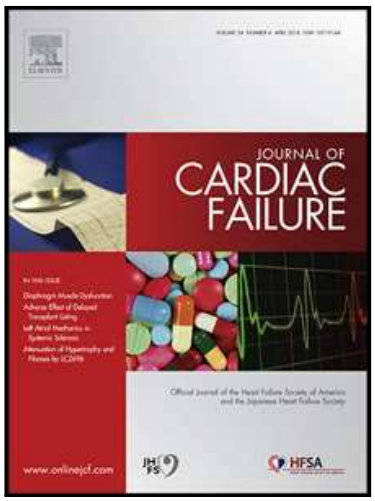
Article:
Bowen, TS, Herz, C, Rolim, NPL et al. (7 more authors) (2018) Effects of endurance training on detrimental structural, cellular, and functional alterations in skeletal muscles of heart failure with preserved ejection fraction. Journal of Cardiac Failure, 24 (9). pp. 603-613. ISSN 1071-9164

https://doi.org/10.1016/j.cardfail.2018.08.009



Effects of endurance training on detrimental structural, cellular, and functional alterations in skeletal muscles of heart failure with preserved ejection fraction

T. Scott Bowen , Christian Herz , Natale P.L. Rolim , Anne-Marie Ormbostad Berre , Martin Halle , Angela Kricke , Axel Linke , Gustavo Justo da Silva , Ulrik Wisloff , Volker Adams

PII: S1071-9164(18)30966-7
DOI: https://doi.org/10.1016/j.cardfail.2018.08.009
Reference: YJCAF 4192

To appear in: *Journal of Cardiac Failure*

Received date: 25 January 2018
Revised date: 4 May 2018
Accepted date: 14 August 2018

Please cite this article as: T. Scott Bowen , Christian Herz , Natale P.L. Rolim , Anne-Marie Ormbostad Berre , Martin Halle , Angela Kricke , Axel Linke , Gustavo Justo da Silva , Ulrik Wisloff , Volker Adams , Effects of endurance training on detrimental structural, cellular, and functional alterations in skeletal muscles of heart failure with preserved ejection fraction, *Journal of Cardiac Failure* (2018), doi: https://doi.org/10.1016/j.cardfail.2018.08.009

Effects of endurance training on detrimental structural, cellular, and functional alterations in skeletal muscles of heart failure with preserved ejection fraction

T. Scott Bowen[1*], Christian Herz[2*], Natale P.L. Rolim[3], Anne-Marie Ormbostad Berre[3], Martin Halle[4], Angela Kricke[2], Axel Linke[5], Gustavo Justo da Silva[3], Ulrik Wisloff[3,6], Volker Adams[5]

School of Biomedical Sciences, University of Leeds, Leeds, United Kingdom[1]; Department of Internal Medicine and Cardiology, Leipzig University-Heart Center, Leipzig, Germany[2]; K.G. Jebsen Center of Exercise in Medicine, Department of Circulation and Medical Imaging, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway[3]; Technische Universität München, Munich Germany[4]; Department of Internal Medicine and Cardiology at the Technische Universität Dresden[5]; *School of Human Movement & Nutrition Sciences, University of Queensland, Australia[6] :*
*Equal Contribution

Running head: Skeletal muscle alterations in HFpEF

Word Count: 2732

Corresponding Author: T. Scott Bowen, PhD

Address: School of Biomedical Sciences, Univ. of Leeds, Leeds, LS2 9JT, United Kingdom,

Email: t.s.bowen@leeds.ac.uk

Telephone: +44 113 343 3834

Abstract

Background: HFpEF is underpinned by detrimental skeletal muscle alterations that contribute to disease severity, yet underlying mechanisms and therapeutic treatments remain poorly established. This study used an animal model of HFpEF to better understand whether skeletal muscle abnormalities were: 1) fiber-type specific; and 2) reversible by various exercise training regimes.

Methods and Results: Lean controls were compared to obese ZSF1 rats at 20 weeks, and 8 weeks later following sedentary, high-intensity interval training, or moderate-continuous treadmill exercise. Oxidative-soleus and glycolytic-extensor digitorum longus (EDL) muscles were assessed for fiber size, capillarity, glycolytic metabolism, autophagy, and contractile function. HFpEF reduced fiber size and capillarity by 20-50% ($P<0.05$) in both soleus and EDL, but these effects were not reversed by endurance training. In contrast, both endurance training regimes in HFpEF attenuated the elevated lactate dehydrogenase activity observed in the soleus. Autophagy was downregulated in EDL and upregulated in soleus ($P<0.05$), with no influence following endurance training. HFpEF impaired contractile forces of both muscles by ~20 % ($P<0.05$) and these were not reversed by training.

Conclusion: Obesity-related HFpEF was associated with detrimental structural, cellular, and functional alterations to both slow-oxidative and fast-glycolytic skeletal muscles that could not be reversed by endurance training.

Word count: 198 of 200

Key words: diastolic dysfunction; exercise training; HFpEF; obesity; soleus: ZSF1

Introduction

The increasing prevalence of heart failure patients with preserve ejection fraction (HFpEF) despite no breakthrough in effective treatments is now recognized as a major unmet problem in cardiovascular medicine [1]. Cardiac-orientated drug interventions in large-scale clinical trials in HFpEF have proven largely ineffective in terms of patient outcomes [2]. This has shifted focus towards possible treatment of “non-cardiac organs”, such as the skeletal muscle, endothelial, and pulmonary systems. Recent studies have now established functional and structural impairments are present in skeletal muscle from both patients and animal models of HFpEF [3-7]. Muscle-specific changes include fiber atrophy, adipose infiltration, oxidative to glycolytic fiber type shift, reduced capillary density, and mitochondrial dysfunction, which have also been closely correlated to the main symptom of exercise intolerance [5-8]. Interestingly, new evidence has confirmed the presence of a specific obesity-related HFpEF patient cohort [9]. Whether skeletal muscle alterations occur in this distinct phenotype is yet to be fully explored. Nevertheless, skeletal muscle maladaptations induced by HFpEF predispose patients towards a rapid depletion of high-energy phosphates and earlier accumulation of fatigue inducing metabolites [10, 11]. Therefore identification of interventions to reverse such processes are a key therapeutic target in cardiovascular medicine.

While evidence remains scarce, numerous skeletal muscle alterations induced by HFpEF can be prevented following exercise training. One study using a hypertensive rat model of HFpEF documented that high-intensity interval training (HIIT) attenuated mitochondrial impairments, which was associated with normalized contractile function and fatigue-resistance [4]. However in this study exercise training was initiated before the onset of HFpEF (i.e. primary intervention) [4], which precluded answering the more clinically-relevant question of whether exercise training reverses muscle impairments in HFpEF.

Further, HFpEF was induced by hypertension alone rather than the multiple co-morbidities (e.g., obesity, diabetes, renal dysfunction, and hypertension) that typically drive the development of this disease in most patients - particular in those with obesity-related HFpEF [9].

This study, therefore, aimed to further characterize the underlying structural, metabolic, and molecular skeletal muscle alterations that occur in obesity-related HFpEF and determine whether endurance training could reverse impairments (i.e. secondary prevention). In this study, we used a recently established cardiometabolic obese-driven rat model of HFpEF [3, 12, 13]. The model closely resembles a typical patient phenotype, where cardiac impairments and exercise intolerance are underpinned by numerous co-morbidities including obesity, type II diabetes, hypertension, and kidney dysfunction [14, 15]. We specifically compared two skeletal muscles with differing metabolic profiles: the oxidative slow-twitch soleus in addition to the glycolytic fast-twitch extensor digitorum longus (EDL), in order to determine whether HFpEF induces fiber-type specific maladaptations.

Methods

Study design and animal model of HFpEF

Procedures and experiments in this study were approved by the Norwegian Animal Research Authority, in accordance the European Directive 2010/63/EU. A full description of the present study design and animal cohort as well as the methods employed were recently described elsewhere [3]. Briefly, obese diabetic Zucker fatty/Spontaneously hypertensive heart failure F1 hybrid (ZSF1) rats (Charles River, Kingston, US) were used as a model to induce HFpEF, which occurs by 20 weeks of age [3, 12, 13]. Non-invasive echocardiography as well as invasive hemodynamics were used to confirm the presence of HFpEF, where left ventricular diastolic impairments and a preserved ejection fraction were confirmed alongside measures of obesity, hypertension, type II diabetes, and

exercise intolerance (as described in detail elsewhere [3]). The first part of the study assessed male obese ZSF1 rats (i.e., HFpEF, n=12) rats at 20 weeks of age compared to their lean strain-matched counterparts (i.e., controls; n=8). In the second part of the study, a further 4 groups were assessed at 28 weeks of age, which included lean rats compared to 3 groups of obese ZSF1 rats that performed either no exercise training (i.e., sedentary; n=13), high-intensity interval training (i.e., HFpEF+HIIT; n=11) or moderate continuous training (i.e., HFpEF+MCT; n=11). Exercise training was performed for 8 weeks (from 20-28 weeks of age), which included HIIT (3 x per week; 4 intervals at 90% peak oxygen uptake ($\dot{V}O_{2peak}$) for 4 min, with 3 min recovery at 60% $\dot{V}O_{2peak}$) or MCT (5 x per week at 60% $\dot{V}O_{2peak}$ for 1 h) for a total of 8 weeks on a treadmill at a gradient of 25°, as previously described [3].

Muscle function

The right EDL and soleus were dissected and mounted vertically mounted in a buffer-filled organ bath between a hook and force transducer (FORT250, World Precision Instruments Inc., Berlin, Germany), with the output continuously recorded and digitized (PowerLab 8/30, ADInstruments, Oxford, UK). *In vitro* muscle function was assessed by platinum electrodes stimulating the muscle with a supramaximal current (700 mA; 500 ms train duration; 0.25 ms pulse width) from a base stimulator (Grass S88, Grass Technologies, Warwick, USA) amplified via a high power bi-polar stimulator (701C, Aurora Scientific Inc., Aurora, Canada). The muscle bundle was set at an optimal length (L_o) equivalent to the maximal twitch force produced, after which bath temperature was increased to 25℃ and a 15 min thermo-equilibration period followed. A force-frequency protocol was then performed at 1, 15, 30, 50, 80, and 120 Hz respectively, separated with 1 min rest intervals. Following a 5 min rest period muscles then underwent a fatigue protocol over 5 min (40 Hz every 2 s). Forces generated during the fatigue protocol were normalized to the initial force generated, to provide a relative assessment of fatigability.

Tissue analyses

One portion of the left EDL and soleus were frozen immediately in liquid N_2 for biochemical analyses. Frozen muscle samples were subsequently homogenized in lysis buffer (50 mM Tris, 150 mM sodium chloride, 1 mM EDTA, 1% NP-40, 0.25% sodium-deoxycholate, 0.1% SDS, 1% Triton X-100; pH 7.4) containing a protease inhibitor mix (Inhibitor mix M, Serva, Heidelberg, Germany), sonicated, and centrifuged at 16,000 g for 5 min. The supernatant was isolated and protein content determined (BCA assay, Pierce, Bonn, Germany). Muscle homogenates (5 - 20 µg) mixed with loading buffer (126 mM Tris-HCl, 20% glycerol, 4% SDS, 1.0% 2-mercaptoethanol, 0.005% bromophenol blue; pH 6.8) were separated by SDS-polyacrylamide gel electrophoresis for 1.5 h at 90 V. Proteins were transferred to a polyvinylidene fluoride membrane (PVDF) and incubated overnight at 4°C with primary antibodies for the LC3 (1:1000, Novus Biologicals, Cambridge, UK), where the relative ratio of LC3 in its content form of II/I was used as a marker of autophagy. Membranes were subsequently incubated with a horseradish peroxidase-conjugated secondary antibody and specific bands visualized by enzymatic chemiluminescence (Super Signal West Pico, Thermo Fisher Scientific Inc., Bonn, Germany) and densitometry quantified using a 1D scan software package (Scanalytics Inc., Rockville, USA). In addition, enzyme activities for lactate dehydrogenase (LDH) were measured spectrophotometrically as a measure of glycolytic metabolism, as previously described [16]. Protein and enzyme activity data are presented as fold change relative to the respective control group. In addition, a portion of the EDL and soleus fixed in 4% PBS-buffered formalin and paraffin-embedded were used for histological analyses of cross-sectional area (CSA). Briefly, sections of 3 µm were cut, mounted on glass cover slips, and stained for H&E for fiber CSA to be calculated. In addition, sections were stained with an antibody against von Willebrand factor (vWF; 1/500, Santa Cruz) for capillaries to be visualised, with the capillary to fiber ratio subsequently determined.

Statistical analyses

Analyses were performed by SPSS version 22 (SPSS Inc., Chicago, IL, USA). Data are presented as mean±SEM. Between-group differences were assessed by unpaired Student's *t*-test at 20 weeks or by one-way ANOVA at 28 weeks, while data presented as ratios were analyzed by appropriate non-parametric tests (Mann-Whitney or Kruskal-Wallis). Muscle function (force-frequency and force-time relationships) was assessed by two-way repeated measures ANOVA (group X frequency or group X time). *Post hoc* comparisons were calculated using Bonferroni-corrected tests.

Results

Animal model of HFpEF

Obese ZSF1 rats developed typical signs of HFpEF at 20 and 28 weeks, independent of exercise training. These data have already been published elsewhere and the reader is referred to this manuscript for more expansive details characterizing the current cohort of rats [3]. Briefly, at 28 weeks compared to lean controls all HFpEF groups (sedentary, MCT, HIIT) demonstrated evidence (P<0.05) of diastolic dysfunction (E/E': 15±1 vs. 25±1, 25±2, 23±1, respectively; P<0.0001), LV hypertrophy (23±1 vs. 28±1, 28±1, 29±1 mg/mm of tibia length, respectively; P<0.0001), obesity (body weight: 465±1 vs. 590±15, 591±17, 599±12 g, respectively; P<0.0001), diabetes (fasting blood glucose; 4.1±0.2 vs. 6.4±0.5, 5.5±0.3, 7.8±0.7 mmol/L, respectively; P<0.0001), and hypertension (systolic blood pressure: 158±14 vs. 181±15, 176±13,183±18 mmHg, respectively; P=0.008), in the presence of normal LVEF (66±2 vs. 75±2, 74±2, 72±3~70 %, respectively). Importantly, exercise capacity as assessed by $\dot{V}O_{2\,peak}$ was reduced (P<0.0001) in sedentary HFpEF rats compared to controls by ~30% (38±1 vs. 49±1 mL $kg^{-1}min^{-1}$, respectively). However, 8 weeks of exercise training resulted in a ~15 % $\dot{V}O_{2peak}$ increase (P=0.004) in both the MCT and HIIT groups compared to sedentary HFpEF rats (44±1 and 44±1 vs. 38±1 mL $kg^{-1}min^{-}$

[1], respectively). All of the changes mentioned above between control and sedentary HFpEF rats were also present at 20 weeks.

In addition, compared to control rats, muscle wet-weights at 20 weeks were reduced in HFpEF for both the EDL (258±29 vs. 174±21 mg, respectively; P<0.0001) and soleus (219±11 vs. 180±19 mg, respectively; P<0.0001). Similarly, at 28 weeks control rats maintained higher (P<0.05) muscle wet-weights compared to all HFpEF groups (sedentary, MCT, HIIT) such that training had no impact on muscle mass for the EDL (241±8 vs. 156±2, 164±3, 161±3 mg, respectively; P<0.0001) or soleus (221±4 vs. 170±4, 184±6, 188±4 mg, respectively; P<0.0001).

Glycolytic and oxidative skeletal muscle alterations in HFpEF

At 20 weeks, we observed numerous structural, metabolic, and molecular alterations in the skeletal muscles of HFpEF rats, both in the glycolytic fast-twitch EDL as well as the oxidative slow-twitch soleus muscle. Representative muscle sections of the EDL are presented in Fig. 1A-B for control and HFpEF rats, respectively. Compared to controls, HFpEF rats demonstrated fiber atrophy by ~50 % (P<0.0001; Fig. 1C) and a reduction in capillarity by ~20 % (P=0.001; Fig. 1D) in the EDL muscle. While not significant, the activity of the glycolytic enzyme LDH tended to be higher in the EDL of HFpEF rats (P=0.07; Fig. 1E), with protein levels of LC3 II/I ratio (an index of autophagy) showing a reduction of ~15 % in HFpEF compared to the control rats (P=0.049; Fig. 1F). These structural and molecular changes likely contributed to the lower force generated in the EDL muscle, with maximal forces reduced by ~15 % (P=0.001; Fig. 1G) while relative fatigue was unaffected (P=0.401; Fig. 1H). Similarly, the predominately oxidative soleus muscle also showed numerous alterations in HFpEF, but to a lesser degree. Representative muscle sections of the soleus from control and HFpEF rats is presented in Fig. 2A-B. Compared to controls, the soleus in HFpEF demonstrated a ~40 and 15 % reduction in

fiber cross sectional area and capillarity, respectively (P<0.0001 and P=0.002, respectively; Fig. 2B-C). In contrast, while LDH activity and LC3 II/I protein levels were not different between groups (P=0.791 and P=0.621; Fig. 2E-F), maximal soleus force was reduced by ~20 % in HFpEF compared to control rats (P=0.006; Fig. 2G) yet fatigability remained unchanged (P=0.767; Fig. 2H).

Can exercise training reverse skeletal muscle impairments in HFpEF?

At 28 weeks, following 8 weeks of either sedentary, HIIT, or MCT regimes, the EDL muscle (see Fig. 3A for representative sections) in HFpEF rats showed no apparent benefit from exercise training: the observed fiber atrophy (P<0.0001) and reduced capillarity (P=0.016) were not reversed compared to control values (Fig. 3B-C), while limited effects were observed on markers of glycolytic metabolism (P=0.316) and autophagy (P=0.066; Fig. D-E). As one would expect, therefore, EDL muscle weakness and fatigability were not influenced with exercise training in HFpEF, with evidence of impaired force production (P<0.004) and greater fatigability (P<0.009) in the HFpEF groups compared to controls (Fig. 3F-G). In line with these findings, the oxidative soleus muscle (see Fig. 4A for representative sections) also showed limited benefits following exercise training in the HFpEF rats: HIIT or MCT were unable to increase fiber cross-sectional area (P=0.003) or capillarity (P=0.010) back towards control values (Fig.4B-C). In contrast, the exercise training regimes attenuated activity of the glycolytic enzyme LDH, which remained elevated in the soleus of sedentary HFpEF rats relative to controls (P=0.004; Fig. 4D). Yet exercise training did not reduce autophagy activation, with protein levels of the LC3 II/I ratio elevated across all three HFpEF groups compared to controls in the soleus (P=0.016; Fig. 4E). As such, the lower contractile forces observed in the soleus from HFpEF animals compared to healthy rats at 20 weeks were not overcome by 8 weeks of exercise training (P=0.003; Fig. 4F), which was also similar for fatigability (P=0.001; Fig 4G).

Discussion

This is the first study to characterize fiber type-specific skeletal muscle alterations in a rat model of HFpEF and to further assess the efficacy of various exercise training regimes to reverse these deficits. The main finding of this study is that HFpEF caused detrimental alterations to both slow-oxidative and fast-glycolytic skeletal muscles including fiber atrophy, impaired capillarity, a shift towards glycolytic metabolism, and autophagy dysregulation, which expands upon our current knowledge collected from both patient and animal studies [3-8]. These structural, metabolic, and proteostasis changes likely contributed, at least in part, to the greater muscle weakness and fatigability that we also observed in HFpEF rats. Somewhat surprisingly 8 weeks of exercise training (either high- or moderate-intensity treadmill running) was unable to attenuate many of these muscle-specific deficits, which likely provides a rationale for why muscle dysfunction could not be overcome. Overall, therefore, these data: 1) provide novel underlying mechanisms that may be responsible for skeletal muscle dysfunction in HFpEF: 2) confirm that glycolytic and oxidative muscles are both affected in HFpEF; 3) demonstrate exercise training to be an ineffective tool in reversing skeletal muscle impairments in this experimental model.

Skeletal muscle dysfunction in HFpEF: oxidative vs. glycolytic

Data from vastus lateralis biopsies have shown that when compared to age-matched controls, patients with HFpEF demonstrate reduced mitochondrial content, lower mitochondrial fusion proteins, a shift from oxidative to glycolytic fibers, and impaired capillarity [5-7]. Recent data have also suggested that a distinct obesity-related HFpEF patient cohort exists [9], but no data are available to support whether skeletal muscle alterations specific to this phenotype develop. In the current study, therefore, we used an obese-specific rat model of HFpEF to provide novel evidence that skeletal muscle impairments also occur in this phenotype, but also extend current knowledge in HFpEF to

show that both oxidative as well as glycolytic muscles are afflicted. While both the soleus and EDL were severely affected by fiber atrophy and lower capillarity, it is important to note that the glycolytic EDL demonstrated greater susceptibility. Indeed, previous data have shown the oxidative soleus to be better protected against impairments induced by heart failure with reduced ejection fraction (HFrEF) compared to glycolytic muscles [17], with our data tending to support this view in HFpEF. Our data also showed a strong trend for the glycolytic enzyme lactate dehydrogenase activity to be increased in HFpEF (both in soleus and EDL), which supports recent magnetic resonance imaging studies where an earlier depletion of high-energy phosphates occurred during the onset of exercise in HFpEF patients [10, 11].

An additional novel finding in the present study was revealing that other molecular signaling pathways may be involved in the muscle remodeling process induced by HFpEF. For example autophagy (as assessed by the ratio of LC3 II/I protein expression) was differentially regulated between fiber types during HFpEF. Autophagy is a catabolic process that degrades cytoplasmic proteins, however basal flux is key to maintain cell homeostasis. Previous studies in mice have shown that either excessive or deficient levels of autophagy can induce skeletal muscle atrophy and functional deficits [18, 19]. Collectively, therefore, our data suggest that HFpEF could modulate autophagy levels in a fiber-type specific manner via a signaling pathway not yet characterized in this disease. We speculate the significant increase of autophagy in the soleus but decrease in the EDL may have contributed, at least in part, to the muscle atrophy and dysfunction observed in HFpEF. Interestingly, previous data have shown that autophagic flux differs between oxidative and glycolytic muscles, which supports the suggestion that HFpEF could modulate this process in a fiber-dependent manner [20]. Moreover our findings also suggest that HFpEF could induce muscle alterations in both a fiber- and time-dependent manner determined by disease progression. For example, the glycolytic EDL was more

susceptible to impairments earlier in the disease (i.e., 20 weeks), whereas the oxidative soleus only exhibited alterations at a later time point (i.e. 28 weeks). Interestingly, only at 28 weeks did the soleus demonstrate a significant increase in LDH activity and autophagic flux in HFpEF rats, which were not apparent at 20 weeks. The mechanism underlying these time-dependent changes remain unclear, however it may be related to the chronic pro-inflammatory state that accompanies the HFpEF phenotype [21], which given enough time would be expected to impact even the most resistant of all muscle fibers.

Exercise training in HFpEF to reverse skeletal muscle impairments

In the present study, exercise training conferred no significant benefits to either glycolytic or oxidative muscles, which was generally consistent in terms of structural, metabolic, autophagic, and functional measures. We did observe that glycolytic enzyme activity of lactate dehydrogenase was significantly attenuated in the soleus muscle of HFpEF rats that performed HIIT or MCT, which suggests a shift towards oxidative metabolism following exercise training. While the effects of exercise training to reverse skeletal muscle impairments in HFpEF remain largely unestablished, previous data in a hypertensive rat model of HFpEF showed endurance training was protective against reductions in soleus mitochondrial content and fatigability [4]. As the current study's main focus was not on muscle mitochondrial adaptations, we cannot rule out the possibility that exercise training induced a shift towards an oxidative phenotype in the trained HFpEF rats, such that improvements in mitochondrial function/content or a shift towards increased oxidative slow-twitch fibers occurred. However, as part of another study using the same HFpEF rats [3], we have previously documented that citrate synthase activity (a marker of mitochondrial content) was not significantly increased in the soleus post-training. These findings, together with those we present here, seem to support therefore that a metabolic inflexibility may exist in this specific animal model with obesity-related HFpEF. Indeed, whether the lack of skeletal muscle adaptations we found in this study is specific to the

current HFpEF experimental model remains unknown, but the potential co-morbidities that occur in these animals such as obesity, diabetes, hypertension and renal dysfunction may prove too overwhelming for exercise training to be beneficial in only 8 weeks. In support, we recently reported the diaphragm was unable to benefit from exercise training in the same model [3], suggesting skeletal muscle adaptations in this model are not achieved with 8 weeks of endurance exercise training. Whether these findings, therefore, translate to patients with a specific obese-related HFpEF phenotype remains to be confirmed, but warrants further attention.

Clinical translation and new insights

In terms of clinical translation, our findings strongly support a number of muscle-specific changes reported to occur in HFpEF patients, as assessed from leg biopsies (e.g., fiber atrophy, reduced capillarity) [5-7], but also extend current knowledge in HFpEF by providing new findings on fiber-type specific adaptations (i.e., oxidative vs. glycolytic) as well as new mechanistic insight into the underlying signaling pathways (e.g., dysregulation in autophagy, upregulation of glycolytic enzymes). Up until now, patient and animal studies in HFpEF had drawn conclusions based upon a single muscle group (i.e., the vastus lateralis in patients and the soleus in animal models) [3-8]. As such, it was unknown whether other limb muscles composed of different fiber types showed similar impairments in HFpEF. The current study, therefore, provides new evidence that shows skeletal muscle remodeling in HFpEF is fiber-type dependent, with fast-twitch glycolytic fibers impacted earlier and to a greater degree. This adds additional insight by suggesting muscle groups composed of fast-twitch fibers should be primarily targeted during cardiac rehabilitation in HFpEF patients, as these may be more susceptible to impairments. Future patient studies should therefore focus on sampling various muscle groups in order to characterize the role of fiber-type specific adaptations in HFpEF.

Perhaps the most novel yet controversial finding of the present study is that it challenges the prevalent view that endurance training can be used as a treatment for muscle dysfunction in HFpEF [22]. In fact, our data support the recent suggestion that more work in the area is required to establish the benefit of exercise training in treating muscle dysfunction in HFpEF [23]. Specifically, here we found endurance training was unable to rescue muscle dysfunction and attenuate detrimental structural remodeling induced by HFpEF, irrespective of the exercise regime performed. While it is clearly established that endurance training in patients with HFpEF improves exercise capacity and quality of life [23-29], the role for this in the disease to rescue muscle dysfunction still remains unclear. Our current experimental data also found 8 weeks of endurance training (either HIIT or MCT) in HFpEF was able to increase $\dot{V}O_{2peak}$ by ~15% (which mirrors the increase seen in patients after training [23], thus supporting clinical translation of our findings). Clearly, however, the lack of improvements we found in both oxidative and glycolytic skeletal muscles post-training in HFpEF would suggest a limited role of improved muscle function as a central mechanism underlying the increased exercise capacity. One key question is whether our animal findings closely reflects the patient scenario? Unfortunately, as of yet, no patient studies have evaluated skeletal muscle biopsies following endurance training in HFpEF. While data from an experimental rat model have shown HIIT attenuates mitochondrial and contractile impairments in the soleus [4], this study induced HFpEF by hypertension alone (rather than multiple comorbidities) and the endurance training was extensive lasting a total of 28 weeks. As such, limitations to both patient and animal studies preclude the degree to which we can conclude how much endurance training benefits skeletal muscle in HFpEF, but clearly this remains a 'hot-topic' of investigation.

By inference, therefore, our data do not support the concept that an increases in $\dot{V}O_{2peak}$ in HFpEF post-training is underpinned by improvements at the skeletal muscle level. In broad

terms, $\dot{V}O_{2peak}$ is determined by products of the Fick principle (i.e. cardiac output and arterial-venous O_2 difference). As our *in vivo* and *in vitro* measures of cardiac function were not improved post-training in HFpEF (which in fact agrees with many patient studies; as reviewed in ref. [23]), we speculate the main mechanism responsible for the increase in $\dot{V}O_{2peak}$ likely resides at the peripheral vascular level [30, 31]. Indeed patients with HFpEF are known to have endothelial dysfunction [32] and we have previously shown endurance training can reverse *in vitro* endothelial dysfunction in aortic rings isolated from hypertensive rats with HFpEF [33]. However, endothelial dysfunction as a main mechanism of limitation in HFpEF remains controversial based on a key patient study showing endurance training over 16 weeks failed to improve endothelial function [27]. Nevertheless, the same authors haven also provided data to support that an increase in the arterial-mixed venous O_2 difference is likely the primary mechanism underlying the higher $\dot{V}O_{2peak}$ achieved post-training in HFpEF [26, 27], which indicates a peripheral limitation is present in HFpEF. Based on these data and together with our findings, we speculate improved microvascular perfusion may be the primary mechanism leading to an improvements in $\dot{V}O_{2peak}$ following endurance training in HFpEF (which is known to be impaired [30, 31]), as this would be predicted to elevate O_2 diffusion delivery from capillary to mitochondria (as dictated by Fick's Law) [34].

Conclusion

Obesity-related HFpEF caused detrimental alterations to both slow-oxidative and fast-glycolytic skeletal muscles, which included substantial fiber atrophy, a reduction in capillarity, a shift towards glycolytic metabolism, autophagy dysregulation, as well as impaired contractile functional measures. The intervention of exercise training (either high- or moderate-intensity treadmill running) was unable to attenuate many of these deficits,

which poses the intriguing question of whether endurance training can be used to combat skeletal muscle impairments induced by obesity-related HFpEF at the patient level.

Acknowledgments

This study was funded by the European Commission, The Seventh Framework Programme for Research (FP7-Health/602405).

Conflicts of interest

The authors report no relationships that could be construed as a conflict of interest.

References

[1] Butler J, Fonarow GC, Zile MR, Lam CS, Roessig L, Schelbert EB, et al. Developing therapies for heart failure with preserved ejection fraction: current state and future directions. JACC Heart failure. 2014;2:97-112.

[2] Sharma K, Kass DA. Heart failure with preserved ejection fraction: mechanisms, clinical features, and therapies. Circ Res. 2014;115:79-96.

[3] Bowen TS, Brauer D, Rolim NPL, Baekkerud FH, Kricke A, Ormbostad Berre AM, et al. Exercise Training Reveals Inflexibility of the Diaphragm in an Animal Model of Patients With Obesity-Driven Heart Failure With a Preserved Ejection Fraction. Journal of the American Heart Association. 2017;6: e006416.

[4] Bowen TS, Rolim NP, Fischer T, Baekkerud FH, Medeiros A, Werner S, et al. Heart failure with preserved ejection fraction induces molecular, mitochondrial, histological, and functional alterations in rat respiratory and limb skeletal muscle. European journal of heart failure. 2015;17:263-72.

[5] Haykowsky MJ, Kouba EJ, Brubaker PH, Nicklas BJ, Eggebeen J, Kitzman DW. Skeletal muscle composition and its relation to exercise intolerance in older patients with heart failure and preserved ejection fraction. The American journal of cardiology. 2014;113:1211-6.

[6] Kitzman DW, Nicklas B, Kraus WE, Lyles MF, Eggebeen J, Morgan TM, et al. Skeletal muscle abnormalities and exercise intolerance in older patients with heart failure and preserved ejection fraction. American journal of physiology Heart and circulatory physiology. 2014;306:H1364-70.

[7] Molina AJ, Bharadwaj MS, Van Horn C, Nicklas BJ, Lyles MF, Eggebeen J, et al. Skeletal Muscle Mitochondrial Content, Oxidative Capacity, and Mfn2 Expression Are Reduced in Older Patients With Heart Failure and Preserved Ejection Fraction and Are Related to Exercise Intolerance. JACC Heart failure. 2016;4:636-45.

[8] Haykowsky MJ, Brubaker PH, Morgan TM, Kritchevsky S, Eggebeen J, Kitzman DW. Impaired aerobic capacity and physical functional performance in older heart failure patients with preserved ejection fraction: role of lean body mass. J Gerontol A Biol Sci Med Sci. 2013;68:968-75.

[9] Obokata M, Reddy YNV, Pislaru SV, Melenovsky V, Borlaug BA. Evidence Supporting the Existence of a Distinct Obese Phenotype of Heart Failure With Preserved Ejection Fraction. Circulation. 2017;136:6-19.

[10] Bhella PS, Prasad A, Heinicke K, Hastings JL, Arbab-Zadeh A, Adams-Huet B, et al. Abnormal haemodynamic response to exercise in heart failure with preserved ejection fraction. European journal of heart failure. 2011;13:1296-304.

[11] Weiss K, Schar M, Panjrath GS, Zhang Y, Sharma K, Bottomley PA, et al. Fatigability, Exercise Intolerance, and Abnormal Skeletal Muscle Energetics in Heart Failure. Circulation Heart failure. 2017;10:e004129.

[12] Hamdani N, Franssen C, Lourenco A, Falcao-Pires I, Fontoura D, Leite S, et al. Myocardial titin hypophosphorylation importantly contributes to heart failure with preserved ejection fraction in a rat metabolic risk model. Circulation Heart failure. 2013;6:1239-49.

[13] van Dijk CG, Oosterhuis NR, Xu YJ, Brandt M, Paulus WJ, van Heerebeek L, et al. Distinct Endothelial Cell Responses in the Heart and Kidney Microvasculature Characterize the Progression of Heart Failure With Preserved Ejection Fraction in the Obese ZSF1 Rat With Cardiorenal Metabolic Syndrome. Circulation Heart failure. 2016;9:e002760.

[14] Lewis GA, Schelbert EB, Williams SG, Cunnington C, Ahmed F, McDonagh TA, et al. Biological Phenotypes of Heart Failure With Preserved Ejection Fraction. Journal of the American College of Cardiology. 2017;70:2186-200.

[15] Shah SJ, Kitzman DW, Borlaug BA, van Heerebeek L, Zile MR, Kass DA, et al. Phenotype-Specific Treatment of Heart Failure With Preserved Ejection Fraction: A Multiorgan Roadmap. Circulation. 2016;134:73-90.

[16] Seiler M, Bowen TS, Rolim N, Dieterlen MT, Werner S, Hoshi T, et al. Skeletal Muscle Alterations Are Exacerbated in Heart Failure With Reduced Compared With Preserved Ejection Fraction: Mediated by Circulating Cytokines? Circulation Heart failure. 2016; 9: e003027.

[17] Li P, Waters RE, Redfern SI, Zhang M, Mao L, Annex BH, et al. Oxidative phenotype protects myofibers from pathological insults induced by chronic heart failure in mice. The American journal of pathology. 2007;170:599-608.

[18] Masiero E, Agatea L, Mammucari C, Blaauw B, Loro E, Komatsu M, et al. Autophagy is required to maintain muscle mass. Cell metabolism. 2009;10:507-15.

[19] Mammucari C, Milan G, Romanello V, Masiero E, Rudolf R, Del Piccolo P, et al. FoxO3 controls autophagy in skeletal muscle in vivo. Cell metabolism. 2007;6:458-71.

[20] Pare MF, Baechler BL, Fajardo VA, Earl E, Wong E, Campbell TL, et al. Effect of acute and chronic autophagy deficiency on skeletal muscle apoptotic signaling, morphology, and function. Biochimica et biophysica acta. 2017;1864:708-18.

[21] Paulus WJ, Tschope C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. Journal of the American College of Cardiology. 2013;62:263-71.

[22] Fleg JL, Cooper LS, Borlaug BA, Haykowsky MJ, Kraus WE, Levine BD, et al. Exercise training as therapy for heart failure: current status and future directions. Circulation Heart failure. 2015;8:209-20.

[23] Tucker WJ, Nelson MD, Beaudry RI, Halle M, Sarma S, Kitzman DW, et al. Impact of Exercise Training on Peak Oxygen Uptake and its Determinants in Heart Failure with Preserved Ejection Fraction. Card Fail Rev. 2016;2:95-101.

[24] Edelmann F, Gelbrich G, Dungen HD, Frohling S, Wachter R, Stahrenberg R, et al. Exercise training improves exercise capacity and diastolic function in patients with heart failure with preserved ejection fraction: results of the Ex-DHF (Exercise training in Diastolic Heart Failure) pilot study. Journal of the American College of Cardiology. 2011;58:1780-91.

[25] Fu TC, Yang NI, Wang CH, Cherng WJ, Chou SL, Pan TL, et al. Aerobic Interval Training Elicits Different Hemodynamic Adaptations Between Heart Failure Patients with Preserved and Reduced Ejection Fraction. Am J Phys Med Rehabil. 2016;95:15-27.

[26] Haykowsky MJ, Brubaker PH, Stewart KP, Morgan TM, Eggebeen J, Kitzman DW. Effect of endurance training on the determinants of peak exercise oxygen consumption in elderly patients with stable compensated heart failure and preserved ejection fraction. Journal of the American College of Cardiology. 2012;60:120-8.

[27] Kitzman DW, Brubaker PH, Herrington DM, Morgan TM, Stewart KP, Hundley WG, et al. Effect of endurance exercise training on endothelial function and arterial stiffness in older patients with heart failure and preserved ejection fraction: a randomized, controlled, single-blind trial. Journal of the American College of Cardiology. 2013;62:584-92.

[28] Kitzman DW, Brubaker PH, Morgan TM, Stewart KP, Little WC. Exercise training in older patients with heart failure and preserved ejection fraction: a randomized, controlled, single-blind trial. Circulation Heart failure. 2010;3:659-67.

[29] Smart NA, Haluska B, Jeffriess L, Leung D. Exercise training in heart failure with preserved systolic function: a randomized controlled trial of the effects on cardiac function and functional capacity. Congest Heart Fail. 2012;18:295-301.

[30] Lee JF, Barrett-O'Keefe Z, Garten RS, Nelson AD, Ryan JJ, Nativi JN, et al. Evidence of microvascular dysfunction in heart failure with preserved ejection fraction. Heart. 2016;102:278-84.

[31] Lee JF, Barrett-O'Keefe Z, Nelson AD, Garten RS, Ryan JJ, Nativi-Nicolau JN, et al. Impaired skeletal muscle vasodilation during exercise in heart failure with preserved ejection fraction. Int J Cardiol. 2016;211:14-21.

[32] Borlaug BA, Olson TP, Lam CS, Flood KS, Lerman A, Johnson BD, et al. Global cardiovascular reserve dysfunction in heart failure with preserved ejection fraction. Journal of the American College of Cardiology. 2010;56:845-54.

[33] Adams V, Alves M, Fischer T, Rolim N, Werner S, Schutt N, et al. High-intensity interval training attenuates endothelial dysfunction in a Dahl salt-sensitive rat model of heart failure with preserved ejection fraction. J Appl Physiol (1985). 2015;119:745-52.

[34] Poole DC, Richardson RS, Haykowsky MJ, Hirai DM, Musch TI. Exercise limitations in heart failure with reduced and preserved ejection fraction. J Appl Physiol (1985). 2018;124:208-24.

Figure legends

Fig 1. Characterization of the glycolytic EDL muscle at 20 weeks of age in control (Con) and to HFpEF rats, with representative muscle sections stained with an antibody against vWF to visualize capillaries presented for control (A) and HFpEF (B) rats (scale bar represents 100 μm). Mean fiber cross-sectional area (CSA) was severely reduced in HFpEF compared to control rats (C), as was the capillary-to-fiber ratio (D). As a marker of glycolytic metabolism, lactate dehydrogenase (LDH) activity was assessed, which tended to be higher in HFpEF rats (E), while LC3 II/I protein levels were assessed to provide an index of autophagy and these were depressed in HFpEF compared to controls (F). *In vitro* muscle function of the EDL was also assessed and this was impaired in HFpEF rats, with lower absolute forces generated compared to controls at higher stimulation frequencies (G) while relative fatigue was not influenced (H). n = 8 for Con and n = 12 for HFpEF. *P<0.05 vs. Con.

Fig 2. Characterization of the oxidative soleus muscle at 20 weeks of age in control (Con) and to HFpEF rats, with representative muscle sections stained with an antibody against vWF to visualize capillaries presented for control (A) and HFpEF (B) rats (scale bar represents 100 μm). Mean fiber cross-sectional area (CSA) was lower in HFpEF compared to control rats (C), as was capillary-to-fiber ratio (D). While lactate dehydrogenase (LDH) activity (E) and LC3 II/I protein levels (F) were not different between groups, *in vitro* absolute muscle forces were lower in HFpEF compared to control rats (G) but relative fatigue remained unchanged (H). n = 8 for Con and n = 12 for HFpEF. *P<0.05 vs. Con.

Fig 3. Characterization of the glycolytic EDL muscle at 28 weeks of age following exercise training on a treadmill for the last 8 weeks, in control (Con) and HpEF rats that were assigned to either sedentary, high-intensity interval training (HIIT), and moderate-continuous training (MCT) groups for 8 weeks. Representative muscle sections (A) for each group are presented and have been stained with an antibody against vWF to visualize capillaries (scale bar represents 100 μm). Mean fiber cross-sectional area (CSA) was severely lower in all HFpEF groups compared to control rats

(B), as was the capillary-to-fiber ratio (C). Lactate dehydrogenase (LDH) was not different between groups, (D), however the protein levels of the autophagy marker LC3 tended to be lower in the HFpEF groups compared to controls (E). Absolute contractile forces were impaired in all HFpEF groups compared to controls (F), while fatigability was also greater in the HFpEF groups compared to controls early during the onset of repeated stimulations (G). *P<0.05 vs. Con.

Fig 4. Characterization of the oxidative soleus muscle at 28 weeks of age following exercise training on a treadmill for 8 weeks, in control (Con) and HpEF rats that were assigned to either sedentary, high-intensity interval training (HIIT), and moderate-continuous training (MCT) groups. Representative muscle sections (A) for each group are presented and have been stained with an antibody against vWF to visualize capillaries (scale bar represents 100 μm). Mean fiber cross-sectional area (CSA) was lower in all HFpEF groups compared to control rats (B), as was the capillary-to-fiber ratio (C). Lactate dehydrogenase (LDH) activity, as an index of reliance on glycolytic metabolism, was elevated only in sedentary HFpEF rats compared to controls (D), however autophagic flux, as measured by LC3 protein content, was increased in all the HFpEF groups (E). While absolute muscle forces were impaired in all HFpEF groups at the higher stimulation frequencies compared to control rats (F), fatigability was shown only to be greater in the HFpEF-HIIT group compared to controls (G). *P<0.05 vs. Con; $^{\#}$P<0.05 HFpEF-HIIT vs. Con.

Figures

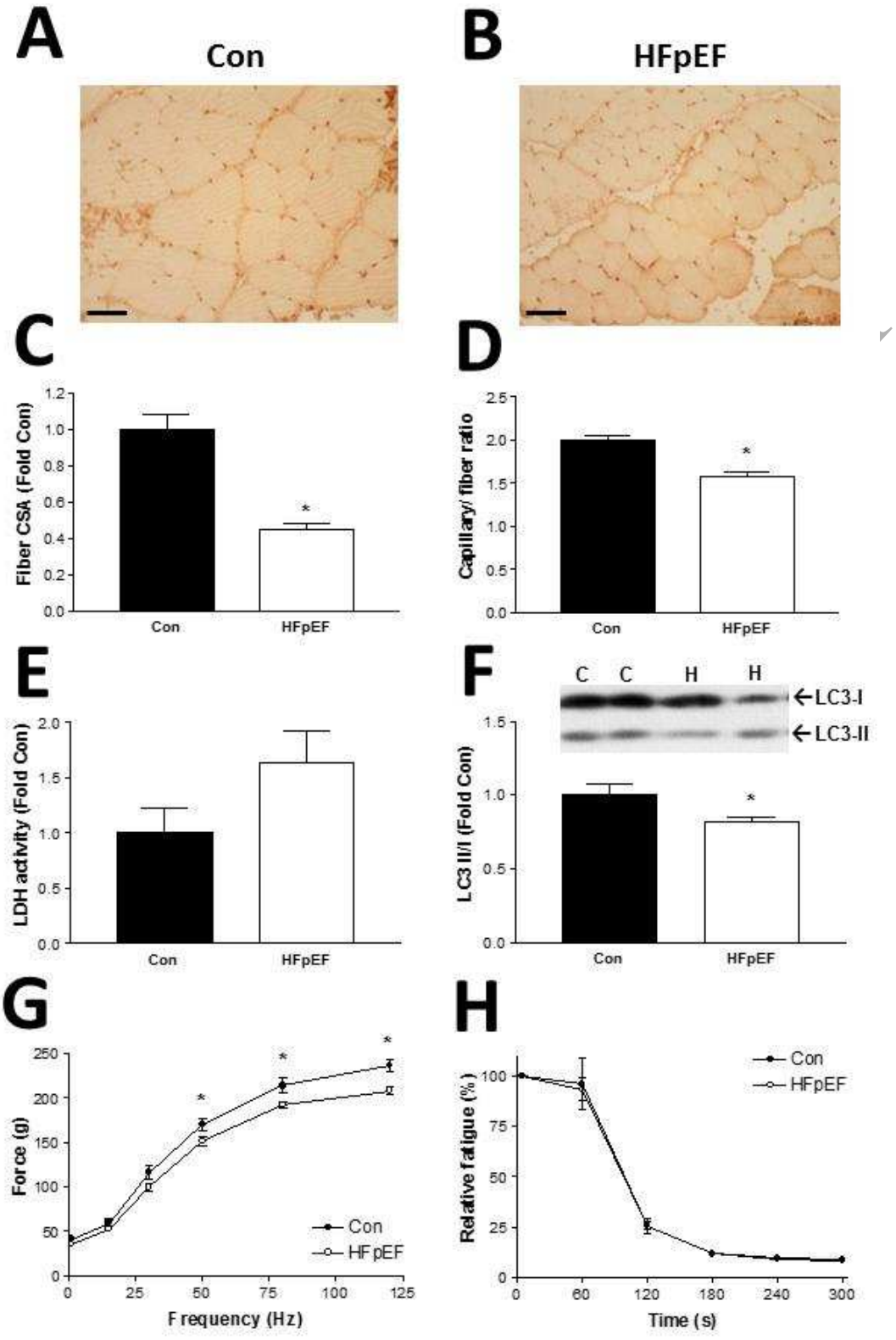


Figure 1.

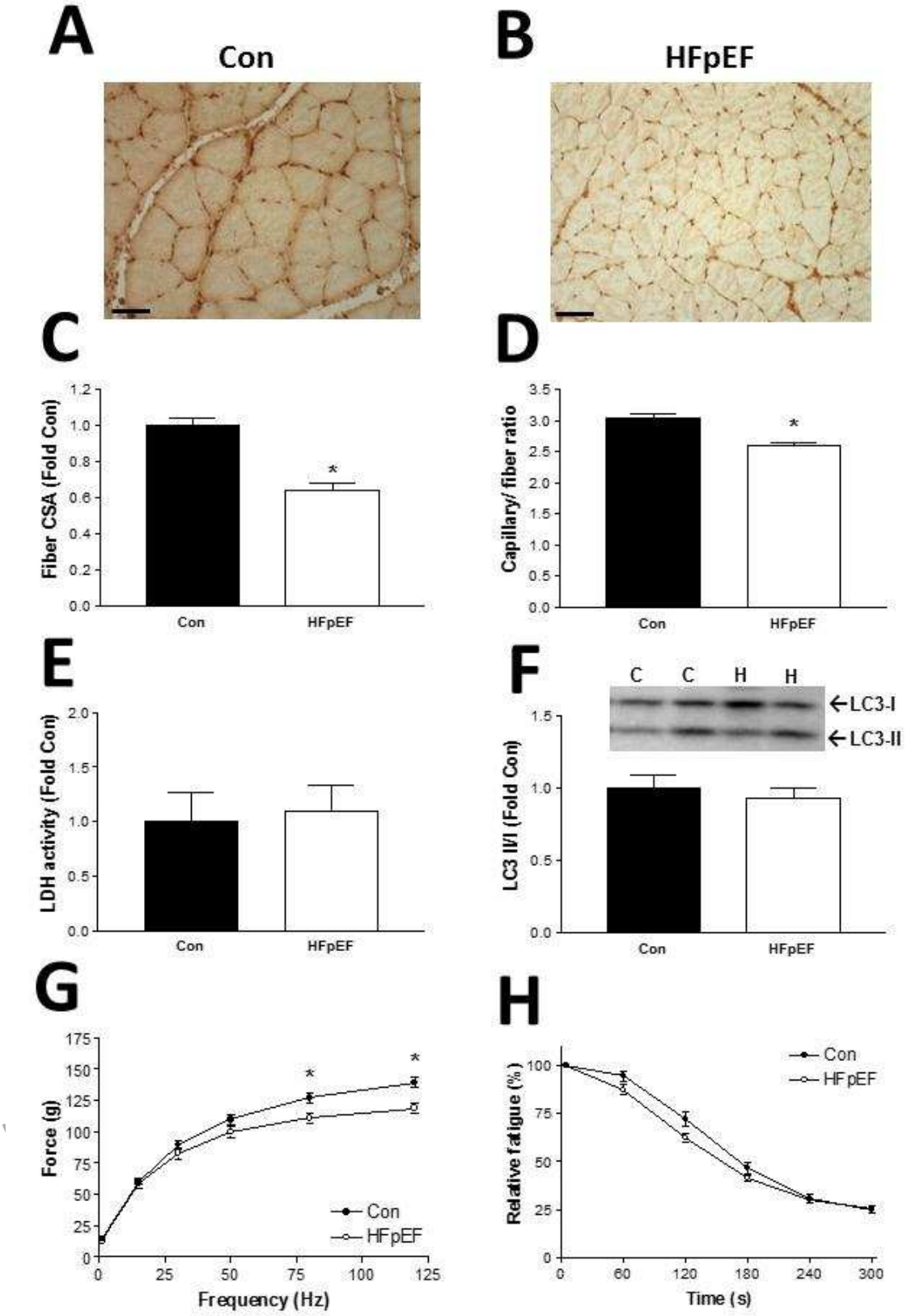

A
Con
B
HFpEF
C
Fiber CSA (Fold Con)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Con
HFpEF
D
Capillary/ fiber ratio
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
Con
HFpEF
E
LDH activity (Fold Con)
2.0
1.5
1.0
0.5
0.0
Con
HFpEF
F
C C H H
←LC3-I
←LC3-II
LC3 II/I (Fold Con)
1.5
1.0
0.5
0.0
Con
HFpEF
G
Force (g)
175
150
125
100
75
50
25
0
*
*
Con
HFpEF
0 25 50 75 100 125
Frequency (Hz)
H
Relative fatigue (%)
100
75
50
25
0
Con
HFpEF
0 60 120 180 240 300
Time (s)

Figure 2.

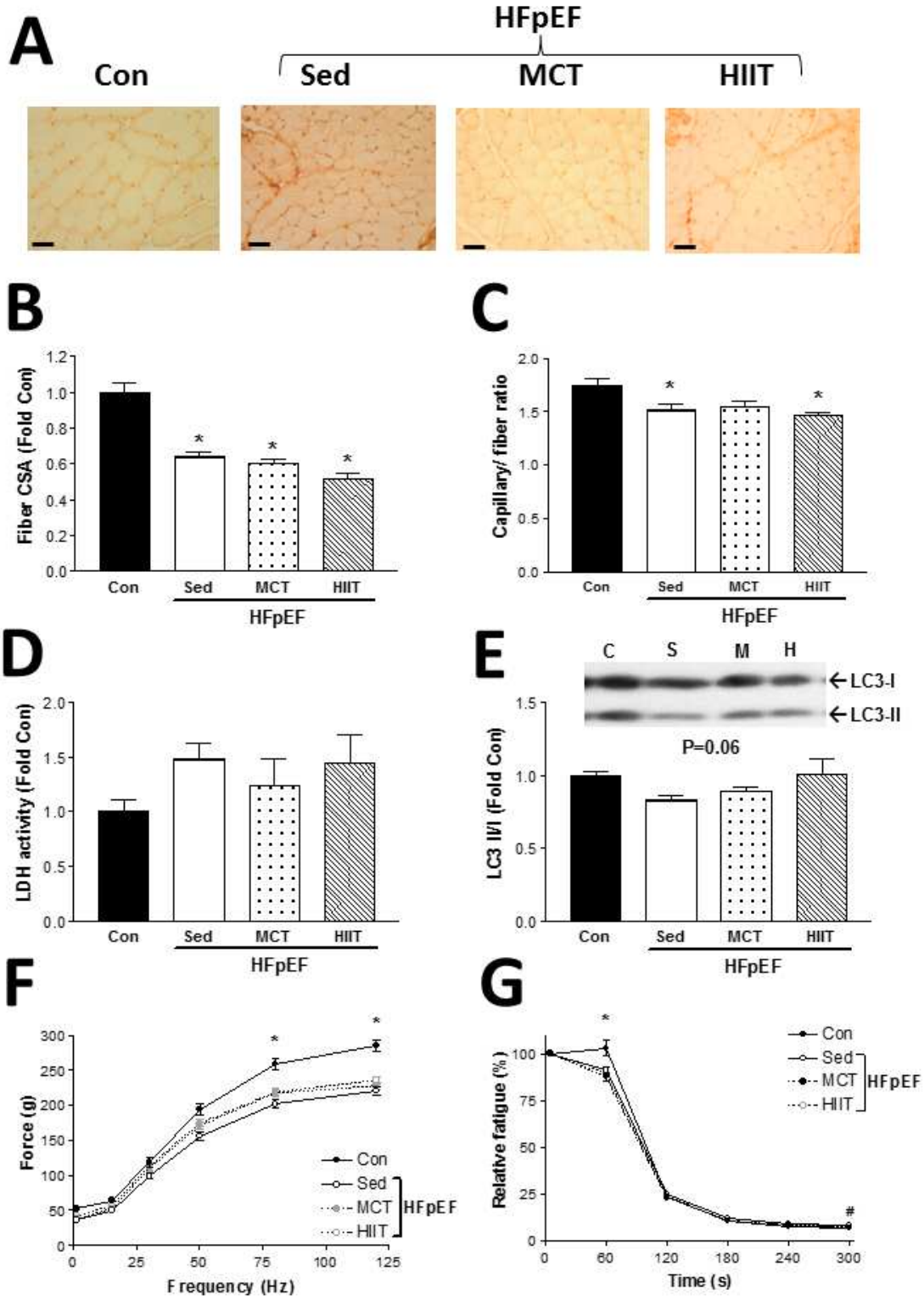

A
HFpEF
Con
Sed
MCT
HIIT
B
Fiber CSA (Fold Con)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
*
Con
Sed
MCT
HIIT
HFpEF
C
Capillary/ fiber ratio
2.0
1.5
1.0
0.5
0.0
*
*
Con
Sed
MCT
HIIT
HFpEF
D
LDH activity (Fold Con)
2.0
1.5
1.0
0.5
0.0
Con
Sed
MCT
HIIT
HFpEF
E
C
S
M
H
←LC3-I
←LC3-II
P=0.06
LC3 II/I (Fold Con)
1.5
1.0
0.5
0.0
Con
Sed
MCT
HIIT
HFpEF
F
Force (g)
300
250
200
150
100
50
0
*
*
0
25
50
75
100
125
Frequency (Hz)
Con
Sed
MCT
HIIT
HFpEF
G
Relative fatigue (%)
100
75
50
25
0
*
#
0
60
120
180
240
300
Time (s)
Con
Sed
MCT
HIIT
HFpEF

Figure 3.

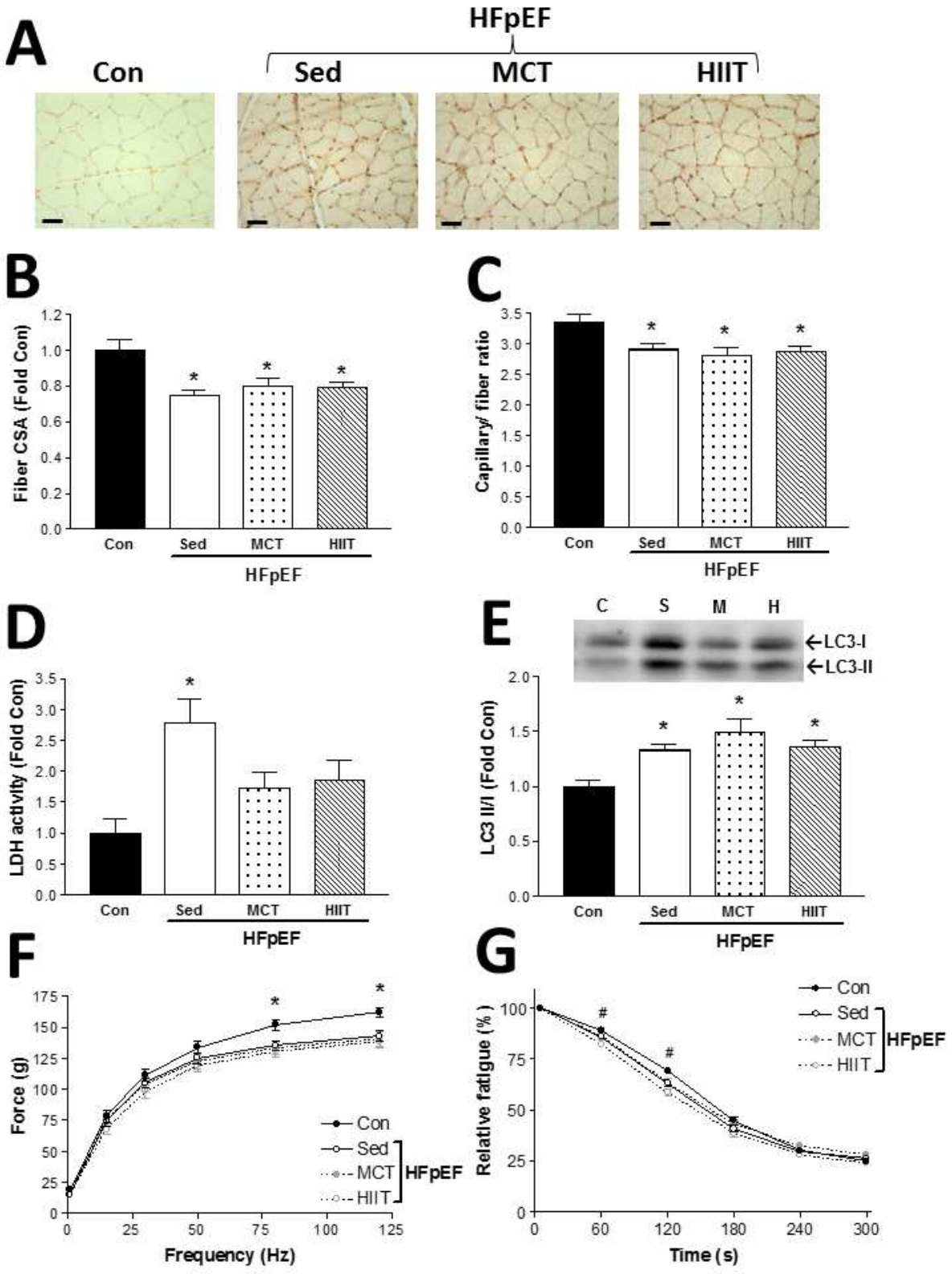

A
HFpEF
Con
Sed
MCT
HIIT
B
Fiber CSA (Fold Con)
Con
Sed
MCT
HIIT
HFpEF
C
Capillary/ fiber ratio
Con
Sed
MCT
HIIT
HFpEF
D
LDH activity (Fold Con)
Con
Sed
MCT
HIIT
HFpEF
E
C
S
M
H
←LC3-I
←LC3-II
LC3 II/I (Fold Con)
Con
Sed
MCT
HIIT
HFpEF
F
Force (g)
Frequency (Hz)
Con
Sed
MCT
HIIT
HFpEF
G
Relative fatigue (%)
Time (s)
Con
Sed
MCT
HIIT
HFpEF

Figure 4.