

The Journal of Physiology

<https://jp.msubmit.net>

**JP-RP-2018-276678R1**

**Title:** Acute and chronic exercise in patients with HFrEF: Evidence of structural and functional plasticity and intact angiogenic signaling in skeletal muscle

**Authors:** Fabio Esposito  
Odile Mathieu-Costello  
Peter Wagner  
Russell Richardson

**Author Conflict:** No competing interests declared

**Author Contribution:** Fabio Esposito: Conception or design of the work; Acquisition or analysis or interpretation of data for the work; Drafting the work or revising it critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work Odile Mathieu-Costello: Acquisition or analysis or interpretation of data for the work; Drafting the work or revising it critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work Peter Wagner: Conception or design of the work; Acquisition or analysis or interpretation of data for the work; Drafting the work or revising it critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work Russell Richardson: Conception or design of the work; Acquisition or analysis or interpretation of data for the work; Drafting the work or revising it critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work

**Running Title:** HFrEF, skeletal muscle structure and function, and exercise training

**Dual Publication:** No

**Funding:** HHS | NIH | National Heart, Lung, and Blood Institute (NHBLI): Russell S Richardson, HL-091830; Veterans Affairs Rehabilitation Research and Development Merit Awards: Russell S Richardson, E6910-R; Veterans Affairs SPiRe Award: Russell S Richardson, E1433-P; Veterans Affairs Senior Research Career Scientist Award: Russell S Richardson, E9275-L

**Acute and chronic exercise in patients with HF<sub>r</sub>EF: Evidence of structural and functional plasticity and intact angiogenic signaling in skeletal muscle**

Fabio Esposito, MD<sup>1</sup>, Odile Mathieu-Costello, PhD<sup>2</sup>, Peter D. Wagner, MD PhD<sup>2</sup>, and Russell S. Richardson, PhD<sup>3,4,5</sup>

<sup>1</sup>Department of Biomedical Sciences for Health, Università degli Studi di Milano, Milan, Italy

<sup>2</sup>Department of Medicine, Division of Physiology, University of California, San Diego

<sup>3</sup>Department of Medicine, Division of Geriatrics, University of Utah, Salt Lake City

<sup>4</sup>Department of Nutrition and Integrative Physiology, University of Utah, Salt Lake City

<sup>5</sup>Geriatric Research, Education and Clinical Center, Salt Lake City VAMC

**Running title:** HF<sub>r</sub>EF, skeletal muscle structure and function, and exercise training

**Key words:** heart failure, skeletal muscle, VEGF.

**Table of Contents category:** Exercise

**FUNDING**

This work was supported by a National Institutes of Health grant from the National Heart, Lung, and Blood Institute (HL-091830), Veterans Affairs Rehabilitation Research and Development Merit Awards (E6910-R, E1697-R, and E2323-I), Veterans Affairs SPiRe Award (E1433-P), and a Veterans Affairs Senior Research Career Scientist Award (E9275-L). We declare no relationship with industry.

**Corresponding author:** Fabio Esposito, M.D., Department of Biomedical Sciences for Health, University of Milan, Via Colombo 71, I-20133 Milan, Italy. Phone: +39-02-5031 4649; Fax: +39-02-5031 4630; E-Mail: [fabio.esposito@unimi.it](mailto:fabio.esposito@unimi.it)

## KEY POINTS SUMMARY

- The vascular endothelial growth factor (VEGF) response to acute submaximal exercise and training effects in patients with heart failure with reduced ejection fraction (HFrEF) were investigated.
- Six patients and six healthy matched-controls performed knee-extensor exercise (KE) at 50% of maximum work-rate before and after (only patients) KE training. Muscle biopsies were taken to assess skeletal muscle structure and the angiogenic response.
- Pre-training, during this submaximal KE exercise, HFrEF exhibited higher leg vascular resistance and greater norepinephrine spillover. Skeletal muscle structure and VEGF response were mostly not different between groups. Post-training, resistance was no longer elevated and norepinephrine spillover was curtailed in the patients. Although, in the trained state, VEGF did not respond to acute exercise, capillarity was augmented. Muscle fiber cross sectional area and %area of type I fibers increased and mitochondrial volume density exceeded that of controls.
- Structural/functional plasticity and appropriate angiogenic signaling were observed in skeletal muscle of patients with HFrEF.

## ABSTRACT

**Objectives** This study examined the response to acute submaximal exercise and the effect of training in patients with heart failure with reduced ejection fraction (HFrEF).

**Background** The acute angiogenic response to submaximal exercise in HFrEF after small muscle mass training is debated. **Methods** The direct Fick method, with vascular pressures,

was performed across the leg during knee-extensor exercise (KE) at 50% of maximum work-rate ( $WR_{max}$ ) in patients (n=6) and controls (n=6) and then post-KE training in patients. Muscle biopsies facilitated the assessment of skeletal muscle structure and vascular endothelial growth factor (VEGF) mRNA levels. **Results** Pre-training, HFrEF exhibited significantly higher leg vascular resistance (LVR) ( $\approx 15\%$ ) and significantly greater norepinephrine (Ne) spillover ( $\approx 385\%$ ). Apart from mitochondrial volume density, which was significantly lower ( $\approx 22\%$ ) in HFrEF, initial skeletal muscle structure, including capillarity, were not different between groups. Resting VEGF mRNA levels, and the increase with exercise, was not different between patients and controls. Post-training LVR was no longer elevated and Ne spillover was curtailed. Skeletal muscle capillarity increased with training, as assessed by capillary-to-fiber ratio ( $\approx 13\%$ ) and number of capillaries around a fiber ( $N_{CAF}$ ) ( $\approx 19\%$ ). VEGF mRNA was now not significantly increased by acute exercise. Muscle fiber cross sectional area and % area of type I fibers both increased significantly with training ( $\approx 18\%$  and  $\approx 21\%$ , respectively), while the % area of type II fibers fell significantly ( $\approx 11\%$ ), and mitochondrial volume density now exceeded that of controls. **Conclusions** These data reveal structural and functional plasticity and appropriate angiogenic signaling in skeletal muscle of HFrEF patients.

## LIST OF ABBREVIATIONS

HFrEF, heart failure with reduced ejection fraction

KE, knee-extensor exercise

LVR, leg vascular resistances

MAP, mean arterial pressure

MVP, mean venous blood pressure

Ne, norepinephrine

NYHA, New York Heart Association

QO<sub>2</sub>, O<sub>2</sub> delivery

SNA, sympathetic nervous system activity

VEGF, vascular endothelial growth factor

WR, work rate

VO<sub>2</sub>, oxygen consumption

## INTRODUCTION

Although heart failure with reduced ejection fraction (HFrEF) is fundamentally a disease that impacts central hemodynamics, a variety of skeletal muscle alterations, including muscle atrophy, a shift in fiber type, impaired oxidative capacity, reduced mitochondrial enzymes, and decreased mitochondrial volume density, are typically associated with this pathology (Mancini *et al.*, 1992; Harrington *et al.*, 1997; Esposito *et al.*, 2010b; Esposito *et al.*, 2015; Kaur *et al.*, 2017; Poole *et al.*, 2017). While, in terms of the peripheral vasculature, HFrEF has also been linked to autonomic and cardiovascular dysregulation that leads to greater sympathetic vasoconstrictor tone (Magnusson *et al.*, 1997; Kaur *et al.*, 2017), decreased capillary-to-fiber ratio, reduced capillary density and smaller capillary diameter (Duscha *et al.*, 1999; Esposito *et al.*, 2010b; Piepoli *et al.*, 2010a; Piepoli *et al.*, 2010b; Poole *et al.*, 2012). These findings imply that in addition to the inability of the heart to appropriately raise pumping capacity during exercise, both skeletal muscle and the peripheral vasculature also contribute to the exercise intolerance associated with HFrEF, a hallmark of this pathology.

To restore exercise tolerance, traditional cardiac rehabilitation has employed whole body (cycle) exercise which recruits a large muscle mass and therefore taxes the central circulation. Although this approach has consistently yielded significant improvements in exercise capacity in patients with HFrEF (Hambrecht *et al.*, 1998; Piepoli *et al.*, 2004; Hollriegel *et al.*, 2016), because whole body exercise induces a complex interaction between central and peripheral hemodynamics, such observations leave doubt as to the role of central and peripheral adaptations in response to exercise training. In an attempt to address this uncertainty, a growing number of studies have employed small muscle mass training, unlikely to stimulate central hemodynamic adaptations (Magnusson *et al.*, 1996; Tyni-Lenne *et al.*, 2001). Although this innovative approach has revealed that isolated muscle training can, indeed, improve whole body exercise capacity in patients with HFrEF (Magnusson *et al.*, 1996; Tyni-Lenne *et al.*, 2001; Barrett-O'Keefe *et al.*, 2014; Poole *et al.*, 2017), the sometimes indirect physiological assessments used in these studies has yet to yield a clear understanding of the skeletal muscle structural and functional response to such exercise training in this population.

Angiogenesis in skeletal muscle is an essential adaptive response to repeated exercise (i.e. training) resulting in an increase in skeletal muscle vascularity that, in turn, enhances O<sub>2</sub> transport (Esposito *et al.*, 2010a). As vascular endothelial growth factor (VEGF) has a high specificity for vascular endothelial cells (Leung *et al.*, 1989), these findings are in line with VEGF being a key angiogenic factor involved in structural and functional adaptations in skeletal muscle associated with exercise (Brodal *et al.*, 1977; Zumstein *et al.*, 1983; Hang *et al.*, 1995; Olfert *et al.*, 2009; Huey *et al.*, 2016). Indeed, previously, both in healthy humans and patients with HFrEF, a significant increase in skeletal muscle VEGF mRNA abundance has been documented in response to an acute small muscle mass exercise stimulus (Gustafsson *et al.*, 1999; Richardson *et al.*, 1999; Esposito *et al.*, 2010a). Furthermore, in healthy humans we have documented that the previously large VEGF mRNA response to acute exercise is significantly attenuated as a consequence of exercise training, adding credence to the concept that VEGF is important in the initial phase of exercise-induced adaptation, but when significant angiogenesis has occurred the need and importance of this mechanism becomes greatly reduced. However, whether this holds true in patients with HFrEF, which is associated with a variety of maladaptations in the periphery, has yet to be determined.

Consequently, this study sought to examine skeletal muscle structure and function and the VEGF response to acute submaximal exercise in patients with HFrEF pre and post exercise training. With the expectation that a negative feedback mechanism should exist to reduce the level of VEGF gene expression as exercise adaptation occurs, the purpose of this study was to a) document the adaptations associated with exercise training in the skeletal muscle bed of patients with HFrEF and b) test the hypothesis that in the face of such exercise training-induced adaptations, the VEGF mRNA increase in patients with HFrEF, in response to acute exercise, would be significantly attenuated.



## METHODS

**Ethical Approval:** After full explanation of the study design and experimental procedures, six male patients with HFrEF and six healthy control subjects gave written informed consent to participate in this study, which was approved by the University of California, San Diego Human Subjects Protection Program (#990152). They were all free to withdraw at any time without jeopardy. The study conformed to the standards set by the 1975 Declaration of Helsinki, except for registration in a database.

**Subjects:** All patients with HFrEF were clinically stable with symptoms compatible with New York Heart Association (NYHA) functional class II-III. Mean left ventricular ejection fraction in the HFrEF patients was  $25 \pm 3\%$ . Patient medications were not altered because of the study, with the exception of beta-blockers that were withheld for 48 hours prior to each experimental day. Particular care was taken to match patients with HFrEF and controls in terms of age, sex, and, especially, physical activity by questionnaire and interview (table 1). Several components of this study have been previously published with a focus on maximal exercise and the impact of exercise training in HFrEF (Esposito *et al.*, 2011) and the VEGF response to acute submaximal exercise in HFrEF (Esposito *et al.*, 2010a). Although some overlap is acknowledged, the current work is novel in that it combines both the acute and chronic responses to submaximal exercise in patients with HFrEF to better understand skeletal muscle plasticity in this population.

**Experimental Design.** An initial graded exercise test was performed on a cycle ergometer to document cycle  $WR_{max}$  and peak  $VO_2$  ( $VO_{2peak}$ ). To determine KE  $WR_{max}$  subjects performed an 8-12 min graded KE test to exhaustion. Several days later, both patients with HFrEF and controls reported again to the laboratory and after a 5 min warm-up performed 30 min of KE at 50% of  $WR_{max}$ . Both the resting and exercised legs were biopsied within one hour of the exercise stimulus. During KE studies, skeletal muscle blood flow and muscle  $\dot{V}O_2$  were

measured. To achieve this, two catheters (radial artery and left femoral vein) and a thermocouple (left femoral vein) were placed using sterile technique to facilitate blood gas and blood flow measurements and O<sub>2</sub> transport calculations, as previously reported (Richardson *et al.*, 1993). These catheter and biopsy studies were then repeated in the HFrEF patients following 8 weeks of supervised KE training (3 times/week, varied intensity - with overall intensity progressively increased based upon biweekly assessments, 50 min/session/leg), as previously described (Richardson *et al.*, 2000; Lawrenson *et al.*, 2003; Lawrenson *et al.*, 2004). Exercise training compliance was evaluated as a % of training sessions attended. Control subjects did not undergo training and, thus, were studied only once with the catheter and biopsy procedures.

**Exercise apparatus.** The knee-extensor ergometer was utilized to produce the acute exercise stimulus, as previously described (Richardson *et al.*, 1995). During exercise, the contraction rate was maintained at 60 repetitions per minute.

**Vascular and metabolic measurements.** The continuous infusion thermodilution approach was used to measure blood flow during exercise, as previously described (Richardson *et al.*, 1993). Femoral arterial and venous blood pressures were continuously monitored at heart level by pressure transducers (model PX-MK099, Baxter). Mean arterial pressure (MAP) and mean venous blood pressure (MVP) were computed by the integration of each pressure curve. Leg vascular resistance (LVR) was calculated as  $(MAP - MVP) / \text{muscle blood flow}$ . Before each blood flow measurement, three to four ml samples of arterial and femoral venous blood were withdrawn from the catheters anaerobically to measure PO<sub>2</sub>, PCO<sub>2</sub>, pH, O<sub>2</sub> saturation, and hemoglobin concentration ([Hb]). All measurements were made on an IL 1306 blood gas analyzer and IL 482 CO-oximeter (Instrumentation Laboratories, Lexington, MA.). Arterial and venous O<sub>2</sub> concentrations were calculated as  $[1.39 \text{ ml O}_2 \times [\text{Hb}] \text{ g}/100 \text{ ml} \times \text{O}_2 \text{ saturation (\%)}] + [0.003 \text{ ml O}_2/100 \text{ ml of blood} \times \text{PO}_2 \text{ (mmHg)}]$ . Arterial-venous O<sub>2</sub> concentration difference was calculated from the difference in radial artery and femoral

venous O<sub>2</sub> concentration measurements. Muscle VO<sub>2</sub> was calculated as the product of blood flow and arterial-venous O<sub>2</sub> difference, while O<sub>2</sub> delivery (QO<sub>2</sub>) was calculated as the product of blood flow and arterial O<sub>2</sub> concentration.

**Norepinephrine spillover.** Epinephrine and norepinephrine (Ne) were extracted from plasma using a cis-diol-specific affinity gel, acylated, derivatized enzymatically and then assayed by competitive ELISA using the microtiter plate format, as previously described (Wadley *et al.*, 2006). The rate of Ne spillover was determined, as described previously (Savard *et al.*, 1989), using the following equation:

$$\text{Ne spillover} = [(C_v - C_a) + C_a (E_e)] * \text{LPF}$$

where C<sub>v</sub> and C<sub>a</sub> are plasma Ne concentrations in the common femoral vein and radial artery, respectively. E<sub>e</sub> is the fractional extraction of epinephrine, measured across the muscle bed, and LPF is the leg plasma flow, determined from leg blood flow and hematocrit.

**KE training.** Following the initial catheter-based studies, subjects returned to the laboratory three times each week for eight weeks to complete supervised KE training of varying intensity for 100 min exercise periods (50 min/leg), as previously described (Richardson *et al.*, 2000).

**Quadriceps Muscle mass.** Utilizing thigh length, circumferences, and skin-fold measurements, thigh volume was calculated to allow an estimate of quadriceps muscle mass, as utilized previously (Jones & Pearson, 1969; Andersen *et al.*, 1985)

**Muscle biopsy.** All biopsies were taken from the *vastus lateralis* muscle at an approximate depth of 3-5 cm, 15 cm proximal to the knee and slightly distal to the ventral midline of the muscle using a Bergstrom needle, as described previously (Bergstrom, 1975). The muscle samples from each biopsy were either immediately frozen in liquid nitrogen and stored at -80°C for subsequent histochemical analysis or immersion-fixed in glutaraldehyde fixative and later processed for electron microscopy and morphometry.

**Histochemistry.** Eight  $\mu\text{m}$ -thick transverse sections were cut at  $-24^{\circ}\text{C}$  on a cryostat (Jung-Reichert Cryocut 1800) and stained for fiber types I and II, and capillaries (Rosenblatt *et al.*, 1987).

**Electron microscopy.** The glutaraldehyde-fixed samples were processed for electron microscopy, as described previously (Mathieu-Costello, 1987).

**Morphometry.** The relative cross-sectional area and number of type I and type II fibers was estimated by point-counting on a light microscope (250x) using an eyepiece square grid test A100 (Weibel, 1979). Capillary density (i.e. capillary number per fiber cross-sectional area), capillary-to-fiber ratio (i.e. capillary number per fiber number), number of capillaries around a fiber ( $N_{\text{CAF}}$ ) and fiber cross-sectional area were measured by point-counting with a light microscope (400x). The volume density of mitochondria per volume of muscle fiber was estimated by point-counting (490x).

**VEGF mRNA levels.** The relative VEGF mRNA levels in skeletal muscle from healthy controls and patients with HFrEF was measured by competitive reverse transcriptase polymerase chain reaction (RT-PCR) analysis according to the method of Zachar (Zachar *et al.*, 1993). The gel was quantified by computer densitometry (GelPro Analyzer, Media Cybernetics, Silver Springs, MD). VEGF mRNA signals were then expressed as the ratio of VEGF target DNA/internal standard in arbitrary units (AU). It should be noted that VEGF protein expression was not assessed as it is well accepted that VEGF undergoes pretranslational regulation (Gustafsson & Kraus, 2001) and also because of the differing time course of VEGF mRNA and VEGF protein expression (1 vs 4 hrs, respectively (Gustafsson & Kraus, 2001), that would have required an additional biopsy, which was not performed for ethical reasons.

**Statistical analysis.** Subject characteristics for patients with HFrEF and healthy controls were

compared using unpaired Student t-tests. Variables assessed in the rested state and following acute exercise were assessed by ANOVA. Differences between groups and conditions were then identified using a Newman-Keuls post-hoc analysis. Despite the relatively small sample size, a priori power calculations, based upon our previously published assessments of the acute VEGF mRNA response to exercise and muscle morphometry (Richardson *et al.*, 1999; Richardson *et al.*, 2000; Esposito *et al.*, 2010a), revealed adequate power (> 0.8) in the major variables of interest. Statistical significance was accepted if  $P < 0.05$ . All data are reported as mean  $\pm$  standard error.

## RESULTS

**Subjects Characteristics:** Prior to exercise training, the only statistically significant different anthropometric characteristic between the patients and controls was body mass, which was higher in the patients with HFrEF. Post-training, body mass was unchanged while quadriceps muscle mass was 15% greater (Table 1).

**Exercise Training Compliance:** The individualized approach to the exercise training with supervision in the laboratory resulted in a 98% compliance to the prescribed exercise regimen. Due to sickness, a single patient with HFrEF did not complete the post training assessments and so was excluded from all analyses.

**Maximal bike and KE pre- and post-KE training:** Cycle exercise  $WR_{max}$  and pulmonary  $VO_{2peak}$  were significantly lower in the patients with HFrEF compared to the controls pre training, however, after training, both  $WR_{max}$  and pulmonary  $VO_{2peak}$  increased to equal that of the (untrained) controls (Table 1). Knee-extensor maximum was also attenuated in the patients with HFrEF, but, as a consequence of the KE training, KE  $WR_{max}$  increased to equal that of the control subjects. Therefore, the initial 50% of KE  $WR_{max}$ , employed as the standard exercise stimulus pre training was, in absolute terms, lower than that for controls, but after training this relative exercise intensity was equal to the controls in both absolute and relative terms (Table 2).

**Muscle metabolic and vascular response to acute submaximal exercise:** As the 50% of  $WR_{max}$  exercise stimulus was, pre exercise training, in absolute terms, lower for the patients with HFrEF, muscle blood flow and muscle  $VO_2$  were lower than that exhibited by controls. Interestingly, prior to the exercise training, patients with HFrEF exhibited a  $\approx 15\%$  higher LVR than the controls which, following the exercise training, was reduced to a level that was not different from the controls (figure 1). Furthermore, a reciprocal change in Ne spillover was documented as a consequence of the exercise training (figure 1). Additional  $O_2$  transport and

metabolic data are presented in Table 2.

**Muscle capillarity and VEGF mRNA:** Prior to exercise training there was no significant difference in capillary-to-fiber ratio or number of capillaries around a fiber between patients and controls (figure 2). As a consequence of training, the patients exhibited a significant increase in capillary-to-fiber ratio and the number of capillaries around a fiber (figure 2). Representative VEGF mRNA gels for patients with HFrEF and controls are presented with the quantification of these signals in Figure 2. In the rested leg VEGF mRNA levels were not different in the controls and the patients with HFrEF both pre and post exercise training ( $0.33 \pm 0.04$ ,  $0.39 \pm 0.03$ , and  $0.38 \pm 0.04$  AU, respectively;  $P > 0.05$ ) (figure 2). One hour following acute knee-extensor exercise, both the controls and the patients with HFrEF pre exercise training exhibited a significant increase in VEGF mRNA levels in the vastus lateralis muscle ( $0.78 \pm 0.07$  and  $0.71 \pm 0.07$  AU in controls and HFrEF pre, respectively; figure 2). This increase in VEGF mRNA abundance, as a consequence of acute exercise, was not different in both the patients with HFrEF and controls. Consequently, the VEGF mRNA exercise-to-rest ratio was not different between controls and patients with HFrEF pre training ( $2.5 \pm 0.5$ , and  $1.8 \pm 0.4$  AU, respectively; figure 2). In contrast, in the trained state, the acute exercise-induced change in VEGF mRNA abundance in the patients with HFrEF ( $0.49 \pm 0.04$  AU) was not significantly different from rest (figure 2) and the VEGF mRNA exercise-to-rest ratio was attenuated compared to the controls and patients pre training ( $1.3 \pm 0.4$  AU; figure 2).

**Capillary density, fiber type, and fiber area:** Prior to exercise training, muscle structural characteristics for both patients with HFrEF and controls were very similar, with no significant difference in capillary density, % area of type I and II fibers, fiber cross sectional area, between patients and controls (figure 3). Interestingly, capillarity, in this case assessed as capillary density, was unchanged by the exercise training performed by the patients with HFrEF, likely because of concomitant muscle fiber changes. Specifically, the % area of type I fibers was significantly increased, while the % area of type II fibers was reduced. Furthermore, the

exercise training induced an increase in fiber cross sectional area.

**Mitochondrial volume density and capillarity:** Prior to exercise training, mitochondrial volume density was significantly lower (22%,  $P < 0.05$ ) in the patients with HFrEF compared to the controls (figure 4). As a result of the exercise training, the patients exhibited a significant increase in mitochondrial volume density. This increase in mitochondrial volume density was such that, post training, there was no longer a difference between patients and controls (figure 4). Interestingly, as illustrated in the inlay in figure 4, there was a significant positive relationship between capillarity, assessed as  $N_{CAF}$ , and mitochondrial volume density when these variables were considered pre and post exercise training in the patients with HFrEF.



## DISCUSSION

To better understand exercise-induced skeletal muscle structural and functional plasticity in patients with HFrEF, this study sought to assess the response to acute submaximal exercise in such patients both before and after KE training and compare them with well-matched controls. This approach yielded several interesting findings. In terms of function, prior to exercise training, assessed at 50% of  $WR_{max}$ , the patients with HFrEF exhibited higher LVR and much greater Ne spillover. Skeletal muscle structure, with the exception of mitochondrial volume density that was significantly lower in the patients with HFrEF compared to the controls, including measures of capillarity and fiber type, was not different between the two groups. Resting and acute exercise-induced VEGF mRNA levels were not different between patients and controls. Post exercise training the patients no longer exhibited elevated LVR and Ne spillover was reduced. Skeletal muscle capillarity increased after exercise training, as assessed by capillary-to-fiber ratio and  $N_{CAF}$ , and VEGF mRNA was no longer increased by the acute exercise stimulus. Muscle fiber cross sectional area was increased by the training and there was an augmented % area of type I fibers and fall in the area of the type II fibers. Finally, exercise training restored mitochondrial volume density, exceeding that of the controls. Collectively, these data provide evidence of structural and functional plasticity and appropriate angiogenic signaling in response to acute exercise in the skeletal muscle of patients with HFrEF.

**Muscle metabolic and vascular response to acute exercise:** A unique component of this study was that the physiological response to the acute KE stimulus was well documented in terms of metabolism and hemodynamics (table 2). An initial important observation was that, pre training, maximal WR and leg  $VO_2$  were significantly lower in the patients with HFrEF than the well-matched controls. Indeed, guided by the concept that training-induced adaptations are predominantly stimulated by relative and not absolute WR, the pre training selection of 50 % of  $WR_{max}$  in both groups resulted in this lower metabolic and vascular challenge for the patients than the controls. Specifically, pre training leg muscle blood flow,  $QO_2$ , and leg  $VO_2$  at 50% of

$WR_{max}$  were 16%, 13%, and 12% lower, respectively, in the patients with HFrEF. However, post training, due to the improvement in maximal exercise capacity of the patients with HFrEF, there was no longer a difference in the absolute WR that equated to 50% of  $WR_{max}$  and, therefore, the metabolic and vascular stress imposed by the acute submaximal exercise was no longer lower than that of the controls and the response of the patients could now be, legitimately, compared to that of the controls.

Neurohumoral activation, including increased sympathetic nervous system activity (SNA), is a hallmark characteristic of advanced HFrEF (Esposito *et al.*, 2010b; Kaur *et al.*, 2017) and, typically, those patients with the greatest SNA have the poorest chance of survival (Cohn *et al.*, 1984). So strong is this link, almost every pharmacologic intervention proven to increase survival in HFrEF interrupts this increase in SNA (Packer *et al.*, 2001). Exercise training, on the other hand, has clear beneficial effects, but data regarding the effect of exercise training on SNA in HFrEF have been equivocal. For example direct SNA measurements with microneurography revealed a reduction in resting SNA in patients with HFrEF following exercise training (Roveda *et al.*, 2003). In contrast, a study, more similar to the current work (Gordon *et al.*, 1997), evaluated patients with HFrEF before and after 8 weeks of two-legged KE training, and although documenting many benefits of chronic exercise in this population, training did not alter resting Ne levels. The current study supports the latter pre training findings, with evidence of elevated Ne spillover and greater LVR in the patients with HFrEF. Of note, the elevated LVR was reversed by KE training and accompanied by a fall in Ne spillover (figure 1). Thus, in contrast to our previous study focused upon maximal exercise (Esposito *et al.*, 2011), the current data support a link between the benefits of exercise training and a reduction in SNA at a submaximal exercise intensity in patients with HFrEF. It is, of course, plausible that other exercise training-induced vascular adaptations, such as improved vascular endothelial function, contributed to the reduction in vascular resistance in the patients with HFrEF, but this is beyond the scope of the current study.

**Skeletal muscle vasculature and VEGF:** Prior to training, both the patients with HFrEF and the controls muscle vascular characteristics were very similar, with no significant differences in capillary-to-fiber ratio or  $N_{CAF}$  or capillary density. As a consequence of training, the patients exhibited a significant increase in both capillary-to-fiber ratio and  $N_{CAF}$ , but, ultimately, there was still no difference between groups in terms of these indices of capillarity (figure 2). In the current study and in our previous work (Esposito *et al.*, 2010a), the VEGF mRNA response to acute exercise in the untrained patients with HFrEF was not different from the healthy controls (figure 2). As VEGF functions as a direct angiogenic factor with a high specificity for vascular endothelial cells (Neufeld *et al.*, 1999; Wahl *et al.*, 2014) these findings are in line with the well-accepted critical role of VEGF in the formation of new blood vessels in human skeletal muscle, including patients with HFrEF, in response to exercise (Gustafsson *et al.*, 2001; Esposito *et al.*, 2010a).

Angiogenesis is an essential adaptive response in skeletal muscle to chronic exercise (i.e. training) resulting in an increase in the number of capillaries per muscle fiber that enhances  $O_2$  transport conductance between the microcirculation and mitochondria (Gustafsson *et al.*, 2001; Esposito *et al.*, 2010a). The present data extend the link between VEGF and capillary proliferation to include the observation that following significant adaptations to exercise training, including angiogenesis, the previously large VEGF mRNA response to acute exercise in patients with HFrEF is significantly attenuated (figures 2). This finding adds credence to the concept that VEGF is important in the initial phase of exercise adaptation in patients with HFrEF, but when significant angiogenesis has occurred, due to training, the need and importance of this mechanism becomes significantly reduced. It should be noted that although VEGF protein levels were not assessed in the current study, previous findings have documented proportional changes in VEGF mRNA and protein expression in this population (Gustafsson *et al.*, 2001). Collectively, these data document the exciting and clinically relevant observation that the capacity of acute exercise to initiate the process of new capillary growth and the maintenance of existing vessels (i.e. increasing VEGF mRNA levels) is still intact in the skeletal muscle of patients with HFrEF.

**Skeletal muscle mass and fiber type plasticity:** Although HFrEF is often associated with muscle atrophy, this was not the case in the current patients in comparison to controls, despite the patients being predominantly defined as NYHA Class III. Indeed, likely due to the greater body mass in the patients, muscle mass actually tended to be greater in the patient group prior to KE training (Table 1). Following the 8 weeks of KE training, this initial tendency was translated into a significantly greater muscle mass in the patients compared to the, untrained, controls (Table 1). HFrEF is often associated with not only muscle atrophy and skeletal muscle dysfunction, but also with a shift in fiber types from “slow”, aerobic, fatigue-resistant (myosin heavy chain I, MHC1) to the “fast” more fatigable fibers (MHC2a and MHC2x) (Mancini *et al.*, 1992; Narumi *et al.*, 2015). Although not statistically different, in this regard, from controls, the current patients with HFrEF were no exception to this theme demonstrating a strong trend to a reduced % area of type I and increased % area of type II fibers in comparison to the controls (figure 4). Of note, considerable effort was made to find control subjects who exhibited not only similar age and anthropometric characteristics, but also a similar level of daily physical activity as the patients with HFrEF (i.e. relatively inactive) as this, in and of itself, may influence muscle structure. In agreement with this concept, KE training significantly increased the % of type I and decreased the % of type II fibers, with the patients, post training, now exhibiting fiber type proportions that were now not different to the controls (figure 4).

**Skeletal muscle mitochondrial volume density:** Interestingly, the only morphometric difference pre training between patients with HFrEF and controls was at the subcellular level with mitochondrial volume density in HFrEF patients being 22% lower than in controls (figure 4). Of further note, and germane to the current focus on the vasculature, when training-induced mitochondrial biogenesis restored mitochondrial volume density to levels in the patients with HFrEF to that of the controls, pre and post training mitochondrial volume densities were significantly correlated with pre and post  $N_{CAF}$  values (figure 4, inlay). This finding reinforces the

likely teleological link between the skeletal muscle metabolic machinery and the vasculature, and implies that this important relationship is still intact in patients with HFrEF (Poole & Mathieu-Costello, 1996).

**Perspective:** The exercise-induced skeletal muscle structural and functional improvements in patients with HFrEF, documented herein, provide evidence of significant peripheral vascular and metabolic plasticity in this population that can be developed with small muscle mass training and then harnessed to benefit whole body exercise capacity. Additionally, these findings highlight the intact nature of skeletal muscle specific processes such as angiogenesis and mitochondrial biogenesis in patients with HFrEF allowing the contributions of these peripheral factors to be targeted, perhaps guiding clinical interventions in the future.

### **Translational Perspectives**

This study has documented skeletal muscle structural and functional plasticity and appropriate angiogenic signaling in response to acute submaximal exercise in patients with HFrEF. In practical terms, small muscle mass exercise training appears to hold significant promise in terms of promoting peripheral adaptation in the apparently still responsive skeletal muscle of patients with HFrEF. These observations may contribute to a more focused, evidence-based non-pharmacological component of HFrEF treatment by physicians. The same strategical approach could be proposed also to other pathological scenarios, such as chronic obstructive pulmonary disease, where the central component of oxygen delivery is compromised but their disuse-induced peripheral maladaptation at the skeletal muscle level could be reversed by small muscle mass physical training.

### **ACKNOWLEDGMENTS**

The authors thank all participants for their committed involvement in this study.

### **CONFLICT OF INTERESTS**

None declared.

## **AUTHOR CONTRIBUTIONS**

FE: conception and design of the work, acquisition, analysis and interpretation of data for the work, and drafting the work;

OMC: conception and design of the work, analysis and interpretation of data, and critical revision for important intellectual content;

PDW: conception and design of the work, analysis and interpretation of data, and critical revision for important intellectual content;

RSR: conception and design of the work, acquisition, analysis and interpretation of data for the work, and critical revision of it for important intellectual content.

We state the following:

- All authors approved the final version of the manuscript;
- All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved;
- all persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

## REFERENCES

- Andersen P, Adams RP, Sjogaard G, Thorboe A & Saltin B. (1985). Dynamic knee extension as model for study of isolated exercising muscle in humans. *J Appl Physiol (1985)* **59**, 1647-1653.
- Barrett-O'Keefe Z, Lee JF, Berbert A, Witman MA, Nativi-Nicolau J, Stehlik J, Richardson RS & Wray DW. (2014). Hemodynamic responses to small muscle mass exercise in heart failure patients with reduced ejection fraction. *Am J Physiol Heart Circ Physiol* **307**, H1512-1520.
- Bergstrom J. (1975). Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scand J Clin Lab Invest* **35**, 609-616.
- Brodal P, Ingjer F & Hermansen L. (1977). Capillary supply of skeletal muscle fibers in untrained and endurance-trained men. *Am J Physiol* **232**, H705-712.
- Cohn JN, Levine TB, Olivari MT, Garberg V, Lura D, Francis GS, Simon AB & Rector T. (1984). Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. *New England J Med* **311**, 819-823.
- Duscha BD, Kraus WE, Keteyian SJ, Sullivan MJ, Green HJ, Schachat FH, Phippen AM, Brawner CA, Blank JM & Annex BH. (1999). Capillary density of skeletal muscle: a contributing mechanism for exercise intolerance in class II-III chronic heart failure independent of other peripheral alterations. *J Am Coll Cardiol* **33**, 1956-1963.

Esposito F, Mathieu-Costello O, Entin PL, Wagner PD & Richardson RS. (2010a). The skeletal muscle VEGF mRNA response to acute exercise in patients with chronic heart failure. *Growth Factors* **28**, 139-147.

Esposito F, Mathieu-Costello O, Shabetai R, Wagner PD & Richardson RS. (2010b). Limited maximal exercise capacity in patients with chronic heart failure: partitioning the contributors. *J Am Coll Cardiol* **55**, 1945-1954.

Esposito F, Reese V, Shabetai R, Wagner PD & Richardson RS. (2011). Isolated quadriceps training increases maximal exercise capacity in chronic heart failure: the role of skeletal muscle convective and diffusive oxygen transport. *J Am Coll Cardiol* **58**, 1353-1362.

Esposito F, Wagner PD & Richardson RS. (2015). Incremental large and small muscle mass exercise in patients with heart failure: evidence of preserved peripheral haemodynamics and metabolism. *Acta Physiol (Oxf)* **213**, 688-699.

Gordon A, Tyni-Lenne R, Jansson E, Kaijser L, Theodorsson-Norheim E & Sylven C. (1997). Improved ventilation and decreased sympathetic stress in chronic heart failure patients following local endurance training with leg muscles. *J Cardiac Fail* **3**, 3-12.

Gustafsson T, Bodin K, Sylven C, Gordon A, Tyni-Lenne R & Jansson E. (2001). Increased expression of VEGF following exercise training in patients with heart failure. *Eur J Clin Invest* **31**, 362-366.



Gustafsson T & Kraus WE. (2001). Exercise-induced angiogenesis-related growth and transcription factors in skeletal muscle, and their modification in muscle pathology. *Front Biosci* **6**, D75-89.

Gustafsson T, Puntchart A, Kaijser L, Jansson E & Sundberg CJ. (1999). Exercise-induced expression of angiogenesis-related transcription and growth factors in human skeletal muscle. *Am J Physiol* **276**, H679-685.

Hambrecht R, Fiehn E, Weigl C, Gielen S, Hamann C, Kaiser R, Yu J, Adams V, Niebauer J & Schuler G. (1998). Regular physical exercise corrects endothelial dysfunction and improves exercise capacity in patients with chronic heart failure.[see comment]. *Circulation* **98**, 2709-2715.

Hang J, Kong L, Gu JW & Adair TH. (1995). VEGF gene expression is upregulated in electrically stimulated rat skeletal muscle. *Am J Physiol* **269**, H1827-1831.

Harrington D, Anker SD, Chua TP, Webb-Peploe KM, Ponikowski PP, Poole-Wilson PA & Coats AJ. (1997). Skeletal muscle function and its relation to exercise tolerance in chronic heart failure. *J Am Coll Cardiol* **30**, 1758-1764.

Hollriegel R, Winzer EB, Linke A, Adams V, Mangner N, Sandri M, Bowen TS, Hambrecht R, Schuler G & Erbs S. (2016). Long-Term Exercise Training in Patients With Advanced Chronic Heart Failure: SUSTAINED BENEFITS ON LEFT VENTRICULAR PERFORMANCE AND EXERCISE CAPACITY. *J Cardiopulm Rehabil Prev* **36**, 117-

Huey KA, Smith SA, Sulaeman A & Breen EC. (2016). Skeletal myofiber VEGF is necessary for myogenic and contractile adaptations to functional overload of the plantaris in adult mice. *J Appl Physiol (1985)* **120**, 188-195.

Jones PR & Pearson J. (1969). Anthropometric determination of leg fat and muscle plus bone volumes in young male and female adults. *J Physiol* **204**, 63P-66P.

Kaur J, Senador D, Krishnan AC, Hanna HW, Alvarez A, Machado TM & O'Leary DS. (2017). Muscle Metaboreflex-Induced Vasoconstriction in the Ischemic Active Muscle is Exaggerated in Heart Failure. *Am J Physiol Heart Circ Physiol*, ajpheart 00375 02017.

Lawrenson L, Hoff J & Richardson RS. (2004). Aging attenuates vascular and metabolic plasticity but does not limit improvement in muscle VO<sub>2</sub> max. *Am J Physiol - Heart Circ Physiol* **286**, H1565-1572.

Lawrenson L, Poole JG, Kim J, Brown C, Patel P & Richardson RS. (2003). Vascular and metabolic response to isolated small muscle mass exercise: effect of age. *Am J Physiol - Heart Circ Physiol* **285**, H1023-1031.

Leung DW, Cachianes G, Kuang WJ, Goeddel DV & Ferrara N. (1989). Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* **246**, 1306-1309.

Magnusson G, Gordon A, Kaijser L, Sylvén C, Isberg B, Karpakka J & Saltin B. (1996). High intensity knee extensor training, in patients with chronic heart failure. Major skeletal muscle improvement. *Eur Heart J* **17**, 1048-1055.

Magnusson G, Kaijser L, Sylvén C, Karlberg KE, Isberg B & Saltin B. (1997). Peak skeletal muscle perfusion is maintained in patients with chronic heart failure when only a small muscle mass is exercised. *Cardiovasc Res* **33**, 297-306.

Mancini DM, Walter G, Reichel N, Lenkinski R, McCully KK, Mullen JL & Wilson JR. (1992). Contribution of Skeletal Muscle Atrophy to Exercise Intolerance and Altered Muscle Metabolism in Heart Failure. *Circulation* **85**, 1364-1373.

Mathieu-Costello O. (1987). Capillary tortuosity and degree of contraction or extension of skeletal muscles. *Microvasc Res* **33**, 98-117.

Narumi T, Watanabe T, Kadowaki S, Takahashi T, Yokoyama M, Kinoshita D, Honda Y, Funayama A, Nishiyama S, Takahashi H, Arimoto T, Shishido T, Miyamoto T & Kubota I. (2015). Sarcopenia evaluated by fat-free mass index is an important prognostic factor in patients with chronic heart failure. *Eur J Intern Med* **26**, 118-122.

Neufeld G, Cohen T, Gengrinovitch S & Poltorak Z. (1999). Vascular endothelial growth factor (VEGF) and its receptors. *Faseb J* **13**, 9-22.

Olfert IM, Howlett RA, Tang K, Dalton ND, Gu Y, Peterson KL, Wagner PD & Breen EC. (2009). Muscle-specific VEGF deficiency greatly reduces exercise endurance in

mice. *J Physiol* **587**, 1755-1767.

Packer M, Coats AJ, Fowler MB, Katus HA, Krum H, Mohacsi P, Rouleau JL, Tendera M, Castaigne A, Roecker EB, Schultz MK & DeMets DL. (2001). Effect of carvedilol on survival in severe chronic heart failure. *New England J Med* **344**, 1651-1658.

Piepoli MF, Davos C, Francis DP, Coats AJ & ExTra MC. (2004). Exercise training meta-analysis of trials in patients with chronic heart failure (ExTraMATCH).[see comment]. *BMJ* **328**, 189.

Piepoli MF, Guazzi M, Boriani G, Cicoira M, Corra U, Dalla Libera L, Emdin M, Mele D, Passino C, Vescovo G, Vigorito C, Villani G, Agostoni P, Working Group 'Exercise Physiology SC & Cardiac Rehabilitation ISoC. (2010a). Exercise intolerance in chronic heart failure: mechanisms and therapies. Part II. *Eur J Cardiovasc Prev Rehabil* **17**, 643-648.

Piepoli MF, Guazzi M, Boriani G, Cicoira M, Corra U, Dalla Libera L, Emdin M, Mele D, Passino C, Vescovo G, Vigorito C, Villani GQ, Agostoni P, Working Group 'Exercise Physiology SC & Cardiac Rehabilitation ISoC. (2010b). Exercise intolerance in chronic heart failure: mechanisms and therapies. Part I. *Eur J Cardiovasc Prev Rehabil* **17**, 637-642.

Poole DC, Hirai DM, Copp SW & Musch TI. (2012). Muscle oxygen transport and utilization in heart failure: implications for exercise (in)tolerance. *Am J Physiol Heart Circ Physiol* **302**, H1050-1063.

Poole DC & Mathieu-Costello O. (1996). Relationship between fiber capillarization and mitochondrial volume density in control and trained rat soleus and plantaris muscles. *Microcirculation* **3**, 175-186.

Poole DC, Richardson RS, Haykowsky MJ, Hirai DM & Musch TI. (2017). Exercise Limitations in Heart Failure with Reduced and Preserved Ejection Fraction. *J Appl Physiol* (1985), jap 00747 02017.

Richardson RS, Knight DR, Poole DC, Kurdak SS, Hogan MC, Grassi B & Wagner PD. (1995). Determinants of maximal exercise VO<sub>2</sub> during single leg knee-extensor exercise in humans. *Am J Physiol* **268**, H1453-1461.

Richardson RS, Poole DC, Knight DR, Kurdak SS, Hogan MC, Grassi B, Johnson EC, Kendrick KF, Erickson BK & Wagner PD. (1993). High muscle blood flow in man: is maximal O<sub>2</sub> extraction compromised? *J Appl Physiol* **75**, 1911-1916.

Richardson RS, Wagner H, Mudaliar SR, Henry R, Noyszewski EA & Wagner PD. (1999). Human VEGF gene expression in skeletal muscle: effect of acute normoxic and hypoxic exercise. *Am J Physiol* **277**, H2247-2252.

Richardson RS, Wagner H, Mudaliar SR, Saucedo E, Henry R & Wagner PD. (2000). Exercise adaptation attenuates VEGF gene expression in human skeletal muscle. *Am J Physiol Heart - Circ Physiol* **279**, H772-778.

- Rosenblatt JD, Kuzon WM, Jr., Pyley MJ, Pynn BR & McKee NH. (1987). A histochemical method for the simultaneous demonstration of capillaries and fiber type in skeletal muscle. *Stain Technol* **62**, 85-92.
- Roveda F, Middlekauff HR, Rondon MU, Reis SF, Souza M, Nastari L, Barretto AC, Krieger EM & Negrao CE. (2003). The effects of exercise training on sympathetic neural activation in advanced heart failure: a randomized controlled trial. *J Am Coll Cardiol* **42**, 854-860.
- Savard GK, Richter EA, Strange S, Kiens B, Christensen NJ & Saltin B. (1989). Norepinephrine spillover from skeletal muscle during exercise: role of muscle mass. *Am J Physiol* **257**, H1812-H1818.
- Tyni-Lenne R, Dencker K, Gordon A, Jansson E & Sylven C. (2001). Comprehensive local muscle training increases aerobic working capacity and quality of life and decreases neurohormonal activation in patients with chronic heart failure. *Eur J Heart Fail* **3**, 47-52.
- Wadley GD, Lee-Young RS, Canny BJ, Wasuntarawat C, Chen ZP, Hargreaves M, Kemp BE & McConell GK. (2006). Effect of exercise intensity and hypoxia on skeletal muscle AMPK signaling and substrate metabolism in humans. *Am J Physiol Endocrinol Metab* **290**, E694-702.
- Wahl P, Jansen F, Achtzehn S, Schmitz T, Bloch W, Mester J & Werner N. (2014). Effects of high intensity training and high volume training on endothelial microparticles and

angiogenic growth factors. *PLoS One* **9**, e96024.

Weibel E. (1979). *Practical methods for biological morphometry*. Academic Press, London-New York-Toronto.

Zachar V, Thomas RA & Goustin AS. (1993). Absolute quantification of target DNA: a simple competitive PCR for efficient analysis of multiple samples. *Nucleic Acids Res* **21**, 2017-2018.

Zumstein A, Mathieu O, Howald H & Hoppeler H. (1983). Morphometric analysis of the capillary supply in skeletal muscles of trained and untrained subjects--its limitations in muscle biopsies. *Pflugers Arch* **397**, 277-283.

**Table 1.** Characteristics of healthy controls and patients with heart failure with reduced ejection fraction (HFrEF) pre and post 8 weeks of knee-extensor exercise training.

	<b>Controls</b>	<b>HFrEF pre</b>	<b>HFrEF post</b>
Age (yrs)	51 ± 8	54 ± 14	54 ± 14
Height (cm)	179 ± 7	182 ± 6	182 ± 6
Body Mass (kg)	90 ± 11	100 ± 4	101 ± 4
Quadriceps muscle mass (kg)	2.4 ± 0.4	2.6 ± 0.1	3.0 ± 0.1**
NYHA class	-	II-III	II-III
KE Maximum Cardiac Output (l/min)	10.9 ± 1.2	9.0 ± 1.2	9.4 ± 1.1
Peak Pulm. Cycle VO <sub>2</sub> (l/min)	2.14 ± 0.10	1.63 ± 0.09 <sup>#</sup>	2.02 ± 0.15*
Peak Pulm. Cycle VO <sub>2</sub> (ml/kg/min)	24.1 ± 1.1	15.3 ± 18.6 <sup>#</sup>	18.6 ± 1.6* <sup>#</sup>
Maximum cycle work rate (W)	148 ± 8	115 ± 13 <sup>#</sup>	141 ± 16*
Cycle Maximum Cardiac Output (l/min)	16.8 ± 0.8	13.6 ± 1.2 <sup>#</sup>	14.3 ± 1.4 <sup>#</sup>
<b>Medications (fraction of users)</b>			
Digoxin	-	5/5	5/5
Diuretics	-	5/5	5/5
Long-acting nitrates	-	3/5	3/5
Statins	-	4/5	4/5
Aspirin	-	3/5	3/5
β-Blockers	-	4/5	4/5
Warfarin	-	2/5	4/5
ACE inhibitors	-	2/5	4/5
Ca <sup>2+</sup> channel blockers	-	2/5	4/5

NYHA, New York Heart Association; KE, knee-extensor; Pulm., pulmonary; VO<sub>2</sub> leg, oxygen uptake; Ca<sup>2+</sup>, calcium. Data are expressed as mean ± SE. \**P*<0.05 post vs pre; <sup>#</sup>*P*<0.05 HFrEF vs controls.



**Table 2.** Vascular and metabolic response to knee-extensor exercise at 50% of maximum work rate ( $WR_{max}$ ) in healthy controls and in patients with heart failure with reduced ejection fraction (HFrEF) pre and post 8 weeks of knee-extensor exercise training.

	<b>Controls</b>	<b>HFrEF pre</b>	<b>HFrEF post</b>
50% $WR_{max}$ (W)	20 ± 3	15 ± 2 <sup>#</sup>	21 ± 3
HR (b/min)	94 ± 3	92 ± 6	90 ± 3
$VO_2$ leg (ml/min)	313 ± 28	275 ± 23.5 <sup>#</sup>	324 ± 17 <sup>*</sup>
Q leg (ml/min)	2450 ± 223	2048 ± 159 <sup>#</sup>	2529 ± 238 <sup>*</sup>
$QO_2$ leg (ml/min)	460 ± 41	401 ± 28 <sup>#</sup>	476 ± 48 <sup>*</sup>
Hb (g/dl)	13.9 ± 0.3	12.7 ± 0.6	13.1 ± 0.5
SaO <sub>2</sub> (%)	96 ± 1	97.1 ± 0.6	97.7 ± 0.4
CaO <sub>2</sub> (ml/100ml)	18.8 ± 0.3	17.3 ± 0.9	18.1 ± 0.8
CvO <sub>2</sub> (ml/100ml)	5.9 ± 0.5	4.4 ± 0.6	5.2 ± 0.9
PaO <sub>2</sub> (mmHg)	99.9 ± 3	95.3 ± 6.2	93.6 ± 3.5
PvO <sub>2</sub> (mmHg)	23.0 ± 1.1	19.6 ± 0.9	20.3 ± 1.2
LVR (mmHg/L/min)	41 ± 6	47 ± 7 <sup>#</sup>	39 ± 6 <sup>*#</sup>
MAP (mmHg)	121 ± 11	110 ± 6	112 ± 7
MAP-MVP (mmHg)	100 ± 12	95 ± 7	95 ± 6
Ne Spillover (nM/min)	0.68 ± 0.2	3.30 ± 0.3 <sup>#</sup>	1.43 ± 0.5 <sup>*#</sup>

$WR_{max}$ , maximum work rate;  $VO_2$  leg, leg oxygen uptake; HR, Heart rate; Q leg, leg blood flow;  $QO_2$  leg, leg oxygen delivery; SaO<sub>2</sub>, arterial O<sub>2</sub> saturation; CaO<sub>2</sub>, arterial O<sub>2</sub> concentration; CvO<sub>2</sub>, venous O<sub>2</sub> concentration; PaO<sub>2</sub>, arterial O<sub>2</sub> partial pressure; PvO<sub>2</sub>, venous O<sub>2</sub> partial pressure; LVR, leg vascular resistance; MAP, mean arterial pressure; MVP, mean venous pressure; Ne, norepinephrine. Data are expressed as mean ± SE; \* $P$ <0.05 (post vs pre); # $P$ <0.05 (HFrEF vs controls).

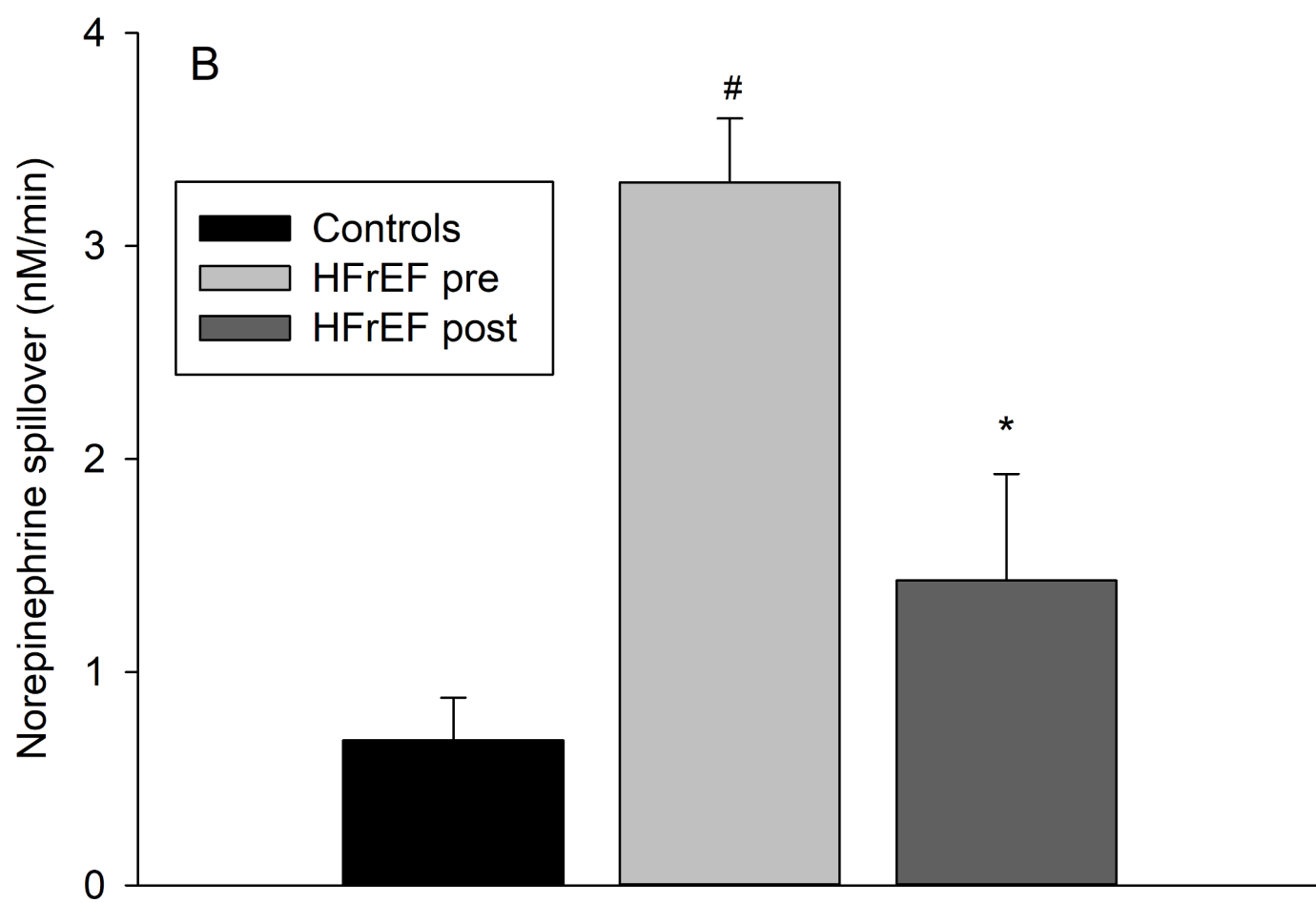
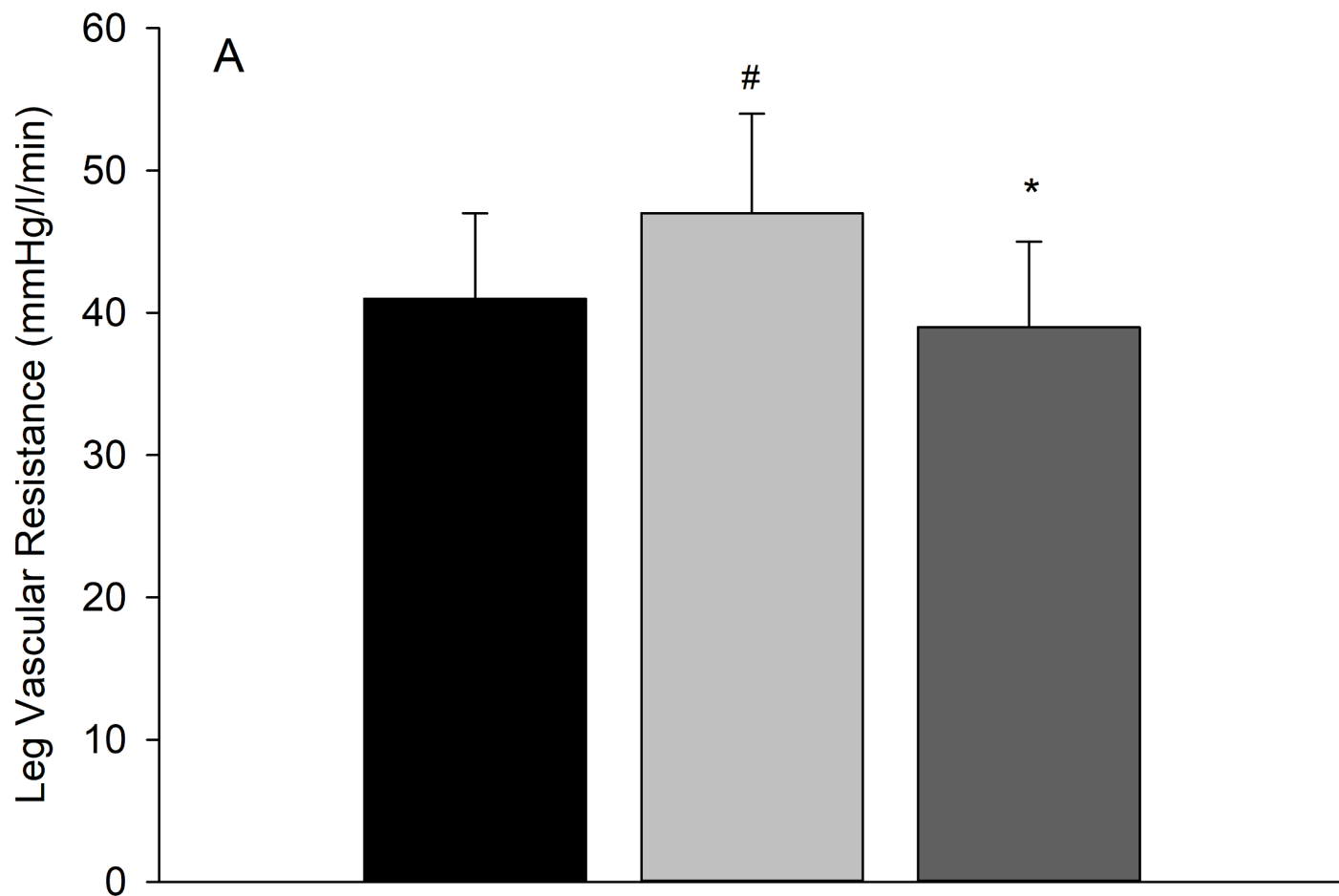
## FIGURE LEGENDS

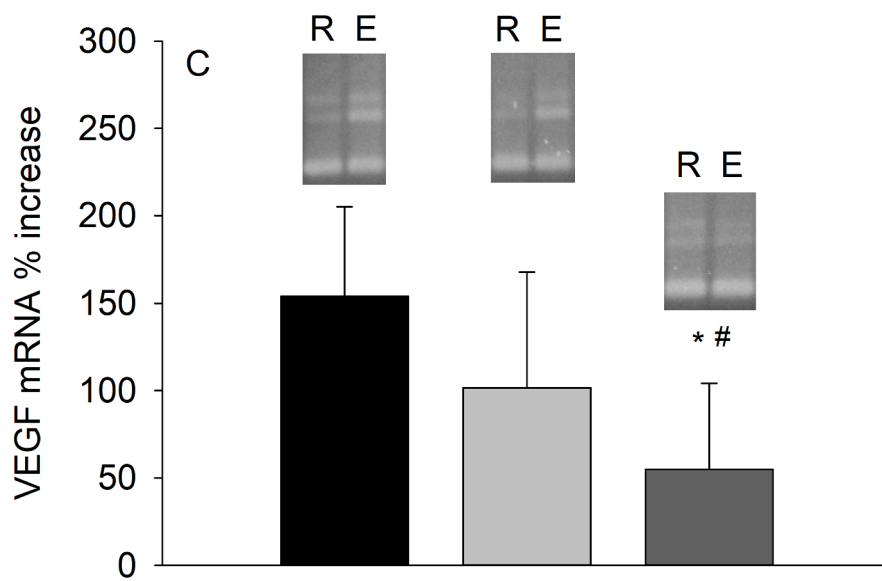
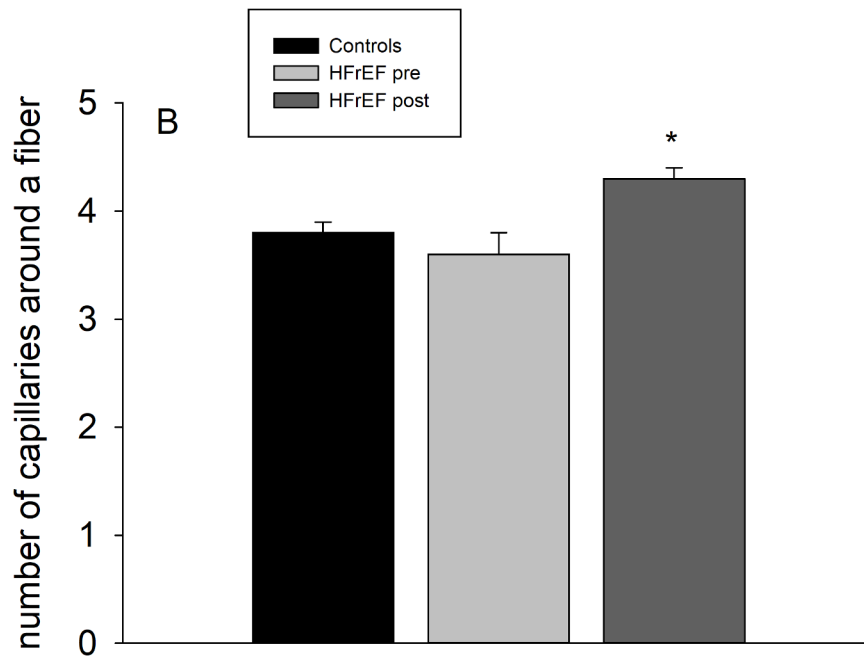
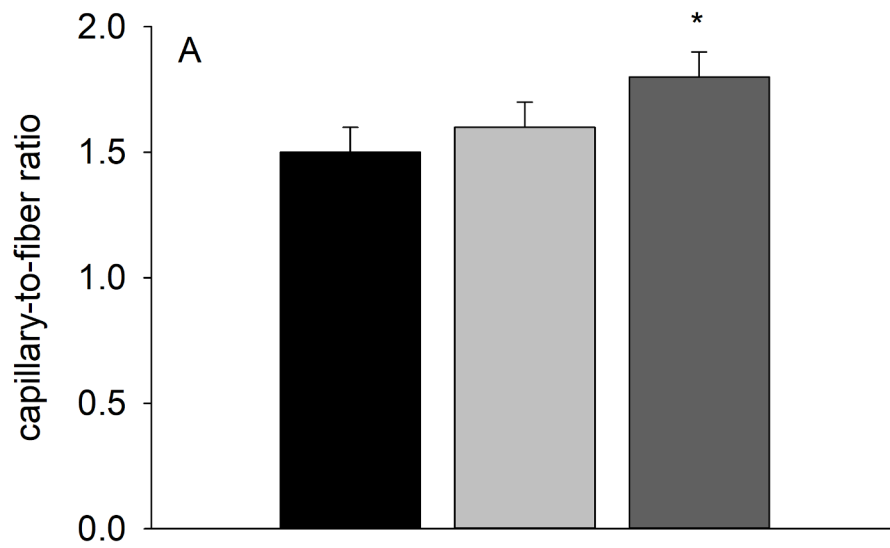
**Figure 1. Leg vascular resistance and norepinephrine spillover across the leg of untrained healthy controls and patients with chronic heart failure with reduced ejection fraction (HFrEF) pre and post knee-extensor exercise (KE) training during KE at 50% of maximal KE capacity. Data illustrated as mean  $\pm$  SE. \*P<0.05 post vs pre; #P<0.05 HFrEF vs controls.**

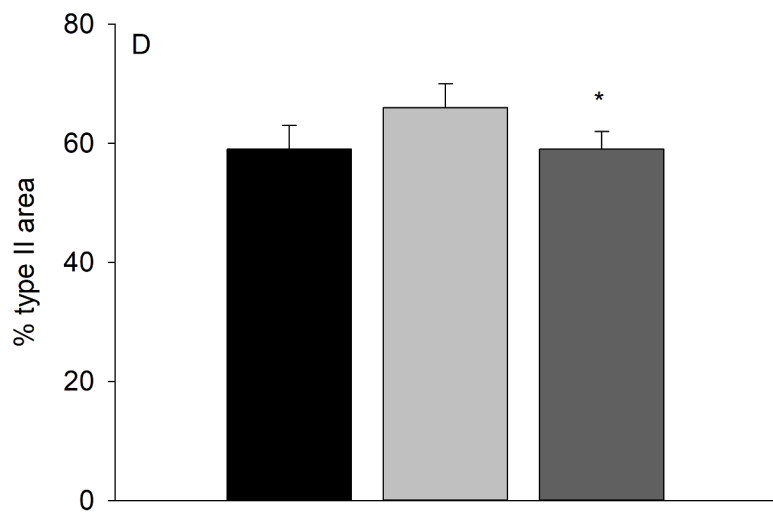
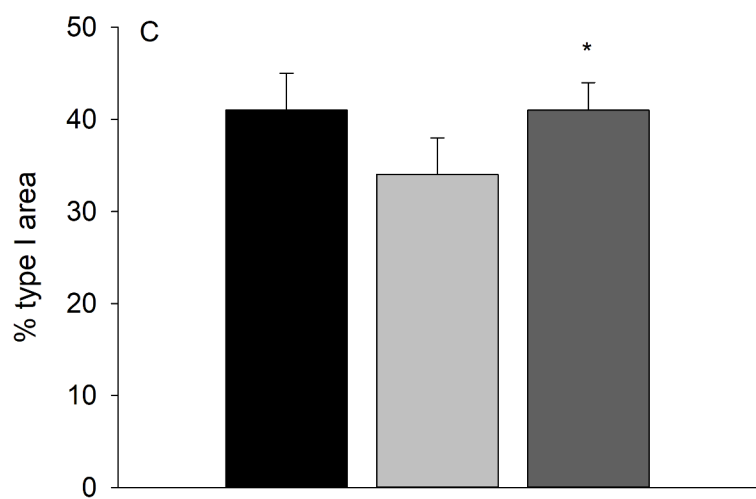
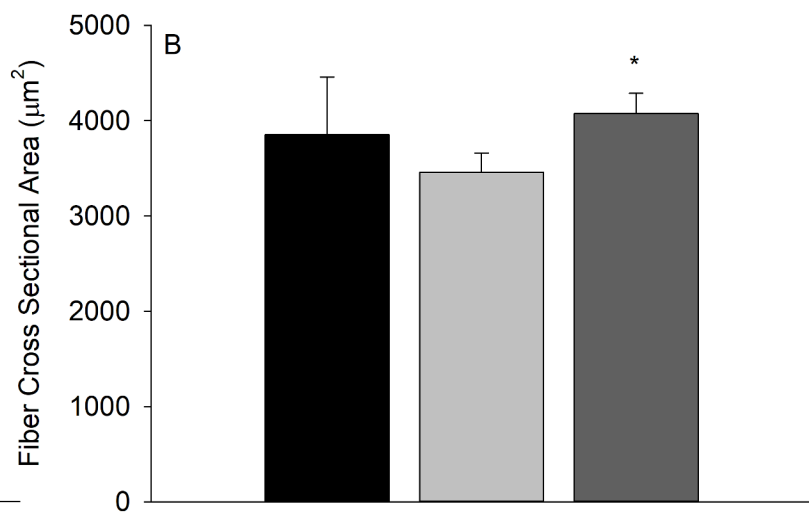
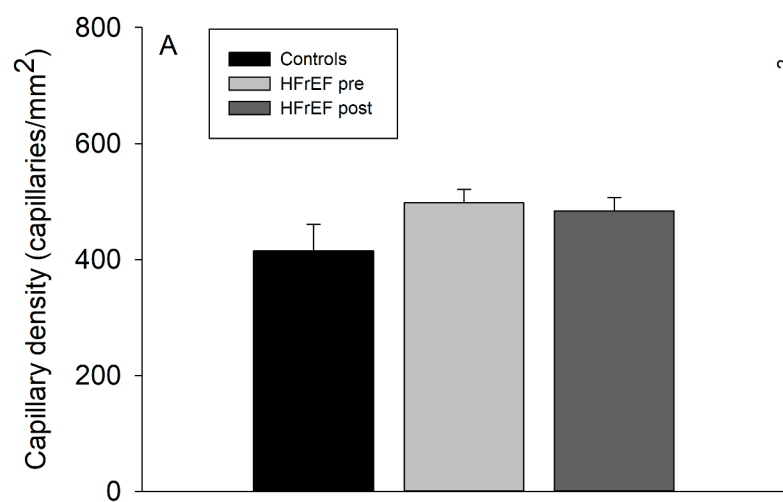
**Figure 2. Skeletal muscle capillary-to-fiber ratio, number of capillaries around a fiber, and the % increase in VEGF mRNA expression following knee-extensor exercise (KE) at 50% of maximal KE work rate in untrained healthy controls and patients with chronic heart failure with reduced ejection fraction (HFrEF) pre and post KE training. Data illustrated as mean  $\pm$  SE. \*P<0.05 post vs pre; #P<0.05 HFrEF vs controls.**

**Figure 3. Skeletal muscle capillary density, fiber cross section area, % area type I fibers, and % area type II fibers in untrained healthy controls and patients with chronic heart failure with reduced ejection fraction (HFrEF) pre and post knee-extensor exercise (KE) training. Data illustrated as mean  $\pm$  SE. \*P<0.05 post vs pre; #P<0.05 HFrEF vs controls.**

**Figure 4. Skeletal muscle mitochondrial volume density in untrained healthy controls and patients with chronic heart failure with reduced ejection fraction (HFrEF) pre and post knee-extensor exercise (KE) training. Inlay illustrates the relationship between capillarity and mitochondrial volume density content pre and post KE training in the patients with HFrEF. Data illustrated as mean  $\pm$  SE. \*P<0.05 post vs pre; #P<0.05 HFrEF vs controls.**







Mitochondrial volume density (%)

