Skeletal muscle fiber characteristics in patients with chronic heart failure: impact of disease severity and relation with muscle oxygenation during exercise


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Short title: Skeletal muscle fiber characteristics in patients with HFpEF

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A.M.H., and R.F.S. conducted all experiments and performed all data collection. V.M.N., T.S., L.B.V., J.K., B.B.L.G., A.M.H., and R.F.S. performed all data analysis. V.M.N. prepared the first draft of the manuscript. All authors edited, and agreed, the final version.
Abstract

Introduction: Skeletal muscle function in patients with heart failure and reduced ejection fraction (HFrEF) greatly determines exercise capacity. However, reports on skeletal muscle fiber dimensions, fiber capillarization, and their physiological importance are inconsistent.

Methods: Twenty-five moderately-impaired patients with HFrEF and 25 healthy control (HC) subjects underwent muscle biopsy sampling. Type I and type II muscle fiber characteristics were determined by immunohistochemistry. In patients with HFrEF, enzymatic oxidative capacity was assessed, and pulmonary oxygen uptake (VO$_2$) and skeletal muscle oxygenation during maximal and moderate-intensity exercise were measured using near-infrared spectroscopy.

Results: While muscle fiber cross-sectional area (CSA) was not different between patients with HFrEF and HC, percentage of type I fibers was higher in HC (46±15% versus 37±12%, respectively, $P=0.041$). Fiber type distribution and CSA were not different between patients in New York Heart Association (NYHA) class II and III. Type I muscle fiber capillarization was higher in HFrEF compared with controls (capillary-to-fiber perimeter exchange (CFPE) index: 5.70±0.92 versus 5.05±0.82, respectively, $P=0.027$). Patients in NYHA class III had slower VO$_2$ and muscle deoxygenation kinetics during onset of exercise, and lower muscle oxidative capacity than those in class II ($P<0.05$). Also, fiber capillarization was lower, but not compared with HC. Higher CFPE index was related to faster deoxygenation ($r_{	ext{normax}}=-0.682$, $P=0.001$), however, not to muscle oxidative capacity ($r=-0.282$, $P=0.216$).
Conclusions: Type I muscle fiber capillarization is higher in HFrEF compared with HC, but not in patients with greater exercise impairment. Greater capillarization may positively affect VO₂ kinetics by enhancing muscle oxygen diffusion.

New & Noteworthy

The skeletal myopathy of chronic heart failure (HF) includes a greater percentage of fatigable type II fibers and, for less impaired patients, greater skeletal muscle fiber capillarization. Near-infrared spectroscopy measurements of skeletal muscle oxygenation indicate that greater capillarization may compensate for reduced blood flow in mild HF by enhancing the diffusive capacity of skeletal muscle. This thereby augments and speeds oxygen extraction during contractions, which is translated into faster pulmonary oxygen uptake kinetics.

Keywords

Exercise capacity; myopathy; fiber type; microvascular; oxidative capacity; oxygen diffusion;
Introduction

Heart failure (HF) is a syndrome characterized by fatigue, breathlessness and exercise intolerance. While these symptoms have been attributed to impaired cardiac function, skeletal muscle abnormalities are also considered as primary contributing factors (48). In fact, muscle mass, muscle strength and muscle oxidative capacity are highly predictive of exercise capacity in patients with heart failure and reduced ejection fraction (HFrEF) (18, 25, 35, 39, 56).

In comparison with healthy individuals, patients with HF demonstrate an unfavorable shift from type I oxidative fibers towards type II glycolytic fibers (37, 56, 62), and lower mitochondrial density (12, 65). However, reports on skeletal muscle oxidative capacity (19, 51, 65), fiber atrophy or loss (12, 37, 44, 55, 69), and capillarization (8, 12, 39, 56, 69), are conflicting or show no differences compared with healthy subjects. This discrepancy may be at least partly explained by inter-individual variability in muscle fiber adaptations to HF. It is therefore important to control inter-individual variability by matching age, gender, and BMI between patient and control subjects (6, 20, 23, 66). However, few studies have matched patients and control subjects for age and gender on an individual basis (37, 43), and even fewer studies maintained matching during statistical analysis (37).

Another important issue is the quantification of skeletal muscle fiber capillarization. Capillary-to-fiber perimeter exchange (CFPE) index is considered as the most appropriate representation of the resistance to diffusion of oxygen from capillary into the myocyte (26). Although CFPE index has been demonstrated to decrease with the severity of chronic
obstructive pulmonary disease (COPD) (10), it has not yet been evaluated in patients with HFrEF. Furthermore, little is known about capillarization differences among fiber types, which is of interest considering the impact of exercise interventions on fiber type adaptation.

In order to evaluate to clinical significance of skeletal muscle fiber characteristics in patients with HF, it is first necessary to develop a better understanding of the impact of skeletal muscle maladaptations on exercise capacity. A non-invasive method to assess the physiological significance of altered skeletal muscle fiber properties is the localized measurement of skeletal muscle oxygenation (SmO₂) during exercise transitions. It has been shown that the temporal characteristics of SmO₂ derived from near-infrared spatially-resolved spectroscopy (NIR-SRS) measurements are indicative of the balance between skeletal muscle O₂ utilization (V̇O₂m) and O₂ delivery (Q̇O₂m) (1, 14). Because decreased capillarization may potentially impair both convective and diffusive O₂ capacity (50) and decreased oxidative capacity impairs the ability for O₂ utilization (7), NIRS measurements can be useful to evaluate the influence of altered muscle fiber characteristics on exercise impairments in patients with HFrEF.

Therefore, the aim of this study is to identify potential targets for improving exercise capacity by 1) assessing skeletal muscle fiber characteristics in moderately-impaired patients with HFrEF in comparison with well-matched healthy control subjects and 2) by evaluating the physiological implications of fiber characteristics for muscle oxygenation during exercise. Our hypothesis is that fiber type distribution shows a lower portion of type I fibers, and that, on average, fiber cross-sectional area and capillarization are preserved in patients with HFrEF.
Furthermore, we suspect that the most impaired patients with HFrEF have compromised skeletal muscle characteristics compared with less severely affected individuals and healthy control subjects. Finally, it is expected that the severity of skeletal muscle derangements is related to slower deoxygenation during exercise, in accordance with limited and slowed oxidative phosphorylation relative to skeletal muscle O₂ supply.

Methods

Subjects

Twenty-five men (≥ 55 y) with HFrEF were recruited. In addition 25 healthy active male subjects were matched to individual patients with HFrEF by BMI (± 2 kg·m⁻²) and age (± 5 y) because of the confounding influence of both on skeletal muscle fiber characteristics (20, 23, 66). Data of healthy subjects have been published before elsewhere (23, 66). Inclusion criteria for patients with HFrEF were stable systolic heart failure attributed to either dilated cardiomyopathy or ischemic heart disease due to myocardial infarction, New York Heart Association (NYHA) functional Class II or III (without change in class or medication ≤ 3 mo prior to inclusion), left ventricular ejection fraction ≤ 40 % (mean LVEF 32 ± 10 % as assessed by echocardiography or magnetic resonance imaging ≤ 2 mo prior), and optimized medical treatment according to current guidelines (40). Patients with HFrEF and healthy subjects were excluded from the study when they presented with recent myocardial infarction (within the
preceding 3 mo, angina pectoris at rest, pulmonary, neurological or orthopedic disorders
limiting the ability to exercise, peripheral vascular disease and/or clinical signs of
decompensated heart failure.

This study represents part of four separate protocols involving exercise training in
patients with chronic HF (Dutch Trial Register: NTR2604), physical activity and protein
synthesis in older men (NTR4492), microvascular function in aging and type 2 diabetes mellitus
(23), and resistance type exercise training and capillarization in older men (66), all of which
were approved by either the Medical Ethical Committee of Máxima Medical Centre, Veldhoven,
the Netherlands, or the Medical Ethical Committee of the Maastricht University Medical
Centre, Maastricht, the Netherlands. The study was conducted according to the Helsinki
Declaration of 1964 and all participants provided written informed consent.

Skeletal muscle biopsy sampling

Muscle biopsy sampling took place in the morning after an overnight fast. Subjects were advised
to avoid strenuous exercise 48 h prior to sampling and arrived at the laboratory by car or public
transportation. After local anesthesia, muscle biopsies (50-80 mg) were obtained from the
middle region of the vastus lateralis muscle of the left leg (15 cm above the patella and
approximately 2 cm away from the fascia) by the percutaneous needle biopsy technique
described by Bergström et al. (5). Muscle biopsies were carefully freed from any visible fat and
blood. Approximately 20 mg of tissue was embedded in Tissue-Tek® (Sakura Finetek,
Zoeterwoude, the Netherlands) and rapidly frozen in liquid nitrogen-cooled isopentane. The remaining part was immediately frozen in liquid nitrogen. Thereafter, parts were stored at -80°C for subsequent transport and analysis.

**Immunohistochemistry**

From the muscle samples, cryosections of 5 μm thickness were cut. Cryosections were thaw mounted on uncoated glass slides. Immunohistochemical staining was performed to determine fiber type specific skeletal muscle capillarization. Slides were taken from the -80°C freezer and thawed for 30 min at room temperature. After fixation for 5 min with acetone (VWR International GmbH, Darmstadt, Germany), samples were air dried for 15 min. Slides were incubated for 45 min with CD31 (dilution 1:50; M0823; DAKO, Glostrup, Denmark), and then washed (3x5 min 0.05% Tween-PBS). After that, 45 min incubation with goat anti-mouse biotin (1:200, BA-2000; Vector Laboratories, Burlingame, CA, USA) was started, and a wash was performed. Slides were then incubated with Avidin Texas Red (A2006, dilution 1:400, Vector Laboratories), antibodies against myosin heavy chain (MHC) I (A4.840, dilution 1:25, Developmental Studies Hybridoma Bank (DSHB), Iowa City, IA, USA), and laminin (polyclonal rabbit anti-laminin, dilution 1:50, Sigma St. Louis, MO, USA) for 45 min, and washed. Finally, GAM IgM AlexaFluor488 and GARIgG AlexaFluor350 (Molecular Probes Eugene, OR, USA) were applied for 30 min. After the final washing, slides were mounted with Mowiol (Calbiochem, La Jolla, CA, USA). The staining procedure resulted in images with laminin in
blue, MHC-I in green, and CD31 in red. Images were automatically captured at 10x magnification with an Olympus BX51 fluorescence microscope with customized spinning disk unit (DSU; Olympus Co. Ltd., Tokyo, Japan) with an ultra-high sensitivity monochrome electron multiplier CCD camera (1,000 × 1,000 pixels, C9100-02, Hamamatsu Photonics Co. Ltd., Hamamatsu City, Japan).

Image acquisition was done by Micromanager 1.4 software, and images were analyzed with ImageJ (U.S. National Institutes of Health, Bethesda, MD, USA). The images were recorded and analyzed by an investigator blinded to subject coding. In all sections, longitudinal fibers were excluded from analysis, and a mean of 220 ± 89 fibers for each individual was counted for fiber type distribution. Muscle fiber CSA and perimeter (P) were automatically determined for each fiber separately using ImageJ software. A minimum of 30 fibers was counted per fiber type for analysis of capillary characteristics. The number of capillaries was manually counted and expressed as capillary contacts (CC; number of capillaries in contact with each fiber), capillary-to-fiber ratio [C/Fi; number of CC divided by sharing factor (SF)], capillary density (CD; C/Fi divided by fiber CSA), and CFPE index (number of capillaries per 1,000 µm perimeter) as previously reported (26). Representative images of histochemical analyses are shown in Figure 1.

Oxidative enzyme activity assays

Skeletal muscle cytochrome c oxidase activity was assessed in 40 mg mixed muscle tissue of patients with HFREF only. For the measurement, a 2.5 % (weight per volume) muscle
homogenate was prepared in SET buffer (based on sucrose (250 nmoL⁻¹), EDTA (2 nmoL⁻¹) and TRIS (10 nmoL⁻¹), pH 7.4). Cytochrome c oxidase activity was measured by spectrophotometrically monitoring the amount of reduced cytochrome c during the cytochrome c oxidase reaction at 550 nm. Cytochrome c oxidase activity was interpreted as a maker for oxidative phosphorylation capacity (33).

**Exercise testing**

Patients with HFrEF performed tests on an electromagnetically braked cycle ergometer (Lode Corival, Lode BV, Groningen, the Netherlands) in an upright seated position. They were instructed to maintain a pedaling frequency of 70 rpm during the exercise phases. Ventilatory and gas exchange measurements (ZAN 680 USB, ZAN Messgeräte, Oberthulba, Germany; calibrated before each test) were recorded breath-by-breath during the entire testing protocols. The maximal exercise protocol started with a 1-min resting period and 4 min of unloaded pedaling before work rate started to increase with an individualized ramp rate aiming to reach exhaustion within 8-12 min (15). The test was terminated when the required pedaling frequency could not be maintained due to volitional exhaustion. Peak work rate was the highest registered work rate, and peak pulmonary oxygen uptake (peak $\dot{V}_{O_2}$) and peak respiratory exchange ratio (RER) were the average values of the final 30-s of the maximal exercise test. The gas exchange threshold (GET) was determined by the mean of the independent assessments of two experienced physicians using the V-slope method (2). Moderate-intensity exercise testing was
performed on a different day than the maximal exercise test. It commenced with a 2-min resting
period, while the right leg was passively held in a predetermined position. This was followed by
a 6-min bout at 80 % of the work rate corresponding to the gas exchange threshold, or at 50 % of
the peak work rate when the GET could not be assessed (29). Subjects were advised to take their
medication as usual and to avoid strenuous exercise (48 h), consuming a meal (2 h), and caffeine
(4 h) before testing.

**Near infrared spectroscopy measurements**

NIRS measurements were performed during moderate-intensity exercise with a wireless
continuous wave (CW) near-infrared spectrophotometer (Portamon, Artinis, Elst, The
Netherlands), which employs modified Beer-Lambert Law and spatially resolved spectroscopy
with 2 wavelengths of emitting light (760 and 841 nm). The device consists of 3 pairs of light
emitting diodes and a detector photo diode, which are configured spatially to provide 3 source-
detector distances (30, 35, and 40 mm). By determining the absorption coefficients derived from
the slopes of light attenuation at different source-detector distances and wavelengths, an
absolute measure of tissue oxygen saturation (StO₂), the tissue saturation index (TSI), can be
Calculated. TSI equals the ratio of oxygenated hemoglobin and myoglobin (O₂HbMb) and the
sum of oxygenated and deoxygenated hemoglobin and myoglobin ([O₂HbMb] + [HHbMb] =
[totalHbMb]), and is expressed as a percentage. It is acknowledged that the separate
contributions of myoglobin and hemoglobin to the NIRS signal cannot be distinguished,
because their absorption spectra are similar. However, Koga et al. have demonstrated that NIRS-derived deoxygenation kinetics, expressed as time constant (i.e. tau), were not influenced by specific deoxygenation characteristics of myoglobin, and can be considered a useful index of local O₂ extraction kinetics (31). A representative plot of TSI during moderate-intensity constant work rate exercise is shown in Figure 2.

The NIRS device was positioned over the distal vastus lateralis of the right leg, 20 cm proximally from the lateral patellar edge, and fixated with adhesive tape and a Velcro strap. Thereafter, it was occluded from ambient light by dark cloth. Data were sampled at 10 Hz, and stored for off-line analysis.

**Exercise data analysis**

Gas exchange parameters for the constant work rate exercise test were averaged into 10-s sampling intervals after removal of outliers (values > 3 SDs from the local mean were omitted) (29).

NIRS data were filtered using a central moving average filter with a window of 11 data points and resampled into 1-s intervals. Absolute values of TSI during moderate-intensity exercise testing were calculated as the average of the last min of the resting phase (TSI<sub>baseline</sub>), and the average of the last min of the exercise phase (TSI<sub>end-exercise</sub>), as described previously (46). A deoxygenation overshoot was defined as a TSI rise of more than 10% of ΔTSI<sub>onset</sub> after the initial decrease during the first three min of exercise (46). The area of the overshoot (Area<sub>overshoot</sub> in %·s)
was calculated as the integral between the measured response and the average TSI of the third
min after onset of exercise, starting from the intersection with the onset curve (1).

**Kinetics analysis**

Mono-exponential modeling of TSI and \( \dot{V}_O_2 \) onset data was performed to attain kinetic values
and to grade submaximal exercise capacity. The rise of \( \dot{V}_O_2 \) (fundamental phase or phase II) and
decay of TSI during onset were calculated by fitting the data to a first-order (mono-exponential)
model using the non-linear (damped) least squares method (Python 2.7, Python Software
Foundation, Beaverton, OR, USA) with the following formulas (29):

\[
Y(t) = Y_{baseline} + A \times (1 - e^{-(t-T_d)/\tau})
\]

(1)

\[
Y(t) = Y_{baseline} - B \times (1 - e^{-(t-T_d)/\tau})
\]

(2)

where \( Y \) equals \( \dot{V}_O_2 \) or TSI, \( Y_{baseline} \) depicts the average value during the last 30 s prior to the
exercise transition, \( A \) indicates the amplitude during exercise onset for \( \dot{V}_O_2 \) (\( \Delta \dot{V}_O_2 \text{exercise} \)), and \( B \)
for TSI (amplitude from TSI_{baseline} to the end of the mono-exponential fit). \( T_d \) is the time delay
and \( \tau \) is the time constant of the mono-exponential function (in seconds). For \( \dot{V}_O_2 \), the
first 20 s after onset were omitted from kinetics analysis, since it is assumed that the rapid
increase of \( \dot{V}_O_2 \) during this period (cardiodynamic phase or phase I) represents increased
pulmonary blood flow rather than microvascular gas exchange (4). For TSI, the time to the start
of the mono-exponential fit (Td) was determined using a matched filter method by sliding a mono-exponentially shaped kernel over the TSI signal, while calculating the cross-correlation (46). The mean response time was calculated as the sum of tau and time delay (MRT = τ + Td) and represents the time to reach 63% of the response from onset of exercise.

269  **Statistical analysis**

270  All data were analyzed using SPSS 24.0.0.0 statistical software (SPSS Inc, Chicago, IL, USA). Results are presented as mean value ± standard deviation (SD). Normality was assessed by skewness and kurtosis of the distribution, and by Shapiro-Wilk tests. For differences between matched pairs of subjects, paired Student’s *t*-tests were used in case of a normal distribution, or Wilcoxon signed rank tests when appropriate (6). In order to assess differences with respect to severity of HF, unmatched comparisons were made between NYHA groups (*i.e.* II and III), by means of unpaired Student’s *t*-tests or Mann-Whitney *U* tests. Associations between categorical data were assessed by Pearson’s chi-square test. Correlations were analyzed with Pearson’s correlation coefficient when data were normally distributed, or otherwise with Spearman’s rho (*r*). A p-value < 0.05 was considered statistically significant for all tests.

278  **Results**

282  Skeletal muscle biopsy sampling was performed in all study participants without any adverse events. All biopsy samples were of adequate size and quality to allow complete
immunohistochemical analysis. Oxidative enzyme activity was only assessed in patients with HFrEF because of differences between the combined study protocols. For similar reasons, exercise tests with measurements of gas exchange and NIR-SRS were only performed in patients with HFrEF and were executed without any untoward events. Skeletal muscle oxygenation (SmO$_2$) measurements by NIR-SRS could not be performed in four participants because of technical issues. Measurements succeeded in all other patients and they showed no paradoxical oxygenation responses (e.g., higher SmO$_2$ during exercise compared with resting phase).

Subject characteristics

Anthropometric and demographic characteristics of patients with HFrEF and HC subjects are presented in Table 1. For the assessment of the influence of severity of HF, the group of patients with HFrEF was divided into groups with NYHA class II ($n = 14$) and class III ($n = 11$). These groups were not significantly different with respect to BMI (Table 1; $P = 0.809$), LVEF (35.6 ± 10.5 versus 29.6 ± 9.8%, $P = 0.150$), or duration of heart failure (Table 1; $P = 0.501$). However, patients with NYHA class II were significantly younger on average (Table 1; $P = 0.003$), and so were HC subjects matched with patients in NYHA class II compared with those matched to patients in NYHA class III (Table 1; $P = 0.027$).

Muscle fiber characteristics
Skeletal muscle fiber characteristics of patients with HFrEF and HC subjects are presented in Table 2. The results showed no significant differences between type I and type II muscle fiber CSA in patients with HFrEF, whereas type II muscle fiber CSA was significantly smaller compared with type I muscle fibers in HC subjects (Table 2; $P < 0.05$). Muscle fiber CSA was not significantly different between the two groups (Fig. 3A; $P > 0.05$). In contrast, patients with HFrEF had a lower proportion of CSA occupied by type I muscle fibers and a lower numerical fiber type distribution than HC subjects (Table 2; $P < 0.05$).

In both groups, CC, C/Fi, and CFPE index were significantly higher in type I compared with type II muscle fibers (Table 3; $P < 0.05$). However, capillary density and CFPE-index for type I muscle fibers were significantly higher in patients with HFrEF compared with HC subjects (Table 3; $P < 0.05$). No significant differences between groups were found for capillarization of mixed or type II muscle fibers (Fig. 3B).

Differentiating for severity of HF showed no significant differences for type I, II and mixed muscle fiber CSA between patients in NYHA class II compared with those in NYHA class III (Table 2). Numerical distribution and percent area of fiber types was also not different between groups. CC, C/Fi and CFPE index were significantly lower for NYHA class III for type I, II and mixed muscle fibers (Table 3; $P < 0.05$), except for CFPE in type II fibers, where the difference was near significant ($P = 0.061$). In addition, HC subjects matched with patients in NYHA class II showed a higher percent area of type I fibers (Table 2; $P = 0.022$), and lower CSA for type II fibers (Table 2; $P = 0.049$). Type I to type II fiber CSA ratio was higher in HC subjects.
compared with patients in NYHA class II (1.25 ± 0.28 versus 1.00 ± 0.21, respectively, \( P = 0.019 \)). Furthermore, CFPE index (Table 3; \( P = 0.005 \)), and CD were significantly lower in HC compared with patients in NYHA class II for type I fibers (Table 3; \( P = 0.008 \)). For mixed fibers, this difference was near-significant for CFPE index (Table 3; \( P = 0.052 \)), and significant for CD (Table 3; \( P = 0.033 \)). The pairing of patients in NYHA class III with matched HC control subjects (Table 2 and 3) showed no statistically significant differences for muscle fiber characteristics.

The C/Fi for type I muscle fibers correlated significantly with type I muscle fiber CSA in patients in NYHA class III (\( r_s = 0.916, P < 0.001 \)), but not in patients in NYHA class II (\( r_s = 0.424, P = 0.131 \)).

**Oxidative enzyme activity**

Skeletal muscle oxidative capacity, as assessed by cytochrome C oxidase activity in muscle biopsy samples of patients with HFrEF, was significantly higher in NYHA class II than in class III (19.8 ± 8.0 versus 13.4 ± 6.4 µmol-[g wet weight]^{-1}.min^{-1}, respectively; \( P = 0.044 \)).

**Response to exercise**

Maximal exercise testing in patients with HFrEF resulted in an average maximum work rate of 128 ± 44 W and peak \( \dot{V}\text{O}_2 \) of 19.2 ± 5.0 mL·min^{-1}·kg^{-1}. The GET could not be determined in three patients (12%). In the remaining 22 patients, the independent observers agreed on the
determination of the GET (mean $\dot{V}_O_2$ 14.1 ± 3.3 mL·min$^{-1}$·kg$^{-1}$). Moderate-intensity constant work rate exercise testing was performed at 57 ± 17 W, which was at 47 ± 10 % of maximal exercise work rate.

Patients in NYHA class II had significantly higher peak$\dot{V}_O_2$ than those in class III (21.5 ± 4.7 versus 15.3 ± 2.3 mL·min$^{-1}$·kg$^{-1}$, respectively, $P < 0.001$). Gas exchange and NIR-SRS parameters for moderate-intensity constant work rate exercise categorized by NYHA class are listed in Table 4. They show that $\dot{V}_O_2$ onset kinetics were slower and end-exercise RER was higher in NYHA class III ($P < 0.05$). Skeletal muscle oxygenation was non-significantly lower for NYHA class III during rest ($P = 0.349$) and exercise ($P = 0.072$). Deoxygenation was more rapid for NYHA class II, evidenced by a smaller time-constant for the mono-exponential decay during onset of exercise. No significant differences between groups were noted for the time-delay of exercise onset, and the incidence or area of the deoxygenation overshoot.

**Relation between exercise parameters and fiber characteristics**

In patients with HFrEF, mixed muscle fiber CSA and proportional area of type I fibers were not related to peak$\dot{V}_O_2$ ($r_s = 0.015$, $P = 0.942$ and $r_s = -0.152$, $P = 0.468$, respectively) or to $\dot{V}_O_2$ onset kinetics ($r_s = -0.179$, $P = 0.391$ and $r_s = -0.015$, $P = 0.945$, respectively). CFPE index for mixed fibers (representing mixed muscle tissue capillarization under the NIRS probe) was not related to peak$\dot{V}_O_2$ ($r_s = 0.206$, $P = 0.323$), but was higher with faster deoxygenation at exercise onset ($\tau$TSI onset: $r_s = -0.682$, $P = 0.001$; Fig. 4a). TSI onset kinetics (i.e. the time constant) also showed a
significant relationship with $\dot{V}_O_2$ onset kinetics ($r_s = 0.709, P < 0.001$), while no significant

correlation was noted for TSI onset kinetics with cytochrome c oxidase activity ($r_s = -0.282, P =

0.216; Fig. 4b), mixed fiber CSA ($r_s = 0.094, P = 0.687$), percent area of type I fibers ($r_s = -0.171,

P = 0.457$), or peak$\dot{V}_O_2$ ($r_s = -0.308, P = 0.175$).

Discussion

This study shows that moderately impaired patients with HFrEF have equal fiber CSA, and a

larger percent area of type II fibers compared with individually matched healthy subjects. In

contrast to our hypothesis, capillary density and CFPE index of type I fibers was greater in

patients with HFrEF compared with HC. When severity of heart failure (categorized by NYHA

class) was taken into account, it was shown that better functional capacity was associated with

greater skeletal muscle oxidative capacity and muscle fiber capillarization, faster $\dot{V}_O_2$ kinetics

and more rapid microvascular deoxygenation during onset of moderate-intensity exercise.

Moreover, faster deoxygenation was significantly correlated with higher muscle fiber

capillarization and not with skeletal muscle oxidative capacity.

Muscle fiber dimensions and fiber type distribution

In the present study we observed no differences for type I, II and mixed muscle fiber CSA in

patients with HFrEF compared with matched healthy control subjects, which is in line with

previous findings (12, 37, 43, 55, 56). In patients with HFrEF with greater exercise impairments
(peak $\dot{V}O_2$ 13-16 mL·min$^{-1}$·kg$^{-1}$, compared with 19.2 ± 5.0 mL·min$^{-1}$·kg$^{-1}$ in this study) and worse cardiac function (LVEF 21-24 %, versus 32 ± 10 % in this study), muscle fiber atrophy and a significant relation of fiber size and peak exercise capacity have been described (39, 63, 67). However, we and others did not observe a relation between muscle fiber dimensions and whole body aerobic exercise capacity (i.e. peak $\dot{V}O_2$ or $\dot{V}O_2$ onset kinetics) (32, 55). Although we cannot exclude that muscle fiber atrophy was counteracted by mild subclinical fluid retention causing muscle fiber swelling, no patients showed any sign of decompensated heart failure, as for instance peripheral edema. It is therefore likely that muscle fiber atrophy is not a compulsory component of moderate HFReF and that other muscle attributes than fiber dimensions are related to exercise intolerance. For instance, Miller et al. have demonstrated lower single fiber myosin protein content in muscle fibers that were larger in HF than in healthy subjects (44), indicating a dissociation between contractile potential and fiber size. Moreover, preservation of muscle fiber dimensions may also indicate that reductions in skeletal muscle mass and exercise performance in moderate HF are accompanied by fiber loss (25, 68).

Remarkably, patients in NYHA class II had significantly larger type II muscle fiber CSA compared with matched healthy controls, and no differences between type I and type II muscle fiber CSA. Therefore, these patients do not show evidence of age-related type II muscle fiber atrophy, which is widely recognized (58) and confirmed in healthy subjects in this and recent studies from our laboratory (23, 66). It could indicate that patients with greater functional capacity expanded the potential for anaerobic metabolism through hypertrophy of glycolytic
muscle fibers. This may enable these patients to better cope with physical demands in case O₂ delivery is compromised by circulatory failure. Support for such a mechanism is provided by the finding that the intensity of daily activities of patients with HFrEF is substantially closer to maximum exercise capacity (i.e. more anaerobically) compared with healthy subjects (60). Confirmation of an emphasizes on anaerobic potential was provided by the finding of a larger proportion of type II muscle fibers in patients with HFrEF, which has been demonstrated in previous studies (37, 39, 43, 56). Similar to Larsen et al. (32), we found that when fiber type distribution was expressed as a portion of muscle fiber area instead of a numerical portion, type II muscle fiber area was still dominant. In agreement, studies using electrophoretical analyses of myosin heavy chain (MHC) isoform composition, demonstrated a decrease of MHC1 content (61, 64, 67), and an increase in MHC2b (67), or MHC2x content in HF (8, 61).

Importantly, recent studies that explicitly matched HC subjects to patients with HFrEF based on physical activity level, did not show a significant difference in muscle fiber type area distribution (12), or MHC isoform distribution (44, 65). Although we did not monitor physical activity, it can be argued that our patients with HFrEF also had a reduced level of physical activity compared with HC subjects.

Other chronic illnesses, like chronic pulmonary obstructive disease (COPD) and chronic renal failure (CRF), have been demonstrated to be accompanied by similarly less oxidative fiber type distribution as reported in HF (16, 52). Despite varying etiology and pathophysiology, deconditioning may be a common determinant of these muscle fiber
alterations in different disease states. However, the common finding of muscle fiber atrophy related to deconditioning in COPD and CHF (17, 53) does not seem to be an indisputable part of the HF syndrome. Although evidence clearly indicates that additional mechanisms (e.g. hypoperfusion, immune activation, sympathetic overstimulation) exert an influence over skeletal muscle function in HF (48), there is no definite understanding of the isolated influence of these mechanisms on structural skeletal muscle fiber maladaptations, and more importantly, how they are interrelated.

Muscle fiber capillary characteristics

The novel finding of a higher capillary count relative to muscle fiber CSA and perimeter (i.e. CD and CPFE index, respectively) in patients with HFrEF compared to HC subjects was confined to type I muscle fibers. The difference was predominantly attributable to patients in NYHA class II, as patients in NYHA class III showed no differences with HC subjects. Higher CD in HF compared with HC has previously been described in mixed fibers of gastrocnemius muscle by Mancini et al. (38), however, CD in vastus lateralis muscle was generally not different in other studies (12, 55, 56, 69). Differences with respect to characteristics (e.g. activity and fitness levels) of patients and healthy subjects could have contributed to this discrepancy. Remarkably, when fiber type specificity of capillarization was reported, C/Fi was reduced with fiber size, allegedly to maintain CD in type I muscle fibers (37, 56, 69). In accordance, we found a strong positive correlation between type I muscle fiber CSA and C/Fi in patients in NYHA class III, which
suggests that in more severe HF redundancy of capillaries (relative to fiber area) is tightly
regulated, probably because type I muscle fibers are more dependent on O₂ supply than type II
muscle fibers. The contrasting poor correlation of C/Fi with type I muscle fiber size in patients
in NYHA class II may suggest greater type I muscle fiber capillarization was regulated to comply
with relatively higher O₂ demand, not fiber area. An alternative or complementary explanation
could be that mild HF is accompanied by a preferential loss of type I fibers with preservation of
the vascular bed, or that favorable skeletal muscle capillarization in individuals, when affected
by heart failure, predisposes to preserve exercise capacity. Whether the physiological
significance of increased capillarization in patients in NYHA class II is a functional adaptation
to optimize the supply of oxygen and substrate to meet skeletal muscle demands by means of
augmented diffusive or convective capacity, is discussed hereafter.

**Muscle fiber characteristics and muscle oxygenation**

As mentioned above, indicators of whole body aerobic exercise capacity were not entirely
explained by muscle fiber size, or percent area of type I fibers in this study. While these muscle
fiber characteristics were also not statistically different between NYHA groups, it was seen that
CFPE index was the only indicator of capillarization in this study to differ between patients with
HFrEF and HC subjects, as well as between NYHA classes. Compared with conventional
capillary indices, CFPE index is thought to be a better estimate of resistance to oxygen diffusion,
because diffusive capacity (D_{O₂, m}) relies more heavily on transport over the muscle fiber
membrane than on the diffusion distance per se (27). It may therefore be suggested that the structural capacity for oxygen diffusion is greater in skeletal muscle tissue of patients with HFrEF when compared with HC subjects.

Interestingly, we showed that higher CFPE index was related to faster deoxygenation of skeletal muscle during the transition from rest to moderate-intensity exercise (i.e. SmO₂ kinetics). Since skeletal muscle oxygenation is an indicator of the ratio of \( \dot{Q}_{O_2,m} \) to \( \dot{V}_{O_2,m} \) (31), faster SmO₂ kinetics in patients in NYHA class II may signify that \( \dot{Q}_{O_2,m} \) was slower relative to \( \dot{V}_{O_2,m} \) compared with patients in NYHA class III. The unlikely consequence would then be that \( \dot{Q}_{O_2,m} \), in the form of microvascular blood flow, is slower with better functional capacity (30, 54). Otherwise, a more rapid increase in \( \dot{V}_{O_2,m} \) relative to \( \dot{Q}_{O_2,m} \) during the onset of exercise would be a more appropriate explanation for faster SmO₂ kinetics in patients in NYHA class II compared with patients in NYHA class III (47). As such, faster SmO₂ kinetics seem to indicate accelerated skeletal muscle oxygen extraction.

That SmO₂ kinetics were shown to be faster with increasing CFPE may lie in the fact that CFPE influences \( D_{O_2,m} \) in two ways. First, by the extent of the capillary exchange surface, and second, through the number of capillaries per muscle fiber outer surface area. That is, a greater CFPE index enlarges the interface for diffusion, plus the greater capillary number per muscle fiber surface area will decrease velocity of red blood cells (RBC) in individual capillaries. In a situation with unchanged muscle RBC flux, transit time of RBC’s along the capillary will thereby increase, allowing greater diffusion time for equilibration of capillary blood with the
myocyte (49). This may suggest that higher capillarization (i.e. CFPE index) in less advanced HF serves as a mechanism to maintain oxygen consumption in the face of reduced blood flow by increasing the possibility for oxygen extraction. Moreover, it can be speculated that lower CFPE index, as seen in more advanced HF (i.e. NYHA class III), is an adaptation to the situation of severely reduced muscle blood flow, wherein increasing diffusion capacity through capillarization is no longer functional because of the already very large RBC transit times. The fact that previous studies have demonstrated increased oxygen extraction and both impaired and enhanced diffusive capacity in patients with HFrEF and animal models of HF (13, 28, 30, 41), may support our contentions.

Although skeletal muscle cytochrome c oxidase activity in this study was higher in patients in NYHA class II, there was no significant correlation between SmO₂ kinetics and cytochrome c oxidase activity, percent area of type I fibers, or fiber cross-sectional area. Therefore, greater skeletal muscle oxidative capacity does not present as a key determinant of accelerated skeletal muscle O₂ extraction at exercise onset. While high skeletal muscle oxidative capacity is probably important in exploiting the diffusive potential of the capillary bed in less affected patients (7), low oxidative capacity in severely impaired patients is thought to be a functional adaptation to reduced O₂ supply (9). Similarly, the speed of activation of the O₂ consuming metabolic pathways in mitochondria (i.e. those supplying ATP for contraction) may also decrease with severity of HF (3, 47, 70), and slow SmO₂ onset kinetics. That is, slower activation of mitochondrial oxidative phosphorylation is thought to manifest itself in a longer
time delay of the temporal profile of SmO₂ (i.e. before actual deoxygenation commences; Fig. 2) (31, 47, 53). However, the time delay was not different between patients with NYHA class II and III. In contrast, the time constant (τ TSI onset), which was shorter in patients in NYHA class II and which formed the basis for our conclusions, is considered a better representation of the actual interplay between the kinetics of $\dot{Q}_{O_2,m}$ to $\dot{V}_{O_2,m}$ once respiration is activated (31, 53).

Taken together, the higher structural capacity for oxygen diffusion in patients in NYHA class II has physiological consequences in that it is associated with faster SmO₂ kinetics, indicating accelerated oxygen extraction. Because faster SmO₂ kinetics were related to faster $\dot{V}_{O_2}$ onset kinetics (associated with a decreased O₂ deficit), the importance of this finding may be that capillary diffusive capacity is an important prerequisite for the functional ability to cope with activities of daily living (49).

Clinical implications

The finding of different skeletal muscle fiber characteristics found in patients with HFrEF in this study may serve as a target to improve exercise capacity. For instance, the lower percentage of type I fibers can potentially be reversed by training interventions aimed at increasing aerobic capacity specifically (e.g. endurance-type exercise training). Furthermore, the higher capillarization in our patients with HFrEF may be important to prime further exercise-induced improvements, because it has been shown that higher CPFE index in healthy individuals predisposes to increase type II fiber CSA to a greater extent when performing resistance-type
exercise training (57). However, patients with lower capillarization are likely still able to induce angiogenesis through exercise training (13), with a specific response to either endurance or resistance type exercise training depending on the primarily affected fiber type (i.e. type I and II, respectively) (11, 34, 36). Moreover, recent evidence suggests that high-intensity aerobic interval training can increase maximal exercise capacity and the response to submaximal exercise by improving microvascular hemodynamics and matching of $\dot{Q}_{O_2,m}$ to $\dot{V}_{O_2,m}$ (59). This may be of particular importance when skeletal muscle cannot increase O$_2$ extraction because of limited convective O$_2$ delivery, as we argue to be the case in more severely impaired patients.

**Study limitations**

Several limitations should be acknowledged. First, this study did not include women or individuals with NYHA class I and IV. Therefore, the results can only be generalized to moderately-impaired male patients with HFrEF. Second, muscle biopsy samples and NIRS measurements were performed on the distal *vastus lateralis*, because this is a representative locomotor muscle of mixed fiber type composition. However, other muscle groups were not investigated and inferences on systemic muscle abnormalities in HFrEF cannot be made. Third, NIR-SRS measurements of SmO$_2$ are confounded by the thickness of the layer of adipose tissue overlying the muscle of interest, thereby invalidating between-subject comparisons (45). However, we assessed kinetic SmO$_2$ parameters (e.g. $\tau_{TSI_{ozone}}$), which are descriptions of the temporal profile of deoxygenation and less likely to be influenced by adipose tissue thickness.
Fourth, because this study did not include measurements of capillary morphology (e.g. path lengths, branching, tortuosity), capillary permeability, or capillary recruitment patterns, the presented role of capillarization in diffusive and convective $O_2$ delivery may be a simplification of the physiology at hand. Finally, the small, but statistically significant, difference in age between patients with HFrEF in NYHA group II and III can be a potential confounder for the observed difference of $\dot{V}_{O_2}$ and deoxygenation kinetics between groups. However, recent studies explicitly comparing young and old subjects showed that aging per se does not slow $\dot{V}_{O_2}$ kinetics (21). Furthermore, older subjects do not appear to have significantly slower deoxygenation (22, 24). Therefore, we consider the observed kinetic differences as reflections of HF severity.

Conclusions

In this study, moderately-impaired patients with HFrEF have equal skeletal muscle fiber CSA and a larger portion of type II fibers when compared with healthy subjects. However, capillary indices related to fiber dimensions are greater in type I fibers, which is primarily attributable to patients with less advanced heart failure, and in whom it may be a compensatory adaptation for reduced muscle blood flow. From measurements of skeletal muscle oxygenation, we conclude that higher capillarization may enhance $O_2$ diffusing capacity which in turn may facilitate $O_2$ extraction and $\dot{V}_{O_2}$ kinetics during exercise.
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567 analysis and publication of data.

568

569 Disclosures

570 The authors declare that they have no conflict of interest.

571

572 References

573 1. Barbosa PB, Bravo DM, Neder JA, Ferreira LF. Kinetics analysis of muscle arterial-
574 venous O(2) difference profile during exercise. Respir Physiol Neurobiol 173: 51–57,
575 2010.


578 3. Behnke BJ, Kindig CA, Musch TI, Koga S, Poole DC. Dynamics of microvascular
579 oxygen pressure across the rest-exercise transition in rat skeletal muscle. Respir Physiol

581 4. Benson AP, Grassi B, Rossiter HB. A validated model of oxygen uptake and circulatory

583 5. Bergstrom J. Percutaneous needle biopsy of skeletal muscle in physiological and clinical


586 7. Diederich ER, Behnke BJ, McDonough P, Kindig CA, Barstow TJ, Poole DC, Musch
587 TI. Dynamics of microvascular oxygen partial pressure in contracting skeletal muscle of


18. Fülster S, Tacke M, Sandek A, Ebner N, Tschöpe C, Doehner W, Anker SD, von...


29. Kemps HMC, de Vries WR, Hoogeveen AR, Zonderland ML, Thijssen EJM, Schep G. Reproducibility of onset and recovery oxygen uptake kinetics in moderately impaired...


52. Rehn TA, Munkvik M, Lunde PK, Sjaastad I, Sejersted OM. Intrinsic skeletal muscle


Figure Captions

**Fig. 1** Representative images of fiber type-specific analysis of skeletal muscle microvascular strainig. A: Laminin (blue); Myosin Heavy Chain I (green); CD31 (red). B: Laminin (blue); CD31 (red). C: Myosin Heavy Chain I (green); CD31 (red). D: CD31 (red) only.

**Fig. 2** Representative plot of a deoxygenation profile during onset of moderate-intensity constant work rate exercise in a patient with heart failure with reduced ejection fraction. The vertical dashed lines indicate (from left to right): start of exercise phase (t = 120 s), and end of exercise phase (t = 480 s). The horizontal lines indicate (from left to right): baseline tissue saturation index (TSI) value, minimum value, and end-exercise value. The curved dashed line represents the best fit of the mono-exponential model to the TSI response during onset. Kinetic data are presented in the graph, where τ is the time constant and Td is the time delay.

**Fig. 3 A-B** Mean muscle fiber cross-sectional area (CSA) (A) and mean muscle capillary-to-fiber perimeter exchange (CFPE) index (B) for patients with heart failure with reduced ejection fraction (HFrEF) in New York Heart Association (NYHA) group II (n = 14) and III (n = 11) and individually matched healthy control (HC) subjects, for muscle fiber type I and II (error bars denote standard error). Difference between bars *P < 0.05
Fig. 4 A-B Correlations between the time constant (tau) of the mono-exponential model of the tissue saturation index (TSI) response during onset of moderate-intensity constant work rate exercise ($r_{spearman} = -0.682, P = 0.001$) and (A) the capillary-to-fiber perimeter exchange (CFPE) index of mixed muscle tissue, and (B) the skeletal muscle oxidative capacity, represented by muscle cytochrome c oxidase (COX) activity ($r = -0.282, P = 0.216$) in patients with heart failure with reduced ejection fraction ($n = 25$).
### Tables

<table>
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<th>Variables</th>
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<th>HFrEF NYHA III</th>
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<th>HC NYHA III match</th>
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<td>70 ± 7</td>
<td>63 ± 6 *</td>
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<td>8 / 3</td>
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<td>- / -</td>
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<td>-</td>
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<td>100 %</td>
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<tr>
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<td>27 %</td>
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<tr>
<td>ACE inhibitor</td>
<td>79 %</td>
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</tr>
<tr>
<td>ARB</td>
<td>14 %</td>
<td>27 %</td>
<td>0 %</td>
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**Table 1** Characteristics of included patients with heart failure with reduced ejection fraction (HFrEF) in New York Heart Association (NYHA) class II and II, and individually matched healthy control (HC) subjects. Data are presented as means ± SD for continuous variables and as numbers (percentages) for dichotomous variables. Body mass index (BMI), ischemic
cardiomyopathy (ICM), dilated cardiomyopathy (DCM), heart failure (HF), pacemaker (PM), implantable cardioverter defibrillator (ICD), cardiac resynchronization therapy (CRT), angiotensin converting enzyme (ACE), angiotensin II receptor blocker (ARB). Difference between NYHA II and NYHA III * P < 0.05.
| Variables       | Fiber type | HFrEF      | HC          | HFrEF      | HC          | HFrEF      | HC          | HFrEF      | HC          | HFrEF      | HC          |
|-----------------|------------|------------|-------------|------------|-------------|------------|-------------|------------|-------------|------------|-------------|-------------|
|                 |            | All        | All         | NYHA II    | NYHA II match | NYHA III  | NYHA III match |
|                 |            | n = 25     | n = 25      | n = 14     | n = 14      | n = 11     | n = 11      | n = 11     | n = 11      | n = 11     | n = 11      |
| CSA (µm²)       | Mixed      | 6,069 ± 1,744 | 5,856 ± 1,205 | 6,342 ± 1,799 | 6,022 ± 1,232 | 5,722 ± 1,690 | 5,643 ± 1,193 |
|                 | Type I     | 6,104 ± 1,552 | 6,190 ± 1,432 * | 6,166 ± 1,386 | 6,566 ± 1,492 * | 6,026 ± 1,809 | 5,711 ± 1,256 |
|                 | Type II    | 6,090 ± 1,989 | 5,514 ± 1,334 | 6,480 ± 2,139 * | 5,391 ± 1,211 | 5,595 ± 1,749 | 5,672 ± 1,521 |
| %Type (%)       | Type I     | 37 ± 12 *  | 46 ± 15     | 38 ± 12    | 50 ± 15     | 37 ± 14    | 42 ± 13    |
|                 | Type II    | 63 ± 12 * † | 54 ± 15     | 62 ± 12 †  | 50 ± 15     | 63 ± 14 †  | 58 ± 13    |
| %Area (%)       | Type I     | 38 ± 12 *  | 49 ± 16     | 37 ± 12 *  | 54 ± 17     | 39 ± 14    | 42 ± 14    |
|                 | Type II    | 62 ± 12 * † | 51 ± 16     | 63 ± 12 * † | 46 ± 17     | 61 ± 14 †  | 58 ± 14    |

Table 2: Skeletal muscle fiber characteristics in patients with heart failure with reduced ejection fraction (HFrEF) with New York Heart Association (NYHA) subgroups (class II and III) and individually matched healthy control (HC) subjects. Values represent mean ± SD. Cross-sectional area (CSA), percent fiber type distribution (%Type), percent fiber type area (%Area). Difference between HFrEF and HC * P < 0.05. Difference between fiber type I and II † P < 0.05. Difference between NYHA II and NYHA III ‡ P < 0.05.
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<th>HC All</th>
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<td></td>
<td></td>
<td>$n = 25$</td>
<td>$n = 25$</td>
<td>$n = 14$</td>
<td>$n = 14$</td>
<td>$n = 11$</td>
<td>$n = 11$</td>
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<td>4.13 ± 0.72 $\dagger$</td>
<td>3.93 ± 0.57</td>
<td>3.40 ± 0.73</td>
<td>3.90 ± 0.89</td>
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<td>Type I</td>
<td>4.32 ± 0.78 $\dagger$</td>
<td>4.24 ± 0.74 $\dagger$</td>
<td>4.61 ± 0.69 $\dagger$ $\dagger$</td>
<td>4.25 ± 0.61 $\dagger$</td>
<td>3.94 ± 0.74 $\dagger$</td>
<td>4.23 ± 0.90 $\dagger$</td>
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<td>Type II</td>
<td>3.54 ± 0.80</td>
<td>3.62 ± 0.74</td>
<td>3.83 ± 0.69 $\dagger$</td>
<td>3.55 ± 0.62</td>
<td>3.17 ± 0.80</td>
<td>3.70 ± 0.89</td>
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<td>C/Fi (number)</td>
<td>Mixed</td>
<td>1.48 ± 0.34</td>
<td>1.51 ± 0.30</td>
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<td>1.32 ± 0.32</td>
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<td>1.72 ± 0.33 $\dagger$</td>
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<td>1.85 ± 0.31 $\dagger$ $\dagger$</td>
<td>1.67 ± 0.28 $\dagger$</td>
<td>1.57 ± 0.30 $\dagger$</td>
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<td>Type II</td>
<td>1.35 ± 0.33</td>
<td>1.38 ± 0.31</td>
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<td>1.36 ± 0.25</td>
<td>1.21 ± 0.34</td>
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<td>CD (cap-mm$^{-2}$)</td>
<td>Mixed</td>
<td>284 ± 83</td>
<td>262 ± 58</td>
<td>305 ± 86 $^*$</td>
<td>257 ± 67</td>
<td>257 ± 72</td>
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<td>Type I</td>
<td>324 ± 73 $^*$ $\dagger$</td>
<td>272 ± 59</td>
<td>340 ± 85 $^*$ $\dagger$</td>
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<td>303 ± 52 $\dagger$</td>
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<td>Type II</td>
<td>263 ± 95</td>
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<td>284 ± 101</td>
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<td>CFPE (cap-1,000 μm$^{-2}$)</td>
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<td>5.70 ± 0.92 $^*$ $\dagger$</td>
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<td>4.87 ± 0.79 $\dagger$</td>
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<td>5.27 ± 0.82 $\dagger$</td>
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<td>4.46 ± 1.02</td>
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<td>4.38 ± 0.86</td>
<td>4.03 ± 0.96</td>
<td>4.46 ± 0.83</td>
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Table 3: Skeletal muscle fiber capillary characteristics in patients with heart failure with reduced ejection fraction (HFrEF) with New York Heart Association (NYHA) subgroups (class II and III) and individually matched healthy control (HC) subjects. Values represent mean ± SD. Capillary density (CD), Capillary contacts (CC), Capillary per fiber (C/Fi), Capillary-to-fiber perimeter exchange (CFPE) index. Difference between HFrEF and HC $^* P < 0.05$. Difference between fiber type I and II $^\dagger P < 0.05$. Difference between NYHA II and NYHA III $\dagger P < 0.05$.  

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<td>401 ± 169</td>
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<td>$\Delta V O_2_{\text{exercise}}$ (mL-min$^{-1}$)</td>
<td>958 ± 256</td>
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<td>RER end-exercise</td>
<td>0.84 ± 0.06</td>
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<td>$\tau \dot{V} O_2_{\text{onset}}$ (s)</td>
<td>52 ± 28</td>
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<td>Skeletal muscle oxygenation</td>
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<td>$\text{TSI}_{\text{overshoot}}$ (%)</td>
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**Table 4** Gas exchange and skeletal muscle oxygenation measurements during moderate intensity constant work rate exercise testing in patients with New York Heart Association (NYHA) class II and III heart failure with reduced ejection fraction (HFrEF). Values represent mean ± SD. Pulmonary oxygen uptake ($\dot{V}_O_2$), respiratory exchange ratio (RER), time constant of mono-exponential model (τ), tissue saturation index (TSI), time delay (Td), mean response time (MRT). Difference between NYHA II and NYHA III * $P < 0.05$, † $P < 0.01$. 

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A

HFrEF HC HFrEF HC

NYHA II NYHA III

CSA (μm²)

B

CFPE index (capillaries per 1,000 μm)

NYHA II NYHA III

Fiber type I

Fiber type II

*