

## Skeletal muscle abnormalities and exercise intolerance in older patients with heart failure and preserved ejection fraction

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**Kitzman DW, Nicklas B, Kraus WE, Lyles MF, Eggebeen J, Morgan TM, Haykowsky M.** Skeletal muscle abnormalities and exercise intolerance in older patients with heart failure and preserved ejection fraction. *Am J Physiol Heart Circ Physiol* 306: H1364–H1370, 2014. First published March 21, 2014; doi:10.1152/ajpheart.00004.2014.—Heart failure (HF) with preserved ejection fraction (HFPEF) is the most common form of HF in older persons. The primary chronic symptom in HFPEF is severe exercise intolerance, and its pathophysiology is poorly understood. To determine whether skeletal muscle abnormalities contribute to their severely reduced peak exercise O<sub>2</sub> consumption ( $\dot{V}O_2$ ), we examined 22 older HFPEF patients (70 ± 7 yr) compared with 43 age-matched healthy control (HC) subjects using needle biopsy of the vastus lateralis muscle and cardiopulmonary exercise testing to assess muscle fiber type distribution and capillarity and peak  $\dot{V}O_2$ . In HFPEF versus HC patients, peak  $\dot{V}O_2$  (14.7 ± 2.1 vs. 22.9 ± 6.6 ml·kg<sup>-1</sup>·min<sup>-1</sup>, *P* < 0.001) and 6-min walk distance (454 ± 72 vs. 573 ± 71 m, *P* < 0.001) were reduced. In HFPEF versus HC patients, the percentage of type I fibers (39.0 ± 11.4% vs. 53.7 ± 12.4%, *P* < 0.001), type I-to-type II fiber ratio (0.72 ± 0.39 vs. 1.36 ± 0.85, *P* = 0.001), and capillary-to-fiber ratio (1.35 ± 0.32 vs. 2.53 ± 1.37, *P* = 0.006) were reduced, whereas the percentage of type II fibers was greater (61 ± 11.4% vs. 46.3 ± 12.4%, *P* < 0.001). In univariate analyses, the percentage of type I fibers (*r* = 0.39, *P* = 0.003), type I-to-type II fiber ratio (*r* = 0.33, *P* = 0.02), and capillary-to-fiber ratio (*r* = 0.59, *P* < 0.0001) were positively related to peak  $\dot{V}O_2$ . In multivariate analyses, type I fibers and the capillary-to-fiber ratio remained significantly related to peak  $\dot{V}O_2$ . We conclude that older HFPEF patients have significant abnormalities in skeletal muscle, characterized by a shift in muscle fiber type distribution with reduced type I oxidative muscle fibers and a reduced capillary-to-fiber ratio, and these may contribute to their severe exercise intolerance. This suggests potential new therapeutic targets in this difficult to treat disorder.

heart failure; exercise; aging

APPROXIMATELY 50% OF HEART FAILURE (HF) patients living in the community have preserved left ventricular (LV) ejection fraction (HFPEF) (33, 52, 68). HFPEF is nearly exclusively a disorder of older persons and is most common in women. The primary symptom in patients with chronic HFPEF is severe exercise intolerance, measured objectively as decreased peak exercise O<sub>2</sub> uptake (peak  $\dot{V}O_2$ ) (2, 3, 5, 26, 30, 34, 35, 41), and this is associated with a reduced quality of life. Despite its

importance, the pathophysiology of exercise intolerance in HFPEF is not well understood.

Several lines of evidence suggest that in older HFPEF patients, noncardiac factors may contribute to reduced peak  $\dot{V}O_2$  and may be major contributors to the improvement in peak  $\dot{V}O_2$  after endurance exercise training (2, 5, 23, 24, 26, 36, 47, 54). It is known that aging results in alterations in skeletal muscle, including a reduction in the relative number of type II fibers (40) and in capillary density (9), and that these are associated with a decline in physical performance (51). Furthermore, it is well established that patients with HF with reduced ejection fraction (HFREF) have multiple skeletal muscle abnormalities (12, 39, 42, 43, 45, 58, 64, 66, 72), including a reduced percentage of type I (oxidative) fibers and a decreased number of capillaries per fiber, and that these contribute significantly to their severe exercise intolerance (49, 64, 65). However, despite the high prevalence of HFPEF and the impact of exercise intolerance as its key chronic symptom, there is no information available regarding skeletal muscle phenotype and its relationship to peak  $\dot{V}O_2$  in HFPEF. Therefore, we performed thigh skeletal muscle needle biopsies in HFPEF patients to test the hypothesis that older HFPEF patients, compared with age-matched healthy controls (HC), have reduced type I oxidative fibers and a decreased capillary-to-fiber ratio, which contribute to their exercise intolerance.

### METHODS

**Participants.** The HFPEF patients in this report are a subset from a previously reported randomized, blinded, placebo-controlled trial evaluating the effect of enalapril on exercise capacity (32). This ancillary study for a muscle biopsy before study entry was offered to 40 consecutive participants, with 22 patients agreeing to participate. As previously described in studies from our laboratory (24–26, 32, 35, 37, 60), HFPEF was defined as symptoms and signs of HF according to the National Health and Nutrition Examination Survey HF clinical score of ≥3 and criteria of Rich et al. (56, 59), preserved resting LV 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 (26, 35, 37). 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 (electrocardiogram, cardiopulmonary exercise, and spirometry), or regularly undertook vigorous exercise (23, 62). This protocol was approved by the Wake Forest School of Medicine Institutional Re-

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view Board for Protection of Human Subjects, and all participants provided written informed consent.

**Exercise testing.** As previously described, exercise testing was performed in the upright position on an electronically braked cycle ergometer using a staged protocol starting at 12.5 W for 2 min, increasing to 25 W for 3 min, and with 25 W per 3-min increments thereafter to exhaustion (24, 26, 29, 35, 37, 44, 60). 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 s, and peak values were averaged from the last two 15-s intervals during peak exercise. A 6-min walk test was performed according to methods previously described by Guyatt et al. (20). The test-retest reliability for 6-min walk distance in our laboratory was good ( $r = 0.90$ ,  $P < 0.001$ ).

**Skeletal muscle biopsy.** As previously described, skeletal muscle biopsies were performed in the early morning after an overnight fast (51). Subjects were asked to refrain from taking aspirin, nonsteroidal anti-inflammatory drugs, and other compounds that may affect bleeding, platelets, or bruising for the week before the biopsy and to refrain from any strenuous activity for at least 36 h before 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 (51). There were no medical complications or other reported adverse events from the procedure.

Visible blood and connective tissue were removed from the muscle specimen, and portions for fiber typing and the capillary-to-fiber ratio were partitioned (51). The muscle portion used for histology analysis was oriented such that the fibers ran longitudinally, were mounted on cork in embedding medium (OCT compound, Miles Laboratory, Naperville, IL), and frozen in isopentane cooled to its freezing point with liquid nitrogen. The muscle portion for capillary density was placed in a histology cassette and quick frozen in isopentane, which was first precooled in liquid nitrogen for 20–30 min. The mounted sample and cassette were stored at  $-80^{\circ}\text{C}$  and transported on dry ice to the laboratories of W. E. Kraus at Duke University School of Medicine for processing (51).

**Fiber typing.** Fiber type histological analyses were performed following published procedures (16, 19, 51). Monoclonal antibodies against myosin heavy chain were used to identify muscle fiber subtypes. Transverse muscle sections of 10  $\mu\text{m}$  thickness, obtained with a cryostat, were mounted on a glass slide ( $\sim 5$  sections/slide). Slides were exposed to the primary antibody (Novocastra, Newcastle, Tyne, UK, and Alexis, San Diego, CA) at 1:20 dilution in PBS for 4 h, rinsed in PBS, and exposed for another 4 h in the secondary antibody (FITC-conjugated IgG rabbit anti-mouse, Sigma). Immunostained cross-sections were analyzed using an inverted Axiovert microscope (Zeiss) equipped with fluorescence filters. Images were digitized using a Photometric charge-coupled device camera and Isee software (Inovision, Durham, NC).

**Capillary-to-fiber ratio.** Endothelial cells were identified using immunohistochemistry and cell-specific monoclonal (CD31) antibodies (15, 16, 51). CD31 is a mouse monoclonal antibody (catalog no. AM232-5M, Biogenex) designed for specific localization of the human endothelium. Slides were brought to room temperature and placed in ice-cold acetone for 2 min and PBS for 5 min. Blocking solution (10% horse serum in PBS) was applied for 1 h at room temperature. Primary antibodies were applied for 1 h followed by sequential incubation with biotinylated anti-mouse IgG and ABC reagent, according to the manufacturer's specifications (Vectastain ABC, Vector Laboratories). Levamisole was added to block endogenous alkaline phosphatase activity, and immune complexes were localized using the chromogenic alkaline phosphatase substrate Vector red (Vector Laboratories). When counterstained with hematoxylin, dehydrated, and mounted with Permunt (Fisher Scientific), the anti-

gen appears red and nuclei appear blue. Murine IgG monoclonal antibody served as a negative control. The capillary-to-fiber ratio (mean number of capillaries/muscle fiber) was measured by counting endothelial cells and muscle fibers in at least three random  $\times 100$  magnification fields per sample (13–15, 31, 51). A minimum of 100 muscle fibers was counted.

**Statistical methods.** Descriptive statistics of the participants for the variables of interest are reported as means and SDs for continuous variables and as the number in each category ( $n$ ) and percentages for categorical variables. Intergroup comparisons of participant characteristics were made by independent-sample  $t$ -tests for continuous variables, by Fisher's exact tests for binomial variables, and by  $\chi^2$ -tests for general categorical variables. Comparisons of all outcome measures between groups were made by analysis of covariance, adjusting for sex. Relationships between muscle fiber type and the capillary-to-fiber ratio and peak  $\dot{V}\text{O}_2$  were assessed by Pearson correlations. Finally, a multivariate regression model was used to assess predictors of peak  $\dot{V}\text{O}_2$ . A two-tailed  $P$  value of  $<0.05$  was required for significance.

## RESULTS

**Subject characteristics.** Patients had characteristics typical of chronic HFPEF with New York Heart Association class II–III symptoms, with greater resting systolic blood pressure, abnormal Doppler LV diastolic function, increased LV mass and mass-to-volume ratio, and concentric hypertrophic LV remodeling compared with HC subjects (Table 1). HFPEF patients were clinically stable and New York Heart Association class II (77%) and class III (23%). Chronic systemic hypertension was present in 73% of HFPEF patients. Key characteristics (age, sex, body mass index, and peak  $\dot{V}\text{O}_2$ ) did not differ between this subset of HFPEF patients and the larger cohort (32). HFPEF and HC patients were well matched for age, although a greater number of women were in the HC group. Body weight and body surface area were similar between the groups, although body mass index was higher in HFPEF compared with HC patients. The sex and body mass index characteristics of the HFPEF group were consistent with previous reports from large population-based studies (33, 52, 68).

**Exercise performance.** Peak exercise  $\dot{V}\text{O}_2$ , exercise time, workload,  $\text{CO}_2$  production, and heart rate were significantly reduced in HFPEF versus HC patients (Table 2). Peak exercise systolic and diastolic blood pressures were significantly increased in HFPEF versus HC patients. The peak exercise respiratory exchange ratio was not different in HFPEF versus HC patients, and the mean was  $\geq 1.13$  in both groups. Furthermore, the 6-min walk distance was significantly reduced in HFPEF compared with HC patients (Table 2).

**Skeletal muscle fiber type distribution and capillary-to-fiber ratio.** Compared with HC subjects, older HFPEF patients had a reduced percentage of type I fibers ( $39.0 \pm 11.4\%$  vs.  $53.7 \pm 12.4\%$ ,  $P < 0.001$ ), a greater percentage of type II fibers ( $61.0 \pm 11.4\%$  vs.  $46.3 \pm 12.4\%$ ,  $P < 0.001$ ), and a reduced type I-to-type II fiber ratio ( $0.72 \pm 0.39$  vs.  $1.36 \pm 0.85$ ,  $P = 0.001$ ; Table 3). The capillary-to-fiber ratio was also significantly reduced in HFPEF compared with HC patients ( $1.35 \pm 0.32$  vs.  $2.53 \pm 1.37$ ,  $P = 0.006$ ; Table 3).

**Relationships between skeletal muscle characteristics and exercise performance.** In univariate analyses with all subjects combined, the capillary-to-fiber ratio ( $r = 0.59$ ,  $P < 0.001$ ; Fig. 1A), percentage of type I fibers ( $r = 0.39$ ,  $P = 0.003$ ; Fig. 1B), and type I-to-type II fiber ratio ( $r = 0.33$ ,  $P = 0.02$ ) were

Table 1. Baseline participant characteristics

	HFPEF Patients	HC Subjects	P Value
<i>n</i>	22	43	
Age, yr	69.8 ± 6.6	69.2 ± 7.4	0.76
Number of women/men, <i>n</i> (%)	18/4 (82)	22/21 (51)	0.03
Number in the white ethnic group, <i>n</i> (%)	19 (86)	41 (95)	0.33
Height, cm	163.9 ± 6.7	170.7 ± 8.3	0.002
Weight, kg	79.9 ± 15.1	78.0 ± 16.9	0.67
Body mass index, kg/m <sup>2</sup>	29.7 ± 5.2	26.7 ± 5.1	0.03
Body surface area, m <sup>2</sup>	1.86 ± 0.18	1.85 ± 0.36	0.90
Systolic blood pressure, mmHg	140 ± 22	124 ± 12	<0.001
Diastolic blood pressure, mmHg	75 ± 9	75 ± 7	0.92
Ejection fraction, %	64 ± 6	65 ± 7	0.67
Left ventricular mass, g	227 ± 58	138 ± 36	<0.001
Left ventricular mass-to-end-diastolic volume ratio	3.7 ± 1.7	1.7 ± 0.4	<0.001
Lateral mitral annulus velocity, cm/s	7.5 ± 1.5	9.5 ± 2.0	<0.001
Early mitral flow velocity/lateral mitral annulus velocity	9.9 ± 2.7	7.2 ± 1.7	<0.001
Diastolic filling pattern, <i>n</i> (%)			
Normal	0 (0)	33 (77)	<0.001
Impaired relaxation	18 (82)	10 (23)	
Pseudonormal	4 (18)	0 (0)	
Restrictive	0 (0)	0 (0)	
B-type natriuretic peptide	70 ± 46	34 ± 13	<0.001
History of hypertension, <i>n</i> (%)	16 (73)		
Diabetes mellitus, <i>n</i> (%)	3 (14)		
New York Heart Association class, <i>n</i> (%)			
II	17 (77)		
III	5 (23)		
Medications, <i>n</i> (%)			
Diuretics	12 (55)		
Angiotensin-converting enzyme inhibitors	0 (0)		
β-Blockers	8 (36)		
Ca <sup>2+</sup> channel blockers	5 (23)		

Values are means ± SD or numbers of patients/subjects per group (*n*) with percentages. HFPEF, heart failure with preserved ejection fraction; HC, healthy age-matched control.

significantly correlated with peak  $\dot{V}O_2$  (in ml·kg<sup>-1</sup>·min<sup>-1</sup>). Among patient characteristics, the strongest univariate correlates of peak  $\dot{V}O_2$  were sex ( $r = 0.55$ ,  $P < 0.001$ ) and age ( $r = -0.31$ ,  $P = 0.02$ ). In a multivariate model that included the variables of age, sex, body surface area, capillary-to-fiber ratio, and percentage of type I fibers as competing predictors of

peak  $\dot{V}O_2$ , both the capillary-to-fiber ratio (partial  $r = 0.34$ ,  $P = 0.02$ ) and percentage of type I fibers (partial  $r = 0.40$ ,  $P = 0.004$ ) were independent predictors of peak  $\dot{V}O_2$ .

Furthermore, in univariate analyses, the capillary-to-fiber ratio ( $r = 0.48$ ,  $P < 0.001$ ), percentage of type I fibers ( $r = 0.35$ ,  $P = 0.006$ ), sex ( $r = 0.31$ ,  $P = 0.01$ ), and age ( $r = -0.40$ ,  $P = 0.001$ ) were significantly correlated with 6-min walk distance. In multivariate analyses, the percentage of type I fibers (partial  $r = 0.28$ ,  $P = 0.046$ ) remained as a significant, independent predictor of 6-min walk distance.

## DISCUSSION

The primary symptom in patients with chronic HFPEF, even when well compensated, is severe exercise intolerance, and it is associated with a reduced quality of life (24, 26, 35); however, its pathophysiology is not well understood. Multiple lines of evidence have suggested that in addition to cardiac function, noncardiac “peripheral” factors are also important contributors to the severe exercise intolerance observed in older HFPEF patients (2, 5, 24, 26, 36, 54). The major novel finding of this study is that compared with HC subjects, older HFPEF patients exhibited a shift in skeletal muscle fiber type distribution with a reduced percentage of slow twitch type I fibers and reduced type I-to-type-II fiber ratio as well as a reduced capillary-to-fiber ratio. Furthermore, both the capillary-to-fiber ratio and percentage of type I fibers were significant, independent predictors of peak  $\dot{V}O_2$ . The intrastudy comparison with age-matched HC subjects indicated that these abnormalities were in addition to alterations that would be expected from aging alone, and comparisons with reports from others (discussed below) indicate that the abnormalities are similar to those seen in HFREF (12, 39, 42, 43, 45, 58, 64–66, 72). Taken together, these findings may help explain why older HFPEF patients have such severely reduced exercise capacity.

To our knowledge, this is the first report of skeletal muscle fiber composition and capillarity in HFPEF and their relationships with peak  $\dot{V}O_2$ . However, our results are believable given what is known from many reports regarding skeletal muscle fiber composition and capillarity in patients with HFREF and other populations. Specifically, several investigators have reported that HFREF patients have decreased type I fibers compared with HC subjects (12, 39, 42, 45, 64, 66, 67) and that this is related to peak  $\dot{V}O_2$  (49). Moreover, several investigators

Table 2. Cardiorespiratory and hemodynamic responses during peak cycle exercise and distance walked in 6 min

	Raw Data		Adjusted Data*		P Value
	HFPEF patients	HC subjects	HFPEF patients	HC subjects	
Peak exercise					
Exercise time, min	9.9 ± 2.6	14.8 ± 4.8	11.8 ± 0.8	14.8 ± 0.6	0.004
Workload, W	73 ± 23	116 ± 39	89 ± 7	115 ± 4	0.002
O <sub>2</sub> uptake, ml/min	1192 ± 281	1743 ± 530	1432 ± 83	1734 ± 55	0.004
O <sub>2</sub> uptake, ml·kg <sup>-1</sup> ·min <sup>-1</sup>	14.7 ± 2.1	22.9 ± 6.6	16.6 ± 1.2	22.8 ± 0.8	<0.001
CO <sub>2</sub> production, ml/min	1332 ± 302	2016 ± 601	1586 ± 97	2007 ± 65	<0.001
Respiratory exchange ratio	1.13 ± 0.07	1.16 ± 0.09	1.12 ± 0.02	1.16 ± 0.01	0.09
Heart rate, beats/min	134 ± 16	148 ± 16	134 ± 4	148 ± 3	0.004
Systolic blood pressure, mmHg	193 ± 24	175 ± 23	201 ± 5	175 ± 3	<0.001
Diastolic blood pressure, mmHg	90 ± 8	77 ± 7	91 ± 2	77 ± 1	<0.001
Ventilatory threshold, ml/min	696 ± 156	929 ± 356	817 ± 65	925 ± 42	0.17
6-min walk distance, m	454 ± 72	573 ± 71	461 ± 17	573 ± 11	<0.001

Raw data are presented as means ± SD. \*Adjusted for sex and presented as least-square means ± SE. P values correspond to adjusted data.

Table 3. Skeletal muscle fiber type distribution and capillary-to-fiber ratio

	Raw Data		Adjusted Data*		P Value
	HFPEF patients	HC subjects	HFPEF patients	HC subjects	
Type I fibers, %	39.0 ± 11.4	53.7 ± 12.4	37.4 ± 2.8	53.8 ± 1.9	<0.001
Type II fibers, %	61.0 ± 11.4	46.3 ± 12.4	62.6 ± 2.8	46.2 ± 1.9	<0.001
Type I-to-type II fiber ratio	0.72 ± 0.39	1.36 ± 0.85	0.64 ± 0.18	1.37 ± 0.12	0.001
Capillary-to-fiber ratio	1.35 ± 0.32	2.53 ± 1.37	1.60 ± 0.26	2.51 ± 0.17	0.006

Raw data are presented as means ± SD. \*Adjusted for sex and presented as least-square means ± SE. P values correspond to adjusted data.

have reported that the capillary-to-fiber ratio is also reduced in HFREF patients compared with HC subjects (42, 43, 58, 64, 72).

These results build on reports from our group and others (2, 5, 23, 24, 26, 47, 54). We (26) have previously reported that compared with age-matched healthy subjects, older HFPEF patients have a reduced peak exercise arteriovenous O<sub>2</sub> difference and that this contributes, along with reduced cardiac output, to their severely reduced peak  $\dot{V}O_2$ . We also found that noncardiac peripheral factors were a major contributor to the improvement in peak  $\dot{V}O_2$  after endurance training (24). In a preliminary analysis, Bhella et al. (2), using <sup>31</sup>P magnetic spectroscopy, reported that during static leg exercise, HFPEF patients had impaired skeletal muscle oxidative metabolism compared with HC subjects. Recently, we (23) reported that older HFPEF patients had reduced percent total and leg lean body mass compared with age-matched HC subjects. Moreover, the slope of the relationship of peak  $\dot{V}O_2$  with percent leg lean mass was markedly reduced in HFPEF patients versus age-matched HC subjects, suggesting that skeletal muscle hypoperfusion or impaired O<sub>2</sub> utilization by the active muscles may play an important role in limiting exercise performance in elderly HFPEF patients. Finally, we (22) recently showed that HFPEF patients have increased thigh intermuscular fat and an intermuscular fat-to-skeletal muscle ratio and that these are related to peak  $\dot{V}O_2$ . Together with the present report, this suggests that older HFPEF patients have significant skeletal muscle abnormalities and that these may be important contributors to their severely reduced peak  $\dot{V}O_2$ .

Although not addressed by our study, potential causes for the skeletal muscle abnormalities we observed include neuroendocrine activation, sympathetic overdrive, oxidative stress, inflammation, abnormal Ca<sup>2+</sup> cycling and excitation-contraction coupling, and deconditioning (49). Chronic elevations in sympathetic neural activity and decreased nitric oxide bioavailability result in increased vasoconstriction and reduced skeletal muscle blood flow. A consequence of skeletal muscle hypoperfusion is that it leads to the generation of ROS, which is an important stimulus for TNF- $\alpha$  activation, systemic inflammation, and skeletal muscle myopathy (49).

We cannot exclude a potential contribution of physical deconditioning to the skeletal muscle abnormalities we observed in our HFPEF patients. However, multiple studies in animal models and humans have indicated that the skeletal muscle abnormalities found in HFREF patients are not due solely to deconditioning (13, 18, 49, 61, 70). Furthermore, deconditioning primarily decreases type II fibers rather than type I fibers (49).

How might these skeletal muscle alterations contribute to exercise intolerance in older HFPEF patients?  $\dot{V}O_2$  kinetics

strongly relate to peak exercise capacity (57). In aging and in HF, muscle blood flow (perfusive and diffusive O<sub>2</sub> delivery) assumes an important role in limiting  $\dot{V}O_2$  kinetics (57). Furthermore, in an animal model, the reduction in maximal  $\dot{V}O_2$  resulted primarily from reduced O<sub>2</sub> delivery (10). Therefore, the reduced capillary-to-fiber ratio in HFPEF patients would be expected to result in a decreased diffusive capacity for O<sub>2</sub> transport to active skeletal muscle during exercise and limit exercise capacity (53). Likewise, increased capillarity and therefore red blood cell volume acts to elevate diffusive O<sub>2</sub> conductance and elevate the arteriovenous O<sub>2</sub> difference, thus elevating peak  $\dot{V}O_2$ .

Compared with type II fibers, type I fibers, which we found to be reduced in HFPEF, have greater oxidative capacity and mitochondrial density and contribute disproportionately to the

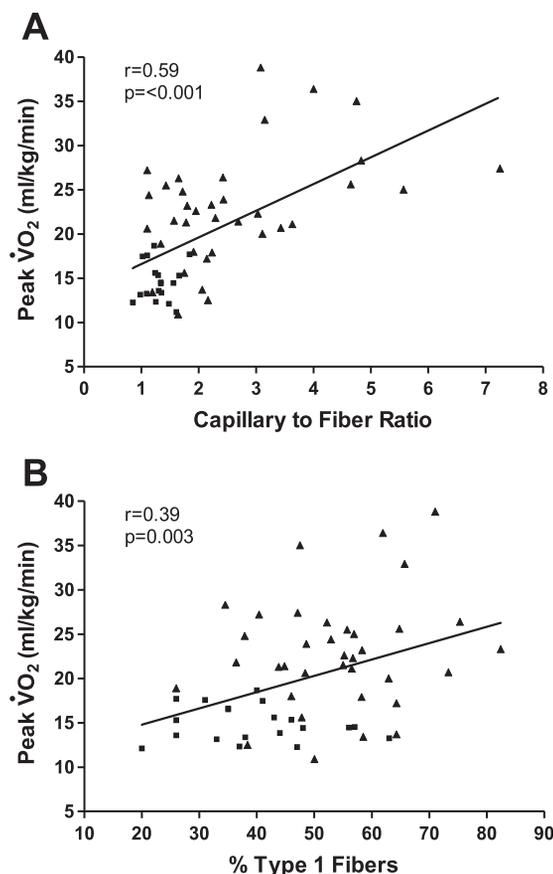


Fig. 1. Relationship of capillary-to-fiber ratio (A) and percentage of type I muscle fibers (B) with peak O<sub>2</sub> uptake ( $\dot{V}O_2$ ) in older patients with heart failure with preserved ejection fraction (■) and age-matched healthy control subjects (▲).

ability to perform sustained aerobic exercise. While speculative, a reduction in the percentage of type I fibers could be associated with reduced oxidative capacity and mitochondrial density and thereby contribute to the reduced peak  $\dot{V}O_2$  in HFPEF. Indeed, Coen et al. (8) recently reported that thigh muscle oxidative capacity (measured as maximal stage 3 respiration of permeabilized fibers) is a major determinant of peak  $\dot{V}O_2$  in healthy older sedentary adults, and, in a preliminary report, Bhella et al. (2) found reduced leg muscle oxidative metabolism by MRI during exercise in HFPEF patients. Furthermore, Drexler et al. (12) reported that the volume density of mitochondria and the surface density of mitochondrial cristae were significantly reduced in HFREF patients compared with HC subjects and that both were positively related to peak  $\dot{V}O_2$ . However, Mettauer et al. (48) reported that despite 60% lower peak  $\dot{V}O_2$ , the maximal vastus lateralis mitochondrial oxidative capacity was similar in HFREF patients compared with sedentary HC subjects. Thus, a prospective study with direct assessments is needed to determine whether HFPEF patients have abnormalities in mitochondrial function and oxidative metabolism and, if so, whether they contribute to their severely reduced peak  $\dot{V}O_2$ .

**Limitations.** Although we found significant correlations, due to the cross-sectional design, we cannot determine whether the reduced percentage of type I fibers and capillary-to-fiber ratio in our HFPEF patients are causes or consequences of their severely reduced peak  $\dot{V}O_2$ .

Sex, a known potential confounder of both exercise capacity and skeletal muscle characteristics, was significantly different between groups. However, we adjusted all analyses for sex, a well-established and accepted method for controlling for the potential confounder's effect in the group comparison (38). Furthermore, all key results, including fiber type, capillary-to-fiber ratio, and their relationships to peak  $\dot{V}O_2$ , were confirmed in an additional case control analysis whereby control subjects and patients were carefully matched by sex. The body mass index was also different between groups. However, our results were unchanged after we adjusted for body mass index.

Our control group was, by design, healthy subjects without comorbidities. As such, we cannot exclude a contribution of the comorbidities that are common in HFPEF, such as hypertension and diabetes (63). However, the pattern of altered skeletal muscle fiber type and capillary-to-fiber ratio we observed in our elderly HFPEF patients is strikingly similar to that reported by others in HFREF patients (12, 39, 42, 43, 45, 49, 58, 64–67, 72), and the fiber type alteration is dissimilar to that seen with aging alone (40).

By study design, HFPEF patients were ambulatory, stable, well compensated, had no recent acute hospitalization, and were physically able to participate in exhaustive exercise testing. Our B-type natriuretic peptide levels were similar to other studies of stable HFPEF patients able to undergo maximal exercise testing (4, 5, 32, 50) and, as we have previously shown (35), were significantly twofold increased in HFPEF patients compared with HC subjects. Doppler LV diastolic function parameters were also abnormal in our HFPEF patients versus HC subjects. However, our results may not apply to HFPEF patients who are sicker, poorly compensated, or less clinically stable.

Finally, another limitation is that peak  $\dot{V}O_2$  was determined as the highest  $O_2$  consumed at volitional exhaustion. Although not performed in the present study, a second square-wave test performed at a higher exercise attained than completed by our subjects during the incremental test would verify unambiguously whether a true maximal  $\dot{V}O_2$  was reached.

**Perspectives.** Regardless of the underlying mechanisms, these skeletal muscle abnormalities may be reversible with exercise training (1, 11, 16, 21, 27, 28, 49, 69, 71). In health and disease, exercise training can reverse the reduction in type I fibers, increase skeletal muscle mitochondrial volume density, and increase capillarity, and these changes are positively related to the training-related improvement in peak  $\dot{V}O_2$  (1, 16, 21, 27, 69). In particular, increases in capillarity and therefore red blood cell volume caused by exercise training would be expected to elevate the diffusive  $O_2$  conductance and arteriovenous  $O_2$  difference. These favorable adaptations might also occur in older patients with HFPEF, since after endurance exercise training, the change in exercise arteriovenous  $O_2$  difference was strongly related to the increase in peak  $\dot{V}O_2$  (24). There are a number of other intervention strategies that may potentially improve skeletal muscle function as well (6, 7, 49). These new potential therapeutic targets may be valuable since trials of exercise intolerance in HFPEF to date have been directed primarily at improving cardiovascular function and have largely been ineffective (17, 32, 46, 55).

**Summary.** Older HFPEF patients have a significantly reduced percentage of slow twitch type I oxidative fibers, type I-to-type II fiber ratio, and capillary-to-fiber area ratio, and these alterations are associated with their severely reduced peak exercise  $\dot{V}O_2$ . Interventions designed to reverse these skeletal muscle abnormalities may improve the severe exercise intolerance in older HFPEF patients.

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## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

## AUTHOR CONTRIBUTIONS

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

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