number (%).

Table 2

Cardiorespiratory and hemodynamic responses during peak treadmill exercise and distance walked in 6 minutes

	HFpEF	HC	HFpEF	HC	p-value
	Raw Data		Adjusted data*		
Peak Pulmonary oxygen uptake, ml/kg/min	15.8 ± 2.7	26.5 ± 6.9	16.0 ± 1.1	26.0 ± 1.3	<0.001
Peak Pulmonary oxygen uptake, ml/min	1574 ± 333	2147 ± 584	1638 ± 78	2013 ± 87	0.003
Ventilatory anaerobic threshold, ml/min	1064 ± 279	1284 ± 383	1106 ± 60	1198 ± 67	0.32
Peak Carbon dioxide production, ml/min	1767 ± 427	2463 ± 711	1841 ± 103	2311 ± 115	0.005
Peak Respiratory exchange ratio	1.12 ± 0.11	1.15 ± 0.09	1.12 ± 0.02	1.14 ± 0.03	0.58
Peak Heart rate, beats/min	142 ± 20	152 ± 18	141 ± 4	153 ± 5	0.09
Peak Systolic blood pressure, mmHg	173 ± 19	182 ± 20	173 ± 4	182 ± 5	0.21
Peak Diastolic blood pressure, mmHg	79 ± 12	76 ± 7	79 ± 2	77 ± 3	0.61
6-minute walk distance, feet	1419 ± 201	1858 ± 284	1428 ± 55	1840 ± 61	<0.001

HFpEF, Heart failure and preserved ejection fraction; HC, Healthy age-matched control. Raw data are presented as Mean ± SD

*Adjusted for gender and presented as least square means ± SE; p-value corresponds to adjusted data.

Table 3

Mitochondrial Characteristics

	HFPEF	HC	p-value	p-value†	p-value‡	p-value#	p-value*
Mfn1 / porin	1.64 ± 0.23	2.13 ± 0.25	0.16	0.21	0.32	0.30	0.52
Mfn2 / porin	1.15 ± 0.20	2.52 ± 0.30	<0.001	<0.001	0.03	0.002	0.03
Porin / gapdh	1.24 ± 0.29	2.29 ± 0.45	0.01	0.004	0.21	0.003	0.07
Citrate Synthase (nmol/min/mg)	98.0 ± 7.6	137.8 ± 13.3	0.01	0.04	0.005	0.06	0.053

HFPEF, Heart failure and preserved ejection fraction; HC, Healthy age-matched control. Raw data are presented as Mean ± SE with unadjusted p-value;

† p-value adjusted for gender

‡ p-value adjusted for body mass index

p-value adjusted for race

* p-value adjusted for race, gender, and BMI; all p-values shown for Porin/gapdh are shown following logarithmic transformation.

Table 4

Relationships of mitochondrial measures with Peak VO_2 and 6 minute walk distance

	Peak VO_2	**6MWD**
Porin / gapdh	R=0.48, p=0.003	R=0.33, p=0.05
Mfn1 / porin	R=0.34, p=0.05	R=0.38, p=0.03
Mfn2 / porin	R=0.40, p=0.02	R=0.37, p=0.03
Citrate Synthase Activity (nmol/min/mg)	R=0.37, p=0.07	R=0.22, p=0.28

Peak VO_2, peak oxygen uptake; 6MWD, 6 minute walk distance; R; Spearman correlation coefficient; p, 2 tailed p-value.