Skeletal muscle alterations are exacerbated in heart failure with reduced (HFrEF) compared to preserved ejection fraction (HFpEF): mediated by circulating cytokines?

Martin Seiler¹,*, T. Scott Bowen¹,*, Natale Rolim², Maja-Theresa Dieterlen³, Sarah Werner¹, Tomoya Hoshi⁴, Tina Fischer¹, Norman Mangner¹, Axel Linke¹, Gerhard Schuler¹, Martin Halle⁵, Ulrik Wisloff², Volker Adams¹

¹ University of Leipzig, Heart Center Leipzig, Department of Cardiology, Leipzig, Germany
² K.G. Jebsen Center of Exercise in Medicine, Department of Circulation and Medical Imaging, Faculty of Medicine, NTNU, Trondheim, Norway
³ University of Leipzig, Heart Center Leipzig, Department of Cardiac Surgery, Leipzig, Germany
⁴ Cardiovascular Division, Faculty of Medicine, University of Tsukuba, Japan
⁵ Department of Prevention, Rehabilitation and Sports Medicine, Klinikum rechts der Isar, Technische Universität München, Munich, Germany; DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany; Else Kröner-Fresenius-Zentrum, Klinikum rechts der Isar der Technischen Universität München, Munich, Germany

* Both authors contributed equally

Running head: Skeletal muscle alterations in HFrEF and HFpEF

Word count (without abstract, including references): 5108

Address for correspondence:

Volker Adams, PhD
University Leipzig - Heart Center Leipzig
Department of Cardiology,
Strümpellstrasse 39,
04289 Leipzig
Germany
Tel: +49 341 865 1671
Fax: +49 865 1461
E-mail: adav@medizin.uni-leipzig.de
**Background:** A greater understanding of the different underlying mechanisms between heart failure patients with reduced (HFrEF) and preserved (HFpEF) ejection fraction is urgently needed to better direct future treatment. However, while skeletal muscle impairments, potentially mediated by inflammatory cytokines, are common in both HFrEF and HFpEF, the underlying cellular and molecular alterations that exist between groups are yet to be systematically evaluated. The present study, therefore, used established animal models to compare whether alterations in skeletal muscle (limb and respiratory) were different between HFrEF and HFpEF, while further characterizing inflammatory cytokines.

**Methods and Results:** Rats were assigned to: 1) HFrEF (ligation of the left coronary artery; n=8); 2) HFpEF (high-salt diet: n=10); 3) Control (Con: no intervention; n=7). HF was confirmed by echocardiography and invasive measures. Soleus tissue in HFrEF, but not HFpEF, showed a significant increase in markers of: 1) Muscle atrophy (i.e., MuRF1, calpain, ubiquitin proteasome); 2) Oxidative stress (i.e., higher NADPH oxidase but lower anti-oxidative enzyme activities); 3) Mitochondrial impairments (i.e., a lower SDH/LDH ratio and PGC-1α expression). The diaphragm remained largely unaffected between groups. Plasma concentrations of circulating cytokines were significantly increased in HFrEF for TNF-α, while IL-1β and IL-12 were higher in HFpEF.

**Conclusions:** Our findings suggest, for the first time, skeletal muscle alterations are exacerbated in HFrEF compared to HFpEF, which predominantly reside in limb rather than respiratory muscle. This disparity may be mediated, in part, by the different circulating inflammatory cytokines that were elevated between HFpEF and HFrEF.

**Keywords:** skeletal muscle, diaphragm, heart failure with reduced ejection fraction (HFrEF), heart failure with preserved ejection fraction (HFpEF), soleus: MuRF1; inflammation
Introduction

Heart failure (HF) is a major and growing public health concern, with estimates suggesting that ~3.6 million patients are diagnosed every year in Europe alone. Of these, approximately 50% of HF patients have a reduced ejection fraction (HFrEF) while the remainder a preserved ejection fraction (HFpEF). Importantly, HFpEF patients fail to respond favourably to many pharmacological interventions that have otherwise proved beneficial to HFrEF patients. This has widespread consequences for the treatment of HF patients, suggesting two apparent cohorts exist that demonstrate a contrasting response to therapeutic interventions. As such, a greater understanding of the different underlying mechanisms acting between HFrEF and HFpEF may help better direct future treatment in this disease.

The main symptom observed in HF patients is exercise intolerance, consequent not only to dyspnoea but also severe skeletal muscle weakness (both in the limb and respiratory systems), with the latter being a robust predictor of quality of life and prognosis (as reviewed in 3). Interestingly, initial evidence from independent studies indicates that some but not all skeletal muscle alterations are similar between HFrEF and HFpEF when compared to controls. For example, HFrEF is well established to induce both a fiber atrophy and isoform shift, reduce contractile protein content, and lower mitochondrial capacity. These impairments are mediated by the combination of reduced anabolic (e.g., IGF-1) and elevated catabolic factors (e.g., atrogenes and proteolytic activity), an increased reactive oxygen species (ROS) load, and greater inflammation driven by elevated concentrations of circulating cytokines (e.g., TNF-alpha, IL-1B). In contrast, only a few studies have investigated skeletal muscle alterations in HFpEF, with initial findings indicating fewer (or even opposite) changes may occur in comparison to HFrEF such as a reduced ROS load and lower proteolytic activity. Therefore, while preliminary evidence supports the suggestion that divergent skeletal
muscle alterations may occur between HFrEF and HFpEF and thus contribute to disease progression, as of yet no direct comparison has been performed to answer this ambiguity.

The present study, therefore, used established animal models to directly compare whether molecular and cellular alterations in skeletal muscle (both limb and respiratory) were different between HFrEF and HFpEF. In addition, we also investigated if circulating inflammatory cytokines were differentially expressed between the two conditions, as these may provide a potential mechanism to explain any such contrasting muscle alterations. A priori we hypothesized that markers of atrophy and oxidative stress are more activated in the limb muscle of HFrEF when compared to HFpEF, whereas in the diaphragm the differences between HFrEF and HFpEF are less pronounced.

METHODS

Animals and experimental procedures

Female Sprague-Dawley rats (8 weeks old; n=8) underwent ligation of the left anterior descending (LAD) coronary artery to induce a myocardial infarction (MI) and were sacrificed 10 weeks later following development of HFrEF (confirmed by echocardiography and histology), as previously described in detail 12. Female Dahl salt-sensitive rats (7 weeks old: n=10) were fed with a high-salt diet (8% NaCl) over 28 weeks to induce diastolic dysfunction and the overall pathophysiology associated with HFpEF (confirmed by echocardiography and invasive pressure measures), as recently described in detail 11. Female Sprague-Dawley rats (n=7) without any intervention also served as controls (Con) and were sacrificed at 18 weeks of age. All rats were exposed to a 12 h light/dark cycle, with food and water provided ad libitum. At sacrifice the costal diaphragm (respiratory muscle) and the soleus (limb muscle), were collected and frozen in liquid nitrogen and stored at -80°C for subsequent analyses. Blood was taken and plasma obtained by centrifugation (10 min at 2500xg) and stored in
 aliquots at -80°C until used. All procedures and experiments were approved by the Norwegian Council for Animal Research and the “Landesbehörde Sachsen”, which was in accordance with Use of Laboratory Animals by the European Commission Directive 86/609/EEC.

**mRNA expression**

The mRNA expression of PGC-1α and IGF-1 was evaluated by quantitative real time RT-PCR and normalized to the expression of hypoxanthine guanine phosphoribosyl transferase (HPRT), as previously described. The following primers were used for the amplification of PGC-1α: 5’-GCCAGTAGATCCTCTTCAAGATC-3’ and 5’-TCACACGCGCTCTTCAATTG-3’, IGF-1: 5’-TCTACCTGGCAGCTCTGCTTGCT-3’ and 5’-CTGAGTCTTTGGGCGATGTCCAGTG-3’; HPRT: 5’-CTCATGGACTGATTATGGACAGGAC-3’ and 5’-GCAGGTCAGCAAAGAACTTATAGCC-3’.

**Protein expression**

Western blot was used to quantify protein expression as previously described. The following antibodies were used: MuRF-1 (1:1000, Abcam, Cambridge, UK), PGC-1α (1:200, Santa Cruz, Heidelberg, Germany), and LC3 (1:1000, Novus Biologicals, Cambridge, UK). Protein expression was normalized to the loading control GAPDH (1:30,000; HyTest Ltd, Turku, Finland), with data presented in arbitrary units (AU).

**Enzyme activity**

Enzymatic activities of succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), creatine kinase (CK), NADPH oxidase, glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase (Cat) were measured using spectrophotometric assays, with specific enzyme activity (Units/mg) calculated.
Proteasome and calpain activity

As previously described \(^{11}\), the activity of chymotrypsin-like, trypsin-like and peptidylglutamyl-peptide hydrolyzing (PGPH) proteasome activity were assayed to provide an index of overall proteasome activity. Calpain activity was also assayed \(^{11}\).

Plasma analyses

A 12-plex rat cytokine assay was used to quantify in duplicates different cytokine concentrations from plasma samples using a Luminex\textsuperscript{®} 200\textsuperscript{TM} analyser (Merck Millipore, Darmstadt, Germany), in accordance to the manufacturer’s protocol (Bio-Rad, Munich, Germany).

Statistical analyses

Data are presented as mean ± SEM. Data were tested for normal distribution (Kolmogorov and Smirnov test) and between-group differences determined by one-way ANOVA, with post hoc test (Tukey-Kramer multiple comparisons test) used where appropriate. Significance was accepted as P<0.05. Analyses were performed by SPSS version 22 (SPSS inc., Chicago, USA).

RESULTS

Animal characteristics

In HFrEF animals that underwent ligation, echocardiography revealed significant systolic dysfunction showing a LVEF of 39±3 % while histological staining demonstrated a LV infarct size of 51±4 %. In addition, fractional shortening was significantly reduced, whereas markers for ventricular dilation (LVEDD, LVESD) were increased (Fig. 1). In contrast, HFpEF animals demonstrated maintained LVEF of 70±4 % but with diastolic dysfunction (82% increase in E/É and 116% increase in LVEDP) in combination with cardiac
hypertrophy, as demonstrated by increased LV wall thickness and heart weight (Fig. 1). As expected, however, Con rats had no significant impairments to systolic or diastolic function.

**Anabolic and catabolic markers**

**Soleus:** Compared to Con, mRNA expression of the anabolic factor IGF-1 was significantly reduced by 66 % and 77 % in HFrEF and HFpEF, respectively (Fig. 2A). The catabolic protein MuRF-1 (a muscle specific ubiquitin E3 ligase) was significantly elevated in HFrEF by 46 % but reduced in HFpEF by 41 %, as compared to Con (Fig. 2B). However, the protein expression of LC3 (a catabolic marker of autophagy) was not different between groups (Fig. 1C). Calpain activity was significantly increased by 120 % and 147 % in HFrEF compared to Con and HFpEF, respectively (Fig. 3A). Similarly, ubiquitin proteasome activity in HFrEF was significantly higher by 245 % and 155 % compared to Con and HFpEF, respectively (Fig. 3B).

**Diaphragm:** Although IGF-1 (Fig. 2D) and LC3 (Fig. 2F) did not differ between groups, the protein expression of MuRF-1 was significantly increased by 176 % and 185 % in HFrEF and HFpEF, respectively, compared to Con (Fig. 2E). No significant difference was detected between groups for proteasome or calpain activity (Fig. 3C-D).

**Mitochondrial indices**

**Soleus:** A 47 % and 51 % significant reduction in the SDH/LDH ratio (an index of oxidative metabolism) was observed in HFrEF compared to Con and HFpEF, respectively (Fig. 4A), while no differences were found in creatine kinase activity between groups (Fig. 4B). Compared to Con and HFpEF, expression of the mitochondrial transcriptional co-activator PGC-1α was significantly reduced in HFrEF (Fig. 5A-B), at both the mRNA and protein level by 45 % and 31 % respectively versus Con.
Diaphragm: No changes between groups were found in the SDH/LDH ratio (Fig. 4C), creatine kinase activity (Fig. 4D), or PGC-1α expression (mRNA and protein) (Fig. 5C-D).

Enzyme activities of ROS modulating proteins

Soleus muscle: Compared to Con and HFpEF, the activity of the ROS generating enzyme NADPH oxidase was significantly increased by 73 % and 133 % in HFrEF, respectively (Fig. 6A). Quantification of ROS scavenging enzymes revealed a significant reduction in the activity of GPX by 35 % and catalase by 47 % in the HFrEF compared to Con, while no changes were observed in HFpEF (Fig. 6B-C). Compared to Con, however, SOD activity was significantly reduced in both HFrEF and HFpEF by 44 % and 60 %, respectively (Fig. 6D).

Diaphragm: No significant difference between groups was found in enzyme activities of NADPH oxidase (Fig. 6E) or GPX (Fig. 6F). Catalase activity, however, was significantly increased by 70 % in HFpEF animals compared to Con, with no change observed in HFrEF (Fig. 6G). In contrast, both HFrEF and HFpEF groups showed a significant increase of 69 % and 78 % in SOD activity compared to Con, respectively (Fig. 6H).

Inflammatory markers

Plasma concentrations of inflammatory cytokines were different between groups, with TNF-α significantly increased by 50 % in HFrEF compared to Con, with no change in HFpEF (Fig. 7A). In contrast, while IL-6 was not different between groups (Fig. 7B), the cytokines 1L-1β and IL12 were significantly increased in HFpEF relative to Con and HFrEF (Fig. 7C-D), specifically by 143 % and 90 % versus Con, respectively.
DISCUSSION

This study used established animal models to reveal a number of novel findings in relation to the different molecular and cellular skeletal muscle alterations that exist between HFrEF and HFpEF, which included:

1) Upregulation in markers of muscle atrophy in HFrEF (i.e., MuRF1, calpain, ubiquitin proteosome) but unchanged or lower levels in HFpEF soleus.

2) Increased oxidative stress in HFrEF (i.e., higher NADPH oxidase with lower anti-oxidative enzyme activities) but not HFpEF soleus.

3) Impaired mitochondrial indices in HFrEF (i.e., a lower SDH/LDH ratio and PGC-1α protein expression) but not HFpEF soleus.

4) Muscle-dependent alterations between HFpEF and HFrEF limited to limb muscle (soleus), with respiratory muscle (diaphragm) remaining largely unaffected.

5) A distinctive circulating inflammatory cytokine response, with increased plasma concentrations of TNF-α in HFrEF but IL-1β and IL-12 in HFpEF.

Overall, therefore, our findings provide initial evidence that skeletal muscle alterations are exacerbated in HFrEF compared to HFpEF, which are mainly isolated to limb (soleus) rather than respiratory (diaphragm) tissue, and that the different circulating inflammatory cytokines detected between phenotypes may be potentially mediating such effects (as summarized in Table 1). As such, our data provide novel insights into the different molecular alterations and potential treatment targets specific to HFpEF and HFrEF.

Skeletal muscle alterations in HF

In recent years it has become evident that HFrEF and HFpEF are two different HF entities with different aetiology yet similar morbidity and mortality outcomes 17. One hallmark of both entities is exercise intolerance, with impairments to skeletal muscle (both limb and respiratory 18) playing a key role in exacerbating the symptoms of breathlessness and fatigue.
As expected, we found numerous skeletal muscle alterations that were similar between HFrEF and HFpEF (Table 1), which included a reduced expression in the anabolic factor IGF-1 and a lower antioxidant enzyme activity of SOD in the soleus, while in the diaphragm a greater expression of the key atrophic marker MuRF1.

As mentioned above, most alterations were restricted to limb (soleus) rather than respiratory (diaphragm) tissue (Table 1), which is in accordance to previous studies. For example, no changes between groups in the diaphragm were observed in relation to anabolic (IGF-1) or catabolic (calpain and ubiquitin proteosome systems, autophagy) factors, mitochondrial markers, and oxidative stress measures (i.e. NADPH oxidase and GPX activity). The main finding in the diaphragm was related to an increased MuRF1 expression in both HF groups, as well as an increased antioxidant enzyme capacity (e.g., SOD). The latter is likely explained by the diaphragm being constantly recruited during respiration, which is exacerbated in HF due to breathlessness, which results in a “training” effect and offsets alterations seen in limb muscle. As such, the remainder of the discussion will focus upon our findings from limb (soleus) skeletal muscle.

**Divergent skeletal muscle alterations between HFrEF and HFpEF**

Skeletal muscle alterations have important clinical implications as they provide a strong surrogate of functional capacity and prognosis in patients with HF. Importantly, we have confirmed a highly diverse skeletal muscle characterization between HFrEF and HFpEF tissue (as summarized in Table 1), which may have important clinical consequences for the future treatment of patients. In HFrEF, for example, we found markers were increased for both atrophy (i.e., MuRF1, calpain and ubiquitin proteasome activity) and oxidative stress (higher NADPH oxidase but lower radical scavenger enzyme activities), while indices of mitochondrial function were further impaired (i.e., lower SDH/LDH ratio and PGC-1α expression). These data support previous HFrEF human or animal studies that found an
increased expression of atrophy related proteins, elevated oxidative stress, as well as impaired mitochondrial respiration. Critically, however, we observed no changes or even a contrasting response in skeletal muscle of HFpEF animals, which included a downregulation in MuRF1 protein expression and unchanged proteolytic activity. This suggests, therefore, that muscle wasting may play a greater role in HFrEF compared to HFpEF, which is supported by data on this topic showing a strong link to mortality in HFrEF patients.

Unfortunately, at present, evidence of skeletal muscle alterations in HFpEF (in both animals and humans) remains limited. The finding that arterio-venous oxygen content difference reserve is an independent predictor of exercise capacity (reviewed in) supports the notion skeletal muscle or microvascular dysfunction is playing a key role in HFpEF, which is supported by patient data showing an increased intramuscular fat deposition, impaired phosphocreatine recovery rates, a fiber type shift and also a reduced capillary-to-fiber ratio. Similarly our group also recently reported numerous skeletal muscle alterations in an animal model of HFpEF, which included fiber atrophy, impaired mitochondrial respiration, a fiber type shift, but unchanged (or even reduced) ROS and proteolytic related markers. The present study, therefore, not only confirms previous findings in HFpEF (from patients and animals), but also advances our current understanding of skeletal muscle alterations in terms of anabolic factors, and additional ROS handling enzyme activities and mitochondrial indices. What still remains unresolved, however, is whether the main factor(s) limiting exercise capacity in both diseases is similar, which specifically includes the role of skeletal muscle dysfunction. Briefly, the potential mechanisms that play a dominant role in limiting exercise tolerance between HFpEF and HFrEF can be broadly categorized as central (i.e., cardiac output, heart rate, and stroke volume) or peripheral (i.e. vascular or intramuscular), as recently reviewed. Our data at least lend support to the argument that intrinsic skeletal muscle impairments may play a greater role in limiting exercise capacity in HFrEF compared to HFpEF. Indeed, other experiments also indicate that greater vascular rather than intramuscular
impairments may occur in HFpEF, as a reduction in diffusive oxygen transport was reported to play a greater role in limiting exercise capacity in HFpEF compared to HFrEF patients \(^26\). Furthermore, it is also known that HFpEF can induce endothelial dysfunction \(^27\), with more recent data showing limb blood flow and vasodilation are impaired during exercise \(^28\). Overall, therefore, while our data indicate that greater skeletal muscle alterations are induced in HFrEF compared to HFpEF, further investigations are warranted in order to determine whether this plays a more dominant role in limiting exercise capacity in one rather than the other disease entity.

**Underlying mechanisms of divergent skeletal muscle alterations in HF**

While it remains unclear what mechanism(s) is responsible for the large variation in skeletal muscle alterations between HFrEF and HFpEF, our data allow us, at least in part, to speculate about some possible key players. One potential mediator may be the transcriptional coactivator PGC-1α, which underpins mitochondrial biogenesis and metabolism \(^29\). Our data revealed that PGC-1α mRNA and protein expression in the soleus was reduced in HFrEF compared to HFpEF and control animals. It is well established that PGC-1α has multiple cellular influences, with it shown to regulate muscle mass by controlling the expression of atrogenes \(^30,31\) and oxidative stress \(^18\). As such, PGC-1α may play a key role in the divergent responses of markers of atrophy, oxidative stress, and mitochondrial activity that we observed between HFrEF and HFpEF.

Alternatively, another mechanism that may underpin the distinctive muscle alterations between HFrEF and HFpEF could be circulating inflammatory cytokines, which were differentially expressed between groups. Specifically, plasma concentrations of TNF-α were increased only in HFrEF, while IL-1β and IL-12 were only increased in HFpEF. Although a systemic inflammation is generally characteristic of HF, it is well established that specific
cytokines have different effects on skeletal muscle. Similar to our findings, TNF-α is increased in patients with HFrEF and is a key factor regulating muscle mass, which can activate the expression of atrogenes such as MuRF-1 and MafBx while suppressing anabolic factors such as IGF-1, while further also mediating oxidative stress. Additionally, TNF-α can down-regulate PGC-1α expression and thus impair mitochondrial function. That we too found such alterations in our HFrEF animals simultaneous with increased plasma TNF-α levels, strongly suggests a causal role of this cytokine in mediating skeletal muscle alterations between HF cohorts - a suggestion further supported by our HFrEF animals having unchanged plasma concentrations of TNF-α alongside fewer skeletal muscle alterations.

Nevertheless, a recent notion has proposed that systemic inflammation is the key trigger of HFpEF, mediating endothelial dysfunction and subsequent myocardial hypertrophy and stiffening. However, unlike HFrEF, current evidence remains scarce and is conflicting in relation to the role of inflammatory cytokines in HFpEF. For example, a comparison between HFrEF and HFpEF patients revealed circulating levels of TNF-α were in fact significantly increased in HFpEF but not in HFrEF, without changes in IL-6 between groups. In accordance with that study we also found IL-6 concentrations to be unchanged in HFrEF or HFpEF plasma, supporting the suggestion that this cytokine may not play a key role in skeletal muscle alterations induced during advanced HF. However, our finding that IL-1β levels were significantly higher in HFpEF compared to HFrEF and control plasma is important, as cell culture experiments have confirmed that IL-1β can reduce myofibrillar content in differentiated myotubes via an increased expression of the atrogenes MuRF1 and MAFbx. This suggests, at least, that IL-1β has the potential to modulate skeletal muscle alterations in HFpEF. In addition, while we also found IL-12 plasma concentrations to be increased in HFpEF compared to HFrEF and controls, robust evidence on the effects of this
cytokine modulating skeletal muscle remain at present unknown, but clearly further research is warranted. Overall, therefore, that HFrEF tissue clearly documented significantly fewer changes than HFrEF does lend support to the “single syndrome notion”, which suggests HF is a continuum with multiple phenotypes between both extremes.\textsuperscript{43}

\textit{Study limitations}

Whether skeletal muscle impairments vary between HFrEF and HFrEF due to aetiology remains to be determined. Naturally, our findings must therefore be viewed with caution in that our HFrEF and HFrEF groups were animals from two different strains that underwent two different procedures to induce HF (detailed in methods). However, as no model is currently established in one strain to induce HFrEF and HFrEF, we feel our findings still provide an important contribution to an area where there remains a paucity of data. In addition, the HFrEF rats were significantly older compared to HFrEF rats (35 vs. 18 weeks), potentially limiting effects seen to age rather than HF. However, our data revealed\textit{greater} (rather than\textit{fewer}) changes in HFrEF compared to HFrEF tissue, which provide strong support age\textit{per se} did not influence our findings.

\textit{Conclusion}

We found skeletal muscle alterations to be exacerbated in HFrEF compared to HFrEF, which were mainly isolated to limb (soleus) rather than respiratory (diaphragm) tissue. That different circulating inflammatory cytokines were also elevated between HFrEF and HFrEF suggest these could potentially mediate such effects.

\textit{Grants.}

TSB is a recipient of a Postdoctoral Research Fellowship from the Alexander von Humboldt Foundation. Support for this study was provided by the European Commission, Framework
Program 7, grant number: EU 602405-2. The authors are supported by grants from the K.G. Jebsen Foundation (UW, NR) Norwegian Research Council (UW), and the Liaison Committee between the Central Norway Regional Health Authority and the Norwegian University of Science and Technology (UW).

Disclosures

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

Reference List


Figure Legends:

**Figure 1.** Echocardiographic evaluation of fractional shortening (A), left ventricular posterior wall thickness (LVPwD) (B), left ventricular end diastolic diameter (LVEDD) (C), left ventricular end systolic diameter (LVESD) (D) of rats with heart failure reduced ejection fraction (HFrEF), heart failure preserved ejection fraction (HFpEF) and controls (con). In addition heart to tibia length was determined in all three groups. Values are shown as mean±SEM.

**Figure 2:** The mRNA expression of the anabolic factor IGF-1 (A,D) and the protein expression of the catabolic factors MuRF-1 (B,E) and LC3 (C,F) in the soleus and diaphragm, from control (con) animals compared to those demonstrating heart failure with reduced (HFrEF) or preserved ejection fraction (HFpEF). Values are shown as mean±SEM and expressed relative to con.

**Figure 3:** Activity of the calpain (A,C) and ubiquitin proteasome (B,D) systems in soleus and diaphragm tissue in animals exhibiting heart failure with reduced (HFrEF) or preserved ejection fraction (HFpEF) relative to controls (con). Values are shown as mean±SEM.

**Figure 4:** The ratio of succinate dehydrogenase (SDH) to lactate dehydrogenase (LDH) enzyme activity (A,C) and creatine kinase (CK) enzyme activity (B,D) measured in the soleus and diaphragm from controls (con) and animals exhibiting heart failure with reduced (HFrEF) or preserved ejection fraction (HFpEF). Values are shown as mean±SEM.

**Figure 5:** The mRNA (A,C) and protein (B,D) expression of PGC-1α in the soleus and diaphragm in control (con) and heart failure with reduced (HFrEF) or preserved ejection fraction (HFpEF) animals. Values are shown as mean±SEM.
Figure 6: Enzymatic activity of NADPH oxidase (A,E), glutathione peroxidase (GPX) (B,F), catalase (Cat) (C,G), and superoxide dismutase (SOD) (D,H) in the soleus and diaphragm from control animals (con) and those of heart failure with a reduced (HFrEF) or a preserved ejection fraction (HFpEF). Values are shown as mean±SEM.

Figure 7: Inflammatory cytokines measured from plasma concentrations in animals that developed heart failure with a reduced (HFrEF) or preserved ejection fraction (HFpEF) relative to controls (con). Values are shown as mean±SEM.
Figure 1

A. Fractional shortening (%)

B. LVPwD (mm)

C. LVEDD (mm)

D. LVESD (mm)

E. Heart weight TL

Con, HFrEF, HFpEF
Figure 2

A. mRNA expression (AU) of IGF-1 in Soleus

- con: 1.0
- HFrEF: 0.9
- HFrpEF: 0.8

p < 0.01
p < 0.05

B. Protein expression (AU) of Mucr1-1 in Soleus

- con: 1.8
- HFrEF: 2.2
- HFrpEF: 2.0

p < 0.05
p < 0.01
p < 0.001

C. Protein expression (AU) of LC3 in Soleus

- con: 1.2
- HFrEF: 1.5
- HFrpEF: 1.0

p < 0.05

D. mRNA expression (AU) of IGF-1 in Diaphragm

- con: 1.2
- HFrEF: 1.5
- HFrpEF: 1.8

p < 0.05

E. Protein expression (AU) of Mucr1-1 in Diaphragm

- con: 3.0
- HFrEF: 3.5
- HFrpEF: 4.0

p < 0.01

F. Protein expression (AU) of LC3 in Diaphragm

- con: 1.5
- HFrEF: 1.8
- HFrpEF: 1.0

p < 0.01
Figure 3

Soleus

A.

Calpain activity (rel. to con)

<table>
<thead>
<tr>
<th></th>
<th>con</th>
<th>HFrEF</th>
<th>HFP EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>0.05</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

B.

Proteasome activity (rel. to con)

<table>
<thead>
<tr>
<th></th>
<th>con</th>
<th>HFrEF</th>
<th>HFP EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>0.05</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

Diaphragm

C.

Calpain activity (rel. to con)

<table>
<thead>
<tr>
<th></th>
<th>con</th>
<th>HFrEF</th>
<th>HFP EF</th>
</tr>
</thead>
</table>

D.

Proteasome activity (rel. to con)

|       | con | HFrEF | HFP EF |

Figure 4

soleus
diaphragm

A.

Ratio SDH/LDH

<table>
<thead>
<tr>
<th></th>
<th>con</th>
<th>HFrEF</th>
<th>HFP EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>0.05</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

B.

CK activity (mU/mg)

|       | con | HFrEF | HFP EF |

C.

Ratio SDH/LDH

|       | con | HFrEF | HFP EF |

D.

CK activity (mU/mg)
Figure 5

**Soleus**

A. PGC-1α mRNA expression (AU)

<table>
<thead>
<tr>
<th></th>
<th>con</th>
<th>HFrEF</th>
<th>HFPpEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNA</td>
<td>15</td>
<td>12</td>
<td>17</td>
</tr>
</tbody>
</table>

B. PGC-1α protein expression (AU)

<table>
<thead>
<tr>
<th></th>
<th>con</th>
<th>HFrEF</th>
<th>HFPpEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>0.5</td>
<td>0.3</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**Diaphragm**

C. PGC-1α mRNA expression (AU)

<table>
<thead>
<tr>
<th></th>
<th>con</th>
<th>HFrEF</th>
<th>HFPpEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNA</td>
<td>1.0</td>
<td>0.9</td>
<td>1.1</td>
</tr>
</tbody>
</table>

D. PGC-1α protein expression (AU)

<table>
<thead>
<tr>
<th></th>
<th>con</th>
<th>HFrEF</th>
<th>HFPpEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>0.4</td>
<td>0.3</td>
<td>0.4</td>
</tr>
</tbody>
</table>

p<0.05, p<0.01, p<0.005
**Figure 6**

**A.** NAD(P)H oxidase activity (U/mg)
- **Soleus**
  - con
  - HFrEF
  - HFP EF

**B.** GPX activity (U/mg)
- **Soleus**
  - con
  - HFrEF
  - HFP EF

**C.** Cat activity (U/mg)
- **Soleus**
  - con
  - HFrEF
  - HFP EF

**D.** SOD activity (% inhibition/μg)
- **Soleus**
  - con
  - HFrEF
  - HFP EF

**E.** NAD(P)H oxidase activity (U/mg)
- **Diaphragm**
  - con
  - HFrEF
  - HFP EF

**F.** GPX activity (U/mg)
- **Diaphragm**
  - con
  - HFrEF
  - HFP EF

**G.** Cat activity (U/mg)
- **Diaphragm**
  - con
  - HFrEF
  - HFP EF

**H.** SOD activity (% inhibition/μg)
- **Diaphragm**
  - con
  - HFrEF
  - HFP EF

Significance levels are indicated as follows:
- **p<0.05**
- **p<0.01**
- **p<0.001**
- **p<0.05**
Figure 7

A. TNF-alpha (rel. to con)

B. IL-6 (rel. to con)

C. IL-18 (rel. to con)

D. IL-12 (rel. to con)
Table 1. Skeletal muscle molecular alterations in heart failure with a reduced (HFrEF) and preserved (HFpEF) ejection fraction compared to controls from the limb (soleus) and respiratory (diaphragm) muscle, as well as measured circulating cytokines.

<table>
<thead>
<tr>
<th></th>
<th>Soleus</th>
<th>Diaphragm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HFrEF</td>
<td>HFpEF</td>
</tr>
<tr>
<td>Anabolic factors</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td></td>
<td>↓ IGF-1</td>
<td>↓ IGF-1</td>
</tr>
<tr>
<td>Catabolic factors</td>
<td>Increased</td>
<td>Decreased</td>
</tr>
<tr>
<td></td>
<td>↑ MuRF1, ↑ Calpain/ proteasome activity; ↔ LC3</td>
<td>↓ MuRF1, ↔ Calpain/ proteasome activity; ↔ LC3</td>
</tr>
<tr>
<td>Mitochondrial indices</td>
<td>Decreased</td>
<td>Unchanged</td>
</tr>
<tr>
<td></td>
<td>↓ SDH/LDH ratio; ↓ PGC-1α; ↔ CK</td>
<td>↔ SDH/LDH ratio; ↔ PGC-1α; ↔ CK</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Increased</td>
<td>Unchanged (or small increase)</td>
</tr>
<tr>
<td></td>
<td>↑ NADPH oxidase, ↓ GPX, ↔ Cat, ↓ SOD</td>
<td>↔ NADPH oxidase, ↔ GPX, ↔ Cat, ↓ SOD</td>
</tr>
<tr>
<td>Plasma inflammatory cytokines</td>
<td>Increased in HFrEF: TNF-α</td>
<td></td>
</tr>
</tbody>
</table>

↑Increased; ↓Decreased; ↔Unchanged: See manuscript text for the defined molecular abbreviations.