Skeletal muscle alterations in chronic heart failure: differential effects on quadriceps and diaphragm

Norman Mangner1*, Bettina Weikert2, T. Scott Bowen1, Marcus Sandri3, Robert Höllriegel1, Sandra Erbs1, Rainer Hambrecht3, Gerhard Schuler1, Axel Linke1, Stephan Gielen4 & Volker Adams1

1Department of Internal Medicine/Cardiology, University of Leipzig—Heart Center, Leipzig, Germany; 2Department of Internal Medicine/Cardiology, Clinic Altenburg, Altenburg, Germany; 3Department of Cardiology and Angiology, Clinic Links der Weser, Bremen, Germany; 4University Hospital—Martin Luther University of Halle/Wittenberg, Halle, Germany

Abstract

Background Chronic heart failure (CHF) results in limb and respiratory muscle weakness, which contributes to exercise intolerance and increased morbidity and mortality, yet the molecular mechanisms remain poorly understood. Therefore, we aimed to compare parameters of antioxidative capacity, energy metabolism, and catabolic/anabolic balance in diaphragm and quadriceps muscle in an animal model of CHF.

Methods Ligation of the left anterior descending coronary artery (n = 13) or sham operation (n = 11) was performed on Wistar Kyoto rats. After 12 weeks, echocardiography and invasive determination of maximal rates of left ventricular (LV) pressure change were performed. Antioxidative and metabolic enzyme activities and expression of catabolic/anabolic markers were assessed in quadriceps and diaphragm muscle.

Results Ligated rats developed CHF (i.e. severe LV dilatation, reduced LV ejection fraction, and impaired maximal rates of LV pressure change; P < 0.001). There was a divergent response for antioxidant enzymes between the diaphragm and quadriceps in CHF rats, with glutathione peroxidase and manganese superoxide dismutase activity increased in the diaphragm but reduced in the quadriceps relative to shams (P < 0.01). Metabolic enzymes were unaltered in the diaphragm, but cytochrome c oxidase activity (P < 0.01) decreased and lactate dehydrogenase activity (P < 0.05) increased in the quadriceps of CHF animals. Protein expression of the E3 ligase muscle ring finger 1 and proteasome activity were increased (P < 0.05) in both the diaphragm and quadriceps in CHF rats compared with shams.

Conclusion Chronic heart failure induced divergent antioxidative and metabolic but similar catabolic responses between the diaphragm and quadriceps. Despite the quadriceps demonstrating significant impairments in CHF, apparent beneficial adaptations of an increased antioxidative capacity were induced in the diaphragm. Nevertheless, muscle ring finger 1 and proteasome activity (markers of protein degradation) were elevated and oxidative enzyme activity failed to increase in the diaphragm of CHF rats, which suggest that a myopathy is likely present in respiratory muscle in CHF, despite its constant activation.

Keywords CHF; Congestive heart failure; Antioxidative enzymes; MuRF-1

Introduction

Impaired skeletal muscle function is frequently observed in patients with chronic heart failure (CHF).1,2 While limb skeletal muscle has traditionally been the main focus, the respiratory muscles are also known to be affected.20,21 The most important muscle of inspiration is the diaphragm, with inspiratory muscle weakness associated with increased morbidity and mortality in CHF patients.20,23 This suggests that the mechanisms underlying diaphragm muscle dysfunction, which still remain poorly understood, represent an important therapeutic target in CHF.
Chronic heart failure patients suffer impairments to energy metabolism, protein synthesis/degradation balance, and calcium handling in limb skeletal muscle,1,12,14,16,22,32 which are likely mediated by neurohumoral alterations, increased local/systemic inflammatory cytokines, and increased oxidative stress.13,18,30 Interestingly, exercise training of limb skeletal muscle in patients with CHF lowers both local/systemic inflammation and oxidative stress, which is associated with improvements in energy metabolism and protein balance.13,18 This suggests that deconditioning of limb muscle is an important mechanism responsible for exercise intolerance in CHF. In contrast, the diaphragm is constantly active during life, which is further exacerbated in CHF patients where an increased work of breathing consequent to pulmonary abnormalities is reported.20,35 Indeed, CHF induces ‘training-like’ benefits in the diaphragm of patients, as demonstrated by an increased oxidative enzyme activity and a fibre-type shift to a slower, more fatigue-resistant profile.29 Nevertheless, that impaired diaphragmatic function is still reported in CHF and associated with impairments in energy metabolism,10 protein synthesis/breakdown,31 and calcium handling,19,26 which suggests that a myopathy may exist that cannot be explained by deconditioning alone. The diaphragm in patients with CHF may therefore be influenced by two competing, but alternative, mechanisms including (i) an altered systemic and local neurohumoral influences and/or (ii) an increased work of breathing/muscle activation.35

As such, a better understanding of the heterogeneity that exists between constantly active and occasionally active muscle groups in CHF would help provide further insight on whether a muscle myopathy is specific to this disease rather than deconditioning per se. Therefore, we compared parameters of antioxidative capacity, energy metabolism, and catabolic/anabolic balance in the constantly active diaphragm and occasionally active quadriceps in an animal model of CHF.

Materials and methods

**Echocardiography and invasive hemodynamics**

Wistar Kyoto rats underwent ligation of the left anterior descending coronary artery to induce CHF (n=13) or sham operation (n=11), as described previously.33 After 12 weeks, echocardiography was performed using a 12-MHz transducer connected to a Hewlett-Packard Sonos-5500 echocardiograph. A short-axis two-dimensional image-guided M-mode view of the left ventricle was acquired. Left ventricular end-diastolic (LV-EDD) and end-systolic (LV-ESD) dimensions, wall thickness of the anterior and posterior walls in diastole and systole, respectively, were measured in the M-mode tracing according to the leading-edge technique. Measurements from five adjacent cardiac cycles were averaged and used for further analysis. Fractional shortening (FS) was calculated according to the formula: FS = [(LV-EDD − LV-ESD)/LV-EDD] × 100. Left ventricular end-diastolic (LV-EDV) and end-systolic volumes (LV-ESV) as well as ejection fraction were calculated in a parasternal long-axis view using the disk method. From these measurements, stroke volume (SV = LV-EDV − LV-ESV) and cardiac output (CO = SV × heart rate) were calculated. For further hemodynamic analysis, maximal rates of LV pressure change (+dp/dt and −dp/dt) were assessed by using a 2.0 F Millar catheter (Millar Instruments Inc., Houston, Texas, USA) placed in the left ventricle via a right-sided transeptal approach under anaesthesia using 2 mg/kg diazepam i.p. (B. Braun Melsungen AG, Melsungen, Germany), 40 mg/kg ketamin (Serumwerk Bernburg AG, Bernburg, Germany), and 2.5 mg/kg xylazin i.m. (Serumwerk Bernburg AG, Bernburg, Germany). Animals were subsequently sacrificed, and the diaphragm and quadriceps were removed. The harvested tissue was immediately snap frozen in liquid nitrogen and stored at −80°C. The local council for animal research approved experimental protocols.

**Quantification of enzymatic activities**

The frozen biopsy samples were homogenized in radioimmunoprecipitation assay buffer for cell lysis and protein extraction (50 mM Tris, 150 mM sodium chloride, 1 mM EDTA, 1% NP-40, 0.25% sodium-deoxycholate, 0.1% sodium dodecyl sulfate, 1% Triton X-100; pH 7.4; all chemicals purchased from Roth, Karlsruhe, Germany) 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 (bicinchoninic acid assay, Pierce, Bonn, Germany). Enzymatic activities of lactate dehydrogenase (LDH),7 citrate synthase (CS),28 cytochrome c oxidase (COX),12 glutathione peroxidase (GPX),11 catalase,6 superoxide dismutase (SOD), manganese SOD (Mn-SOD),7 and nicotinamide adenine dinucleotide phosphate-oxidase (NAD(P)H-oxidase)9 were measured according to standard protocols, and specific activities were calculated.

**Quantification of protein expression**

Frozen tissue samples were homogenized in radioimmunoprecipitation assay buffer containing a mixture of protease inhibitor (inhibitor mix M, Serva, Heidelberg, Germany) and protein expression that was quantified by western blot using specific antibodies to NAD(P)H oxidase (Abcam, Cambridge, UK), MuRF-1 (generous gift of Dr S. Labeit, University Mannheim, Germany), and MAFbx (generated in rabbits against the following peptide sequence CYPKREQGYDTLQL, Eurogentec, Seraing, Belgium). After incubation with a horseradish peroxidase-conjugated secondary antibody, specific bands were visualized by enzymatic chemiluminescence (Super Signal West

DOI: 10.1002/jcsm.12034
Different effects of CHF in quadriceps and diaphragm

Pico, Pierce, Bonn, Germany) and densitometry quantified by a one-dimensional scan software package (Scanalytics, Rockville, USA). Loading differences were controlled by reprobing the blot with an antibody against Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) (Hytest, Turku, Finland).

RNA-isolation and quantification of messenger RNA-expression

Total RNA was isolated from diaphragm and quadriceps muscle tissue using RNeasy (Qiagen, Hilden, Germany) and reverse transcribed into complementary DNA using random hexamers and Sensiscript reverse transcriptase (Qiagen, Hilden, Germany). An aliquot of the complementary DNA was used for quantitative reverse transcription PCR applying the LightCycler™ (Roche Diagnostics Inc). The expression of specific genes was normalized to the expression of 18S ribosomal RNA. The following primers and conditions were used 18S ribosomal RNA: 5′-ATACAGGACTCTTTCCAGGCCC-3′ and 5′-CGGGACACTCGAAGGACC-3′ at 62°C annealing; IGF-1: 5′-TCTACCTGGCATGCTTGCTG-3′ and 5′-CTGAGTCTTGGCATGTCAGTG-3′ at 62°C annealing.

Proteasome activity

The peptidase activities of the proteasome in the cytosolic fraction of quadriceps and diaphragm homogenates were determined as recently described.3 Chymotrypsin-like and trypsin-like activities were assayed using the fluorogenic peptides Suc-LLVY-AMC and Bz-VGR-AMC, respectively (Biomol, Hamburg, Germany).

Statistical analysis

Statistical analysis was carried out using SPSS version 20 (IBM, Chicago, IL, USA). Data are expressed as mean±t standard error of the mean. Between-group comparisons were made by independent t-test. When variables were not normally distributed or the variance was not equal, the Mann–Whitney U Test was used. Correlation analyses were performed using Pearson correlation. A two-sided probability value of <0.05 was considered statistically significant.

Results

Baseline characteristics and development of heart failure

Animal characteristics along with echocardiographic and invasive hemodynamic data are presented in Table 1. Compared with shams, ligated rats demonstrated a significant increase in the weights of the left and right ventricles and also of the moist lungs. In addition, LV-EDD, LV-ESD, LV-EDV, and LV-ESV were significantly enlarged in ligated rats, consistent with severe left ventricular dilation. Therefore, FS and LV-EF were severely impaired in ligated rats, further confirmed by impaired +dp/dt and −dp/dt. Collectively, these data demonstrate that the rats that underwent the ligation procedure had developed CHF.

Table 1 Baseline characteristics of CHF and control animals

| Parameter          | CHF n = 13 | Control n = 11 | P  |
|--------------------|------------|----------------|----|
| Body mass (g)      | 488.5 ± 14.3 | 486.5 ± 16.4  | n.s.|
| Wet mass (g)       |             |                |    |
| Left ventricle     | 1.06 ± 0.03 | 0.94 ± 0.05    | <0.05|
| Right ventricle    | 0.27 ± 0.01 | 0.21 ± 0.02    | <0.001|
| Lung               | 2.3 ± 0.1   | 2.0 ± 0.6      | 0.05 |
| LV-EDD (mm)        | 9.8 ± 0.3   | 6.8 ± 0.2      | <0.001|
| LV-ESD (mm)        | 8.2 ± 0.3   | 3.6 ± 0.2      | <0.001|
| FS (%)             | 15.8 ± 1.3  | 46.0 ± 1.2     | <0.001|
| LV-EDV (mL)        | 0.44 ± 0.05 | 0.26 ± 0.03    | <0.05 |
| LV-ESV (mL)        | 0.31 ± 0.03 | 0.09 ± 0.02    | <0.001|
| LV-EF (%)          | 30.0 ± 2.5  | 64.8 ± 2.2     | <0.001|
| SV (mL)            | 0.14 ± 0.02 | 0.16 ± 0.02    | n.s.|
| CO (mL/min)        | 30.8 ± 5.5  | 48.2 ± 8.0     | 0.08 |
| RV-EDD (mm)        | 4.2 ± 0.2   | 3.5 ± 0.1      | <0.01|
| IVC (mm)           | 4.0 ± 0.2   | 2.9 ± 0.2      | <0.01|
| dp/dt max          | 4.4 ± 0.3   | 5.7 ± 0.5      | <0.05|
| dp/dt min          | −3.1 ± 0.2  | −4.5 ± 0.4     | <0.01|

CHF, chronic heart failure; CO, cardiac output; FS, fractional shortening; dp/dt max/min, left ventricular pressure change maximum and minimum; IVC, inferior vena cava; LV-EDD, left ventricular end-diastolic diameter; LV-EDV, left ventricular end-diastolic volume; LV-EF, left ventricular ejection fraction; LV-ESD, left ventricular end-systolic diameter; LV-ESV, left ventricular end-systolic volume; n.s., not significant; RV-EDD, right ventricular end-diastolic diameter; SV, stroke volume.

Reactive oxygen species-producing enzyme and antioxidative enzyme activity

The protein expression and activity of NAD(P)H oxidase, a potent reactive oxygen species (ROS)-producing enzyme in skeletal muscle, were increased in quadriceps by 27% and 170%, respectively (P < 0.05, Figure 1A and C). Neither protein expression nor activity of NAD(P)H oxidase was different in the diaphragm of CHF and sham (Figure 1B and D).

No difference was evident between CHF and sham rats for catalase activity in the quadriceps (Figure 2A) or in the diaphragm (Figure 2B). Interestingly, however, GPX activity was reduced by 36% in the quadriceps muscle (P < 0.05; Figure 2C) but increased by 36% in the diaphragm of CHF rats compared with shams (P < 0.01; Figure 2D). Although total SOD activity was not different between groups in the quadriceps (Figure 2E) and diaphragm (Figure 2F), the activity of Mn-SOD was severely reduced by 93% in the quadriceps muscle (P < 0.001; Figure 2G) but increased by 278% in the diaphragm of CHF rats compared with shams (P < 0.05; Figure 2H).
Parameters of oxidative and glycolytic metabolism

In the quadriceps, a significant increase in LDH activity by 28% was evident in CHF rats compared with shams \((P < 0.05; \text{Figure 3A})\), whereas no significant differences were found in the diaphragm \((\text{Figure 3B})\). Activity of COX, an indicator of aerobic metabolism, was reduced by 53% in the quadriceps muscle of CHF animals compared with shams \((P < 0.01; \text{Figure 3C})\) but was not significantly different in the diaphragm \((\text{Figure 3D})\). To determine whether reduced COX activity was the result of quantitative rather than qualitative impairments, we assessed the activity of CS—an indicator of mitochondrial volume density. There were no significant changes in CS activity in both quadriceps muscle \((\text{Figure 3E})\) and diaphragm \((\text{Figure 3F})\). Consequently, COX to CS ratio was significantly reduced by 31% in the quadriceps muscle of CHF animals \((P < 0.05; \text{Figure 3G})\), indicating a blunted aerobic energy production, whereas no significant difference was detectable in the diaphragm between both groups \((\text{Figure 3H})\).

Catabolic activation via E3 ligases and the proteasome system and messenger RNA expression of an anabolic marker

Protein expression of MuRF-1 was increased by 346% in the quadriceps \((P < 0.01; \text{Figure 4A})\) and by 115% in the diaphragm of CHF rats compared with shams \((P < 0.05; \text{Figure 4B})\). In contrast, MAFbx protein expression was decreased by 33% in the quadriceps muscle of CHF rats compared with shams \((P < 0.05; \text{Figure 4C})\), whereas no differences were observed in the diaphragm between groups \((\text{Figure 4D})\).

Two different proteasome activities were measured. In quadriceps, chymotrypsin-like activity was significantly increased in CHF compared with sham by 79% \((P < 0.05; \text{Figure 5A})\), whereas trypsin-like activity only showed a trend towards increased activity in CHF animals by 48% \((P = 0.09; \text{Figure 5C})\). In the diaphragm of CHF animals, chymotrypsin-like activity was also significantly increased compared with sham by 567% \((P < 0.01; \text{Figure 5B})\). This dramatic increase was mainly caused by a very low activity in the diaphragm of the controls. Trypsin-like activity was unchanged in the diaphragm \((\text{Figure 5D})\).

The local messenger RNA expression of the anabolic protein IGF-1 was not different between CHF rats compared with shams in the quadriceps or diaphragm \((\text{Figure 4E and F})\).

Correlation analysis

In the quadriceps muscle, activity of Mn-SOD, COX, and MuRF-1 protein expression correlated to parameters of left ventricular remodelling and dysfunction: \([\text{Mn-SOD: LV-ESD } r = -0.79, P < 0.01], \text{LV-EF } r = 0.85, P < 0.001]; \text{COX: LV-ESD } r = -0.59, P = 0.01], \text{LV-EF } r = 0.69, P < 0.01]; \text{MuRF-1: LV-ESD } r = 0.70, P < 0.05], \text{LV-EF } r = -0.74, P < 0.001)]. Furthermore, an impaired antioxidative capacity as measured by Mn-SOD activity was
correlated to an elevated MuRF-1 protein content in quadriceps \( (r = -0.54, P < 0.05) \).

Manganese-SOD activity and MuRF-1 protein expression in the diaphragm were correlated to impaired left ventricular ejection fraction (Mn-SOD: \( r = -0.63, P < 0.05 \); MuRF-1: \( r = -0.51, P = 0.05 \)).

For further univariate correlation analyses, please refer to Table S1.

### Discussion

The present study demonstrates that significant muscle heterogeneity exists between the diaphragm and quadriceps in terms of antioxidative capacity and energy metabolism but similar activation of protein degradation pathways following the onset of CHF. Compared with shams, the occasionally active limb muscle (i.e. quadriceps) was seen to be more impaired compared with the constantly active diaphragm in terms of antioxidant and metabolic capacities following the development of CHF, which confirms that the diaphragm is likely more protected against a deconditioning effect. However, the diaphragm still also demonstrated a shift towards increased catabolism, despite an elevated antioxidant capacity and normal metabolism. Collectively, therefore, these data provide evidence supporting the notion that a peripheral muscle specific myopathy exists in CHF, where even constantly active muscles such as the diaphragm are vulnerable.
Is a training effect induced in the diaphragm during chronic heart failure?

The increase in radical scavenger enzyme activity in the diaphragm observed in the present study is similar to that reported in the peripheral muscles of CHF patients following an exercise intervention. Linke and colleagues observed a decreased GPX activity in the vastus lateralis of CHF patients at baseline, which increased by ~40% after 6 months of aerobic exercise training. Furthermore, exercise training increases GPX and Mn-SOD activities in the diaphragm of healthy mice and rats.

Regarding ROS production, we investigated activity and protein expression of NAD(P)H oxidase, which increased in the quadriceps of CHF animals but not in the diaphragm. These data indicate that there might be higher ROS concentrations in quadriceps due to enhanced production (via NAD(P)H) and diminished detoxification (lower GPX and Mn-SOD activities). In contrast, the diaphragm might be protected against ROS accumulation due to its constant activity that likely increased the antioxidative capacity and prevented an increased ROS production, at least via NAD(P)H oxidase. In the vasculature, exercise training has been proven to reduce NAD(P)H expression and activity. The unchanged activity of NAD(P)H oxidase in the diaphragm of CHF animals is contrary to the findings following an acute inflammatory challenge or immediately after acute myocardial infarction where an increased NAD(P)H activity was observed. However, the mechanisms leading to diaphragm dysfunction may change during the development of CHF.
With regard to energy metabolism, no significant changes in the enzyme activity of COX, CS, and LDH were detectable in the diaphragm of CHF animals compared with shams in our study. Only a few studies have investigated the topic of oxidative and glycolytic enzyme activities in the diaphragm of CHF patients. Tikunov and colleagues investigated human diaphragm biopsies and found a higher CS activity and decreased LDH activity in CHF relative to controls, suggesting an improvement in oxidative metabolism. In addition, De Sousa et al. described a reduced LDH activity in the diaphragm of CHF rats after 8 months of ascending aorta clipping but no significant changes in CS activity. However, in the same study in situ maximal adenosine-5-diphosphate-stimulated respiration assessed in saponin-skinned fibres was reduced by ~35%, suggesting disease-induced qualitative impairments to mitochondrial function.

In both humans and animals, a fibre-type shift towards increased type-I and decreased type-IIb fibres is described in the diaphragm in heart failure. Furthermore, the shift towards a slower, more fatigue-resistant fibre type is also seen in limb muscles of CHF subjects following an exercise intervention, which is accompanied by an improved COX activity supporting the benefits an increased breathing workload may have on fibre-type distribution and metabolism. Indeed, an increased COX activity was described several years ago in the diaphragm of healthy sheep following increased workload. Interestingly, we found unaltered COX activity in the diaphragm of our CHF animals compared with controls, which may be the result of a complex balance between up-regulation/protection (e.g. via increased breathing workload and/or antioxidative capacity) and down-regulation/inhibition (e.g. via reactive oxygen species leading to COX inhibition). Collectively, however, our data support the suggestion that the diaphragm in CHF likely experiences a training effect—although it seems these beneficial effects are not enough to fully prevent the molecular, cellular, and functional impairments that occur in the diaphragm.

Protein degradation of skeletal muscle in chronic heart failure

Catabolic wasting is a serious consequence of CHF. Activation of the ubiquitin-proteasome system has been shown to contribute to cardiac cachexia and limb muscle

---

**Figure 4** Catabolic and anabolic factors in quadriceps (left panel) and diaphragm (right panel). For detailed information refer to the text. *P < 0.05; §P < 0.01 MuRF-1, muscle ring finger 1; MAFbx, muscle atrophy F-box; IGF-1, insulin-like growth factor 1; CHF, chronic heart failure.
dysfunction.\textsuperscript{14,16} We observed increased MuRF-1 protein expression and increased proteasome activity in the diaphragm. An increase in MuRF-1 has been described in the diaphragm of CHF rats, and this was associated with reduced diaphragmatic force.\textsuperscript{31} Furthermore, a loss of myosin due to activation of caspase-3 and the proteasome was observed in those rats,\textsuperscript{32} but the protease inhibitor drug \textit{bortezomib} was able to prevent this reported catabolic activation and, thereby, partially restore force.\textsuperscript{31} In line with our results, therefore, it seems that despite the diaphragm being constantly active, this muscle group still remains relatively sensitive to the protein degradation pathway, which has an increased activity in CHF.

In contrast to the diaphragm, however, the quadriceps is only occasionally recruited—mostly for locomotion. Thus, peripheral muscles such as the quadriceps are affected not only by systemic and local alterations induced by CHF but also by inactivity, which exacerbates atrophy and exercise intolerance. The ubiquitin-proteasome system has been identified as the principal regulator of limb skeletal muscle catabolism in CHF.\textsuperscript{24} In our study, the protein expression of MuRF-1 and proteasome activity was dramatically increased in the quadriceps of CHF animals compared with controls. This increase of MuRF-1 was correlated to an impaired local antioxidative capacity. In a yeast two-hybrid system, 81 genes were identified as potential MuRF-1 targets coding for myofibrillar proteins, multiple enzymes coordinating energy metabolism, and finally for components of the ribosome.\textsuperscript{34} Therefore, elevated MuRF-1 protein expression may lead to increased protein degradation—a suggestion supported by the increased proteasome activity in our study. Furthermore, the decreased antioxidative capacity in the quadriceps (as indicated by reduced activity of GPX and Mn-SOD in the present study) may promote catabolic activation, since it is known that expression of MuRF-1 is induced by reactive oxygen species\textsuperscript{17} and inflammatory cytokines.\textsuperscript{4} Surprisingly the protein expression of MAFbx was reduced in CHF quadriceps, which may be related to a compensatory effect whereby the large increase in MuRF-1 could have down-regulated MAFbx. A previous study has shown MAFbx in CHF patients to remain unchanged, whereas MuRF-1 was increased in muscle specimens of the \textit{vastus lateralis}.\textsuperscript{14,16} With regard to anabolism, the local messenger RNA expression of IGF-1 has been reported to be reduced in the \textit{vastus lateralis} of CHF patients\textsuperscript{14}; however, this was not the case in our animal model. This may be explained by anabolic parameters being time dependent, which may only be detected later in the course of the disease.

**Conclusion**

In an animal model, we have shown that the antioxidative and metabolic capacities are heterogeneous in their response to CHF between the diaphragm and quadriceps, but similar activation of protein degradation pathways was evident in both muscles. While the quadriceps (occasionally active) had impairments to antioxidative capacity, energy metabolism, and protein balance following CHF, the diaphragm (constantly active) demonstrated not only an increased antioxidant capacity and normal metabolism but also an impaired protein balance. Collectively, therefore, our data provide further support that a peripheral muscle myopathy
exists in CHF, with the more recruited respiratory muscle only partially protected compared with the limb muscles from the systemic and deconditioning effects of this disease.

Acknowledgements

The authors certify that they comply with the ethical guidelines for authorship and publishing of the Journal of Cachexia, Sarcopenia and Muscle (von Haehling S, Morley JE, Coats AJS, and Anker SD). Ethical guidelines for authorship and publishing in the Journal of Cachexia, Sarcopenia, and Muscle. J Cachexia Sarcopenia Muscle 2010; 1:7–8).

References

1. Adams V, Döring C, Schuler G. Impact of physical exercise on alterations in the skeletal muscle in patients with chronic heart failure. Front Biosci 2008;13:302–11.
2. Adams V, Linke A, Kränkel N, Erbs S, Gielen S, Möbius-Winkler S, et al. Impact of regular physical activity on the NAD(P)H oxidase and angiotensin receptor system in patients with coronary artery disease. Circulation 2005;111:555–62.
3. Adams V, Linke A, Wisloff U, Doring C, Erbs S, Kranke N, et al. Myocardial expression of MuRF-1 and MAFbx after induction of chronic heart failure: effect on myocardial contractility. Cardiovasc Res 2007;73:120–9.
4. Adams V, Mangner N, Gasch A, Krohne C, Gielen S, Hörnig S, et al. Induction of MuRF1 is essential for TNF-[alpha]-induced loss of muscle function in mice. J Mol Biol 2008;384:48–99.
5. Anker SD, Negassa A, Coats AJ, Afzal R, Gielen S, Möbius-Winkler S, et al. Prognostic importance of weight loss in chronic heart failure and the effect of treatment with angiotensin-converting-enzyme inhibitors: an observational study. Lancet 2003;361:1077–83.
6. Bazzy AR, Kim YJ. Effect of chronic respiratory load on cytochrome-oxidase activity in diaphragmatic fibers. J Appl Physiol 1992;72:266–71.
7. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 1971;44:276–87.
8. Bergmeyer HU. Measurement of catalase activity. Biochemische Zeitschrift 1955;327: 255–8.
9. Bowen TS, Mangner N, Werner S, Gaser L, Sullivick N, Schrepper A, et al. Diaphragm muscle weakness in mice is early-onset post myocardial infarction and associated with elevated protein oxidation. J Appl Physiol 1985;59:1404–10.
10. De SE, Veksler V, Bigard X, Mateo P, Serrurier B, Ventura-Clapier R. Dual influence of disease and increased load on diaphragm muscle in heart failure. J Mol Cell Cardiol 2001;33:699–710.
11. Flohe L, Gunzler WA. Assays of glutathione peroxidase. Methods Enzymol 1994;240: 114–21.
12. Gielen S, Adams V, Linke A, Erbs S, Möbius-Winkler S, Schubert A, et al. Exercise training in chronic heart failure: correlation between reduced local inflammation and improved oxidative capacity in the skeletal muscle. Eur J Cardiovasc Prev Rehabil 2005;12:393–400.
13. Gielen S, Adams V, Möbius-Winkler S, Linke A, Erbs S, Yu J, et al. Anti-inflammatory effects of exercise training in the skeletal muscle of patients with chronic heart failure. J Am College Cardiol 2003;42:861–8.
14. Gielen S, Sandri M, Kozarek I, Kratzsch J, Teupser D, Thiery J, et al. ‘Exercise training attenuates MuRF-1 expression in the skeletal muscle of patients with chronic heart failure independent of age: the randomised Leipzig exercise intervention in chronic heart failure independent of age—RALE’ study. Eur J Cardiovasc Prev Rehabil 2004;11:609–15.
15. Hambrecht R, Fehn E, Yu J, Niewoehner D, Weigl C, Hilbrich L, et al. Effects of endurance training on mitochondrial ultrastructure and fiber type distribution in skeletal muscle of patients with stable chronic heart failure. J Am College Cardiol 1997;29:1067–73.
16. Höllriegel R, Beck EB, Linke A, Adams V, Möbius-Winkler S, Mangner N, et al. Anabolic effects of exercise training in patients with advanced chronic heart failure (NYHA IIIb): impact on ubiquitin-protein ligases expression and skeletal muscle size. Int J Cardiol 2012;157:271–7.
17. Li YP, Chen Y, Li AS, Reid MB. Hydrogen peroxide stimulates ubiquitin-conjugating activity and expression of genes for specific E2 and E3 proteins in skeletal muscle myotubes. Am J Physiol – Cell Physiol 2003;285:C806–12.
18. Linke A, Adams V, Schulze PC, Erbs S, Gielen S, Fiehn E, et al. Antioxidative effects of exercise training in patients with chronic heart failure. Increase in radical scavenger enzyme activity in skeletal muscle. Circulation 2005;112:1763–70.
19. MacFarlane NG, Darnley GM, Smith GL. Cellular basis for contractile dysfunction in the diaphragm from a rabbit infarct model of heart failure. Am J Physiol – Cell Physiol 2000;278:C739–46.
20. Mancini DM, Henson D, LaMacca J, Levine S. Respiratory muscle function and dyspnea in patients with chronic congestive heart failure. Circulation 1992;86:909–18.
21. Mangner N, Linke A, Oberbach A, Kullnick Y, Gielen S, Sandri M, et al. Exercise training prevents TNF-alpha induced loss of force in the diaphragm of mice. PLoS One 2013;8:e52274.
22. Mangner N, Matsuo Y, Schuler G, Adams V. Cachexia in chronic heart failure: endocrine determinants and treatment perspectives. Endocrine 2013;43:253–65.
23. Meyer FJ, Borst MM, Zügck C, Kirschke A, Schellberg D, Kubler W, et al. Respiratory muscle dysfunction in congestive heart failure: clinical correlation and prognostic significance. Circulation 2001;103:2153–8.
24. Murton AJ, Constantin D, Greenhall PL. The involvement of the ubiquitin proteasome system in human skeletal muscle remodeling and atrophy. BBA 2008;1782:730–43.
25. Oh-ishi S, Kizaki T, Ooikawa T, Sakurai T, Izawa T, Nagata N, et al. Endurance training improves the resistance of rat diaphragm to exercise-induced oxidative stress. Am J Respir Crit Care Med 1997;155:795–81.
26. Peters DG, Mitchell HL, McCune SA, Park S, Williams JH, Kandarian SC. Skeletal muscle sarcoplasmic reticulum Ca2+-ATPase gene expression in congestive heart failure. Circulation Res 1997;81:703–10.
27. Powers SK, Criswell D, Lawler J, Martin D, Ji LL, Herb RA, et al. Regional training-induced alterations in diaphragmatic oxidative and antioxidant enzymes. Respiration 1994;59:227–37.
28. Srere PA. Reactions on the cycle: citrate synthase. In Colowick, S.P. and Kaplan, N.O. (eds) Methods in Enzymology. New York: Academic Press; 1969. pp. 3–5.

29. Tikunov B, Levine S, Mancini D. Chronic congestive heart failure elicits adaptations of endurance exercise in diaphragmatic muscle. Circulation 1997;95:910–6.

30. Torre-Amione G, Kapadia SR, Benedict C, Oral H, Young JB, Mann DL. Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: a report from the studies of left ventricular dysfunction (SOLVD). J Am College Cardiol 1996;27:1201–6.

31. van Hees HWH, Li YP, Ottenheijm CAC, Jin B, Pigmans CJC, Linkels M, et al. Proteasome inhibition improves diaphragm function in congestive heart failure rats. Am J Physiol – Lung Cell Mol Physiol 2008;294: L1260–8.

32. van Hees HWH, van der Heijden HFM, Ottenheijm CAC, Heunks LMA, Pigmans CJC, Verheugt FWA, et al. Diaphragm single-fiber weakness and loss of myosin in congestive heart failure rats. Am J Physiol – Heart Circulatory Physiol 2007;293: H819–28.

33. Wisloff U, Loennechen JP, Currie S, Smith GL, Ellingsen O. Aerobic exercise reduces cardiomyocyte hypertrophy and increases contractility, Ca2+ sensitivity and SERCA-2 in rat after myocardial infarction. Cardiovascular Res 2002;54:162–74.

34. Witt SH, Granzier H, Witt CC, Labeit S. Murf-1 and Murf-2 target a specific subset of myofibrillar proteins redundantly: towards understanding Murf-dependent ubiquitination. J Mol Biol 2005;350:713–22.

35. Wong E, Selig S, Hare DL. Respiratory muscle dysfunction and training in chronic heart failure. Heart, Lung & Circulation 2011;20:289–94.