ORIGINAL RESEARCH ARTICLE

Muscular changes in animal models of heart failure with preserved ejection fraction: what comes closest to the patient?

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Abstract

Aims   Heart failure with preserved ejection fraction (HFpEF) is associated with reduced exercise capacity elicited by skeletal muscle (SM) alterations. Up to now, no clear medical treatment advice for HFpEF is available. Identification of the ideal animal model mimicking the human condition is a critical step in developing and testing treatment strategies. Several HFpEF animals have been described, but the most suitable in terms of comparability with SM alterations in HFpEF patients is unclear. The aim of the present study was to investigate molecular changes in SM of three different animal models and to compare them with alterations of muscle biopsies obtained from human HFpEF patients.
Methods and results   Skeletal muscle tissue was obtained from HFpEF and control patients and from three different animal models including the respective controls—ZSF1 rat, Dahl salt-sensitive rat, and transverse aortic constriction surgery/deoxycorticosterone mouse. The development of HFpEF was verified by echocardiography. Protein expression and enzyme activity of selected markers were assessed in SM tissue homogenates. Protein expression between SM tissue obtained from HFpEF patients and the ZSF1 rats revealed similarities for protein markers involved in muscle atrophy (MuRF1 expression, protein ubiquitinylation, and LC3) and mitochondrial metabolism (succinate dehydrogenase and malate dehydrogenase activity, porin expression). The other two animal models exhibited far less similarities to the human samples.
Conclusions   None of the three tested animal models mimics the condition in HFpEF patients completely, but among the animal models tested, the ZSF1 rat (ZSF1-lean vs. ZSF1-obese) shows the highest overlap to the human condition. Therefore, when studying therapeutic interventions to treat HFpEF and especially alterations in the SM, we suggest that the ZSF1 rat is a suitable model.

Introduction

About 50% of heart failure patients are afflicted with heart failure with preserved ejection fraction (HFpEF) exhibiting exercise intolerance [due to alterations in the skeletal muscle (SM)],[1] congestion, oedema, and increased fibrosis, a figure that is projected to increase due to the changing risk factor landscape, in particular the ageing population. Comparison of the overall mortality between patients with HFpEF and patients with heart failure with reduced ejection fraction

(HFrEF) is discussed controversial, some reporting similar rates of hospitalization,[2,3] whereas others report higher rates in HFrEF.[4–6] Patients with HFpEF have more comorbid conditions such as hypertension, diabetes mellitus, and obesity (reviewed in Gevaert *et al.*[7]). Unfortunately, classic treatment regimens that have proven effective in patients with HFrEF have failed to improve survival in HFpEF.[8–10] Up to now, no clear medical treatment advice for HFpEF is available.

The urgent clinical symptom of heart failure patients (HFrEF and HFpEF) is exercise intolerance leading to reduced quality of life.[11–13] Research during the last years confirmed that reduced exercise capacity is at least partially related to alterations in the peripheral SM, including molecular changes like the switch of the fibre type, muscle atrophy, and mitochondrial energy production (reviewed in Adams *et al.*[14]). Recent studies revealed that exercise training could improve those alterations in the peripheral SM of both, HFrEF,[15–18] and HFpEF.[19]

The limited number of authentic HFpEF animal models poses a major limitation for the investigation of its pathophysiology and potential therapies for HFpEF. Available rodent models of HFpEF, including the ageing accelerate mouse,[20] the Dahl salt-sensitive (DSS) rat,[19,21] the Zucker fatty/spontaneously hypertensive heart failure F1 hybrid (ZSF-1) rat,[22,23] the db/db mouse,[24] and the transverse aortic constriction (TAC) surgery/deoxycorticosterone acetate (TAC/DOCA) mouse[25] have been described. However, which one is most suitable in terms of comparability with SM alterations in HFpEF patients remains unclear.

Therefore, aim of the present study was to investigate molecular changes in SM of 3 different animal models and to compare them with alterations occurring in muscle biopsies obtained from human HFpEF patients. This study helps identifying the most suitable animal model mimicking the condition in HFpEF patients with respect to SM alterations.

Methods and Materials

Patients and animal models

Heart failure with preserved ejection fraction patients and healthy controls

HFpEF patients, analysed in the present study (*n* = 15), were randomly selected from the subgroup of HFpEF patients included into the OptimEx trial[26] of which SM biopsy specimens of the quadriceps muscle were available at the begin of the study (*n* = 42). The healthy control group (*n* = 9) included patients, who were randomly selected from the healthy control group randomized for the LEICA trial.[27] Biopsies of the quadriceps muscle were available at the beginning of the study. Patients recruited for the OptimEx trail had to be stable and

under optimal medication for the last 6 weeks (detailed patient inclusion criteria see clinicaltrainls.gov—NCT02078947).

Transverse aortic constriction surgery/deoxycorticosterone acetate mouse model

Male C57BL/6J mice at 12 weeks of age underwent TAC surgery with concomitant DOCA pellet implantation (*n* = 7). Sham operated mice with placebo pellet implantation served as control mice (*n* = 6). Minimally invasive TAC was performed as described previously with modification.[25] In brief, mice were anaesthetized using a single injection of ketamine and xylazine (120 and 12 mg/kg, intraperitoneal), and a 5-mm horizontal incision was made at the first left intercostal space. The thymus was temporarily retracted to visualize the aortic arch, and a 7–0 silk suture was passed under the aorta between the right innominate and left carotid arteries. The suture was ligated around a blunted 27-gauge needle, and the needle was quickly removed. Sham animals underwent the same procedure without ligation around the aorta. The chest wall and skin were closed. An additional incision was made in the right flank of the animal, and a subcutaneous pocket was created by blunt dissection. A DOCA (DOCA 50 mg per pellet, 21 d release) or placebo pellet (Innovative Research of America) was implanted. The skin was closed, and the mice were allowed to recover in a ThermoCare warmer. Sham animals underwent the same procedure without ligation around the aorta or implantation of a DOCA pellet. Four weeks after TAC/DOCA or sham operation, the animals were sacrificed and the extensor *digitorum longus* (EDL) muscles were removed and snap frozen in liquid nitrogen.

Dahl salt-sensitive rat model

Female DSS rats (*n* = 23) were randomized at the age of 7 week into the following groups: (i) control: fed with a chow diet containing 0.3% NaCl (*n* = 12); (ii) HFpEF: fed with a chow diet containing 8% NaCl (*n* = 11). Rats were exposed to identical conditions in a 12 h light–dark cycle, with food and water provided ad libitum. After 20 weeks, echocardiography and invasive haemodynamic measurements were performed to elucidate the degree of diastolic dysfunction. Rats were subsequently sacrificed, and SM tissue (EDL muscle) was removed and snap frozen in liquid nitrogen for molecular analysis. All procedures and experiments were approved by the Norwegian Council for Animal Research, which was in accordance with Use of Laboratory Animals by the European Commission Directive 86/609/EEC.

ZSF1 rat model

Female ZSF1 lean (control, *n* = 14) and ZSF1 obese (*n* = 14) rats were purchased from Charles River (Charles River Laboratories, USA) and included into the study. Rats were exposed to identical conditions in a 12 h light–dark cycle, with food and water provided ad libitum. At the age of 32 weeks the development of HFpEF was confirmed by

echocardiography and invasive haemodynamic measurements. Rats were subsequently sacrificed, and SM tissue (EDL muscle) was removed and snap frozen in liquid nitrogen for molecular analysis. All animal procedures were approved by the local animal research council, TU Dresden, and the *Landesbehörde Sachsen* (TVV 42/2018).

A schematic summary of all animal and patient groups included into the present study is shown in *Figure 1*.

Protein expression

For western blot analysis, SM tissue was homogenized in Relax buffer (90 mmol/L HEPES, 126 mmol/L potassium chloride, 36 mmol/L sodium chloride, 1 mmol/L magnesium chloride, 50 mmol/L EGTA, 8 mmol/L ATP, 10 mmol/L creatine phosphate, pH 7.4) containing a protease inhibitor mix (Inhibitor mix M, Serva, Heidelberg, Germany) and sonicated. Protein concentration was determined (BCA assay, Pierce, Bonn, Germany) and aliquots (5–20 μg) were separated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis. Proteins were transferred to a polyvinylidene fluoride membrane and incubated overnight at 4°C using the following primary antibodies: Mitochondrial porin (1:1000; Abcam, Cambridge, United Kingdom), MuRF-1 (1:1000; Abcam), Mafbx (1:1000; Abcam), PGC1-α (1:200; Santa Cruz,

Heidelberg, Germany), Telethonin (1:1000; Abcam), gp91 (1:1000; Abcam), ubiquitin linkage-specific K48 (1:1000; Abcam), superoxide dismutase (SOD) 1 (1:200; Santa Cruz, Heidelberg, Germany), SOD 2 (1:200; Santa Cruz, Heidelberg, Germany), LC-3B (1:1000; Sigma-Aldrich, St. Louis, Missouri), Myosin heavy chain (MHC) (1:1000; Sigma-Aldrich, St. Louis, Missouri), TNFα (1:500, Thermo Fisher Scientific, Waltham USA). Membranes were subsequently incubated with a horseradish peroxidase-conjugated secondary antibody and specific bands were visualized by enzymatic chemiluminescence (Super Signal West Pico, Thermo Fisher Scientific Inc., Bonn, Germany). Bands were densitometrically quantified using a Bio1D software package (Version 15.08b. Vilber Lourmat, France). Measurements were normalized to GAPDH (1/30000; HyTest Ltd, Turku, Finland). All data are presented as fold change relative to the respective control group.

Enzyme activity measurements

For enzyme activity measurements, the SM tissue was homogenized in Relax buffer and aliquots were used for enzyme activity measurements. Enzyme activities for lactate dehydrogenase (LDH), malate dehydrogenase (MDH), creatine kinase (CK), succinate dehydrogenase (SDH) were measured spectrophotometrically as previously described in detail.[28–31]

Figure 1 An overview of the study design. Skeletal muscle biopsies (Quadriceps muscle) obtained from heart failure with preserved ejection fraction (HFpEF) patients and healthy controls were compared with skeletal muscle tissue [extensor *digitorum longus* (EDL) muscle] obtained from three different HFpEF animal models: ZSF1 rat model (control: ZSF1-lean rats; HFpEF: ZSF1-obese rats, 31 weeks); Dahl salt-sensitive (DSS) rats [control: low salt (0.3%) in drinking water, HFpEF: high salt (8%) in drinking water for 21 weeks]; TAC/DOCA mouse model (control: sham operated mouse, HFpEF: TAC operations with DOCA releasing pellet for 4 weeks). TAC, transverse aortic constriction; DOCA, deoxycorticosterone acetate.
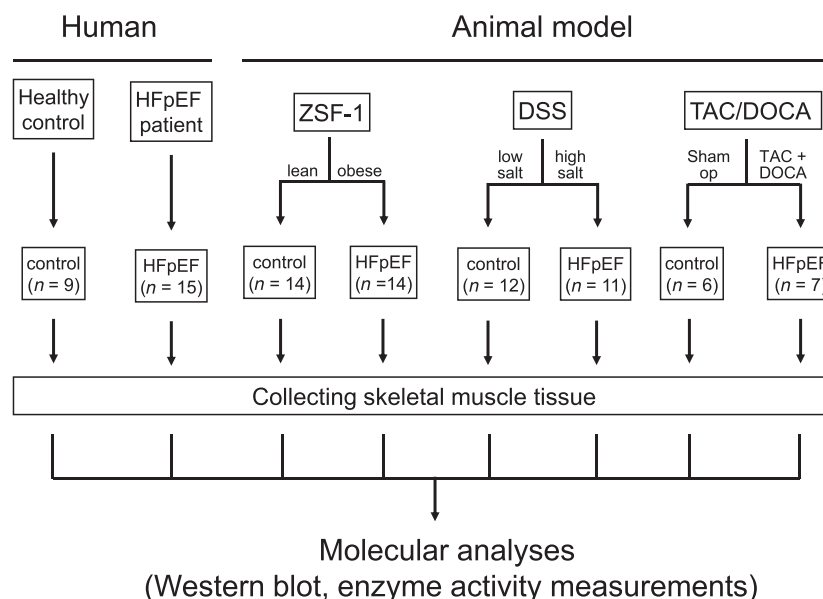
Enzyme activity data are presented as the fold change relative to the respective control group.

Statistical analysis

Data are presented as mean ± SEM. Differences between the HFpEF and the respective control group were analysed using Student t-test. Analyses were performed by GraphPad Instat. *P*-values <0.05 were considered statistically significant.

Results

Patient and animal characteristics

Characteristics of all subjects included into the present comparison document that patients and animals exhibit clear signs of HFpEF compared with their respective controls (*Table 1*).

In the human cohort, HFpEF patients presented diastolic dysfunction (E/é ratio 20.4 ± 1.8 vs. 8.2 ± 0.8, $P < 0.001$) and raised levels of circulating NT-pro brain natriuretic peptide (1,673 ± 671 vs. 73 ± 1, $P < 0.05$) compared with the healthy control group. No differences were observed in age, body mass index, systolic blood pressure, presence of diabetes mellitus and, most importantly, left ventricular ejection fraction (LVEF 64 ± 1 vs. 63 ± 1).

Regarding the HFpEF cohorts of the 3 different animal models, all presented with a significantly elevated heart weight, a higher mean arterial pressure, an increased E/é ratio and an elevated left ventricular end-diastolic pressure (LVEDP) when compared with the controls. It has to be mentioned, that HFpEF-groups of all animal models presented with a reduced LVEF; however, still in the preserved range of >50%. With respect to exercise intolerance a reduced VO_{2-}max was evident in the ZSF1-obese (ZSF1-lean: 49.1 ± 0.8 vs.

Table 1 Patient and animal characteristics

Human samples	Control; $n = 9$	HFpEF patient; $n = 15$
Age (years)	64.0 ± 2.7	69.7 ± 1.6
BMI (kg/m^2)	27.8 ± 1.0	33.1 ± 1.4**
Systolic BP (mmHg)	132 ± 3	138 ± 3
NT-pro BNP (pg/mL)	71 ± 2	915 ± 198***
Diabetes mellitus (%)	11.1	33.3
LVEF (%)	64 ± 2	64 ± 1
E/é ratio	9.6 ± 0.9	20.1 ± 1.3***

ZSF1 rat	Control (ZSF1-lean); $n = 14$	HFpEF (ZSF1-obese); $n = 14$
Body weight (g)	265 ± 4	559 ± 9***
Heart weight (mg/mm TL)	23.54 ± 0.31	35.51 ± 0.54***
Mean arterial BP (mmHg)	104.8 ± 5.6	135.6 ± 3.0***
LVEF (%)	79.2 ± 1.1	74.3 ± 1.2**
E/é ratio	21.63 ± 0.41	27.87 ± 0.65 ***
LVEDP (mmHg)	15.26 ± 0.86	21.92 ± 1.45 ***

Dahl salt-sensitive (DSS) rat	Control (low salt); $n = 12$	HFpEF (high salt); $n = 11$
Body weight (g)	292 ± 5	283 ± 5
Heart weight (mg)	940 ± 10	1,250 ± 20**
Systolic BP (mmHg)	149 ± 5	213 ± 5**
LVEF, %	82.7 ± 1.1	69.2 ± 1.8*
E/é ratio	10.6 ± 1.5	19.4 ± 1.1 *
LVEDP, mmHg	5.6 ± 0.9	13.0 ± 2.6 *

TAC/DOCA mouse	Control $n = 6$	HFpEF, $n = 7$
Body weight (g)	29.7 ± 0.5	28.9 ± 0.8
Heart weight (mg/mm TL)	7.15 ± 0.12	9.76 ± 0.21***
Systolic BP (mmHg)	87.9 ± 1.4	127.4 ± 2.5***
LVEF (%)	53.4 ± 1.0	50.9 ± 0.9
E/é ratio	34.81 ± 1.45	42.70 ± 1.70**
LVEDP (mmHg)	3.32 ± 0.48	6.23 ± 0.63**

BMI, body mass index; BNP, brain natriuretic peptide; BP, blood pressure; HFpEF, heart failure with preserved ejection fraction; LVEDP, left ventricular end-diastolic pressure; LVEF, left ventricular ejection fraction; TAC/DOCA, transverse aortic constriction/deoxycorticosterone acetate.
*$P < 0.05$,
**$P < 0.01$.
***$P < 0.001$ vs. control.

ZSF1-obese: 38.1 ± 1.2; $P < 0.001$)[23] and a reduced exercise capacity was detected in the DSS rats feed wit high salt.[32] Also the TAC/DOCA mice exhibited exercise intolerance since maximal running distance was significantly impaired as reported earlier.[25]

Anabolic proteins

Expression levels of proteins involved in protein degradation, either via the ubiquitin proteasome system (MuRF1 or MafBx) or the autophagy pathway (LC3-I/LC3-II ratio) were analysed (*Figure 2*). Similar patterns were observed between the human SM and the SM obtained from ZSF1 rats (*Figure 2* A-D). Regarding the protein expression of MuRF1 (*Figure 2A*), a significant elevation was seen in the HFpEF samples obtained either from humans (con: 1.00 ± 0.19 vs. HFpEF: 1.76 ± 0.22; $P < 0.05$ x-fold vs. con), ZSF1 (con: 1.00 ± 0.06 vs. HFpEF: 1.22 ± 0.07 x-fold vs. con; $P < 0.05$) and DSS rats (con: 1.00 ± 0.05 vs. HFpEF: 1.15 ± 0.03 x-fold vs. con; $P < 0.05$) compared with the control samples, respectively. No increase in MuRF1 protein expression was evident in the TAC/DOCA HFpEF model.

Regarding the protein expression of MafBx (*Figure 2B*), only the human SM showed enhanced expression levels (con: 1.00 ± 0.09 vs. HFpEF: 1.45 ± 0.15 x-fold vs. con; $P < 0.05$). None of the animal models of HFpEF presented an altered MafBx protein expression.

The main task of MuRF1 and MafBx is to attach ubiquitin molecules to proteins, thereby marking them for degradation by the UPS system. Therefore, we analysed the expression of ubiquitinylated SM proteins. As shown in *Figure 2C*, a significant increase of ubiquitinylated proteins was seen in the HFpEF group of humans (con: 1.00 ± 0.05 vs. HFpEF: 1.15 ± 0.05 x-fold vs. con; $P < 0.05$) and ZSF1 rats (con: 1.00 ± 0.09 vs. HFpEF: 1.44 ± 0.11 x-fold vs. con; $P < 0.05$). A trend was observed in the TAC/DOCA HFpEF model ($P = 0.06$), whereas no change was evident in the DSS HFpEF model.

Besides the UPS system, autophagy is another important protein degradation pathway, with LC3-I and LC3-II being markers of increased autophagy. Analysing the ratio of LC3-I/LC3-II (*Figure 2D*) a significant downregulation by 33% was observed in the human samples (con: 1.00 ± 0.15 vs. HFpEF: 0.67 ± 0.04 x-fold vs. con; $P < 0.05$). A tendency of a lower LC3-I/LC3-II ratio was also seen in the ZSF1 model

Figure 2 Protein expression of MuRF1 (A), MafBx (B), protein ubiquitinylation (C) and LC3 (D) was quantified by western blot analysis in skeletal muscle tissue obtained from humans [heart failure with preserved ejection fraction (HFpEF) and controls] and three different HFpEF animal models [ZSF1, DSS, transverse aortic constriction/deoxycorticosterone acetate (TAC/DOCA)]. Values are shown as mean ± SEM expressed as *x*-fold vs. control. Statistical comparison was made between control and HFpEF of the respective model. Representative examples of Western blots are depicted on top of the figure (C, control; H, HFpEF).
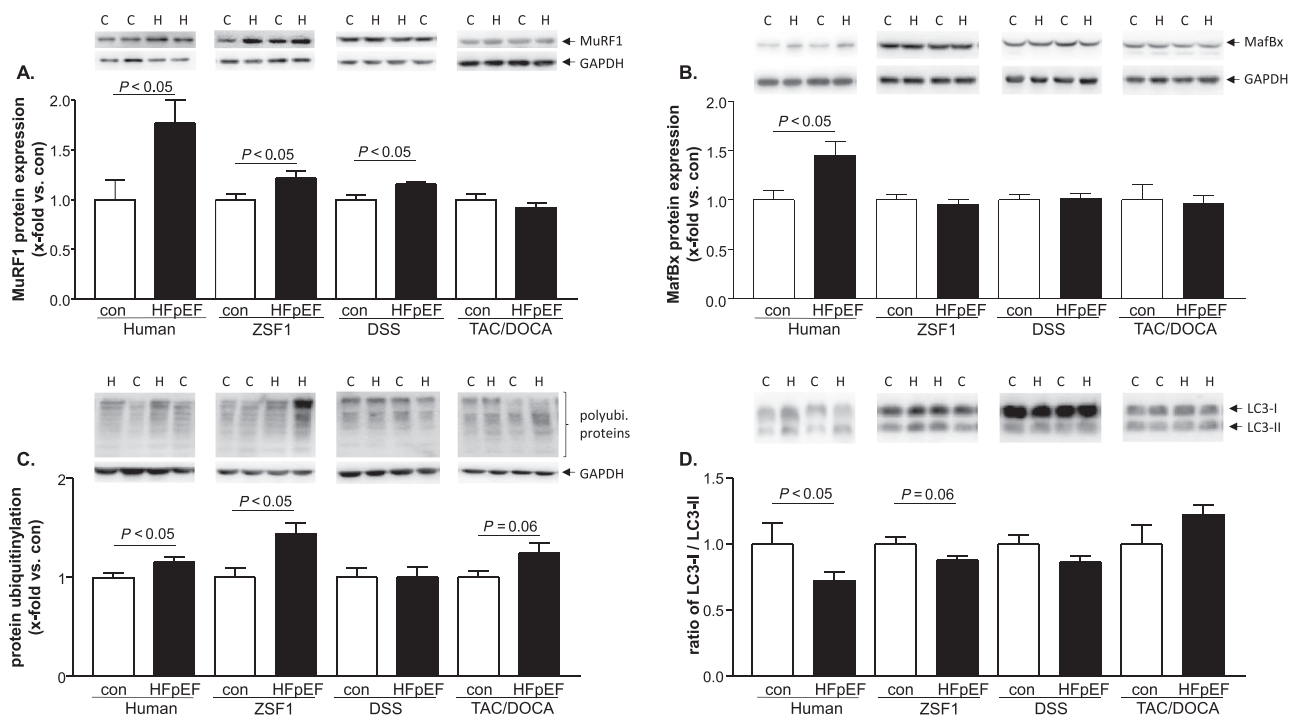
(con: 1.00 ± 0.05 vs. HFpEF: 0.85 ± 0.02 x-fold vs. con; P = 0.06). No difference was detected in the DSS and TAC/DOCA model of HFpEF.

Structural proteins

Quantifying the protein expression of structural proteins like myosin heavy chain (MHC, *Figure 3A*), actin (*Figure 3B*) and telethonin (*Figure 3C*) a significant upregulation was only detected for actin (con: 1.00 ± 0.03 vs. HFpEF: 1.17 ± 0.06 x-fold vs. con; P < 0.05) and telethonin (con: 1.00 ± 0.05 vs. HFpEF:

1.34 ± 0.11 x-fold vs. con; P < 0.05) in the HFpEF group of the DSS rat. In the human samples as well as in SM samples obtained from the ZSF1 and TAC/DOCA animals no changes were observed (*Figure 3* A-C).

Mitochondrial proteins and metabolic enzymes

The assessment of mitochondria and enzymes involved in energy production and transfer in the SM revealed similarities between alterations in humans and ZSF1 animals (*Figure 4*). The expression of porin, a protein of the outer mitochondrial

Figure 3 Protein expression of myosin heavy chain (A), actin (B), and telethonin (C) was quantified by western blot analysis in skeletal muscle tissue obtained from humans [heart failure with preserved ejection fraction (HFpEF) and controls] and three different HFpEF animal models [ZSF1, DSS, and transverse aortic constriction/deoxycorticosterone acetate (TAC/DOCA)]. Values are shown as mean ± SEM expressed as x-fold vs. control. Statistical comparison was made between control and HFpEF of the respective model. Representative examples of Western blots are depicted on top of the figure (C, control; H, HFpEF).
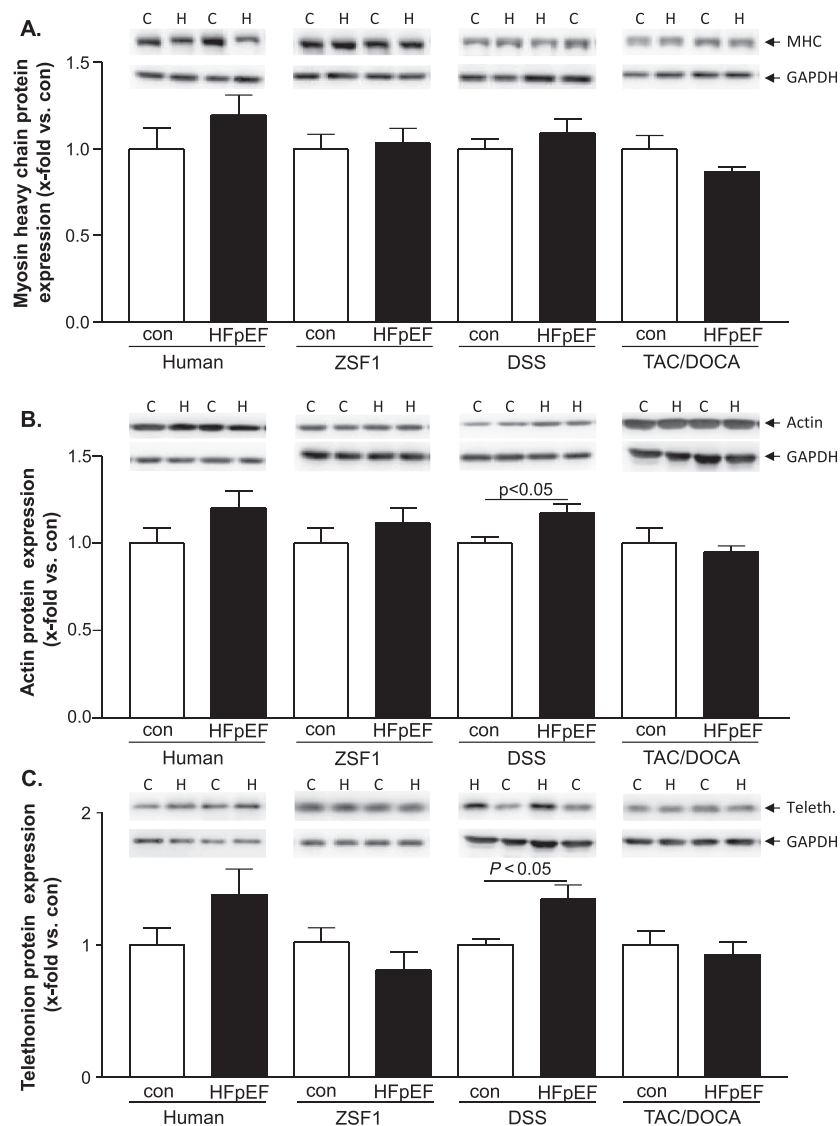
Figure 4 Protein expression of porin (A) and PGC1a (B) was quantified by western blot analysis in skeletal muscle tissue obtained from humans [heart failure with preserved ejection fraction (HFpEF) and controls] and three different HFpEF animal models [ZSF1, Dahl salt-sensitive (DSS), transverse aortic constriction/deoxycorticosterone acetate (TAC/DOCA)]. In addition, specific enzyme activity of succinate dehydrogenase (SDH) (C), malate dehydrogenase (MDH) (D), creatine kinase (CK) (E) and lactate dehydrogenase (LDH) (F) was measured. Values are shown as mean ± SEM expressed as x-fold vs. control. Statistical comparison was made between control and HFpEF of the respective model. Representative examples of Western blots are depicted on top of the figure (C, control; H, HFpEF).
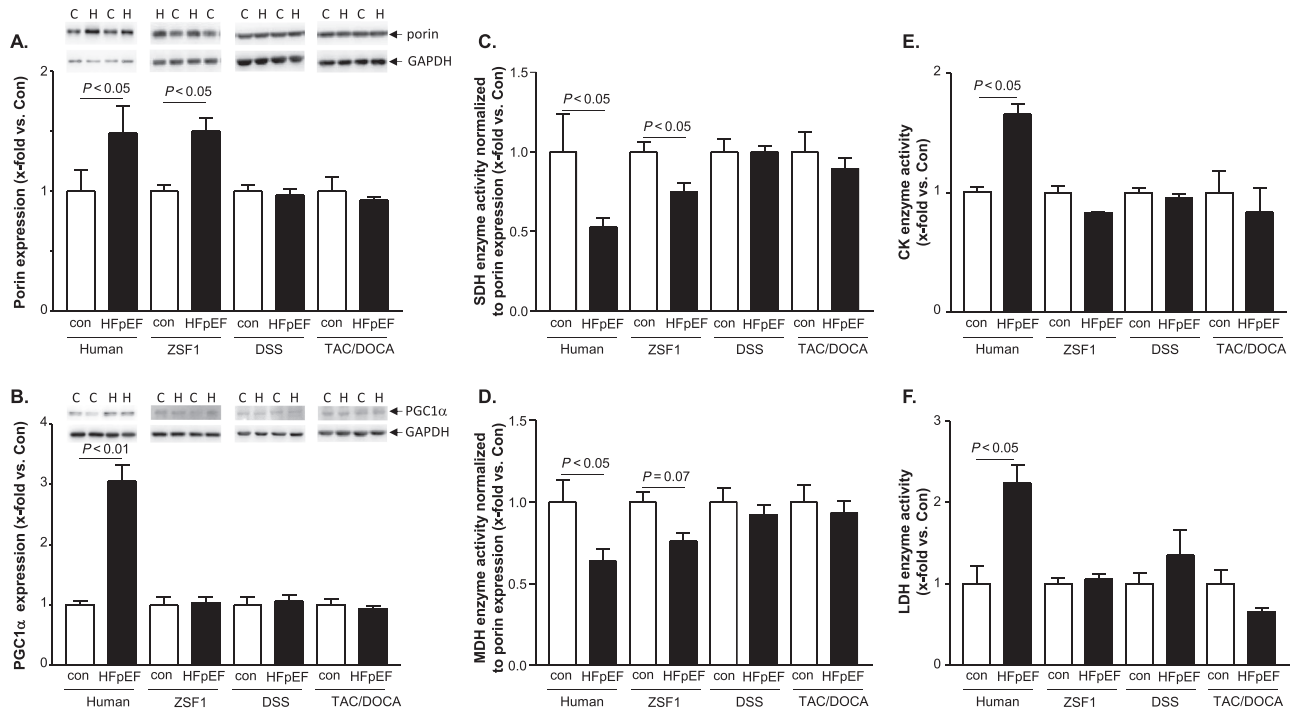
membrane and marker to estimate mitochondrial content, was significantly elevated in SM samples of human HFpEF and ZSF1 rats (48% and 50%, respectively), whereas no changes were observed between control and HFpEF in DSS and TAC/DOCA animals (*Figure 4A*). In the human HFpEF samples, the increased mitochondria content might be triggered by enhanced PGC1α expression while no changed PGC1α expression was detected in the SM across all 3 animal models (*Figure 4B*). Enzyme activities of SDH and MDH revealed a significant decrease in SM samples obtained from HFpEF patients (*Figure 4C, D*). This decrease was also observed in HFpEF samples of ZSF1 rats (*Figure 4C, D*). With respect to creatine kinase (*Figure 4E*) and LDH (*Figure 4F*) a significant increase was only detected in human SM samples of HFpEF patients. None of the animal models showed alterations between control and HFpEF (*Figure 4E, F*).

## Proteins involved in reactive oxygen species production and inflammation

The presence of reactive oxygen species (ROS) represents an imbalance of ROS production and clearance by scavenger proteins. An important protein involved in ROS generation

is the NADPH oxidase, whereas SOD catalyzes the dismutation of superoxide anion free radical ($O_2^{\bullet-}$) into molecular oxygen and hydrogen peroxide (H_2O_2). Assessment of subunit gp91 of the NADPH-oxidase (*Figure 5A*), SOD1 (*Figure 5B*), and SOD2 (*Figure 5C*) revealed no differences between HFpEF and control in SM either from human samples or all investigated animal models. Because it is well documented in the current literature that the development of HFrEF is associated with increased inflammation in the peripheral SM,[33] we analysed protein expression of TNFα. An increase by 84% was detected in the human HFpEF SM (con: 1.00 ± 0.01 vs. HFpEF: 1.84 ± 0.31; $P < 0.05$) (*Figure 5D*) while no changes were observed in all three animal models (*Figure 5D*).

Discussion

For the investigation of novel treatment options for HFpEF, it is of upmost interest to identify suitable animal model mimicking human pathophysiology in the peripheral SM as tool for translational research. Therefore, the present study compared molecular alterations in the peripheral SM of three

Figure 5 Protein expression of NADPH oxidase subunit gp91[phox] (A) SOD1 (B), SOD2 (C) and TNFα (D) was quantified by western blot analysis in skeletal muscle tissue obtained from humans [heart failure with preserved ejection fraction (HFpEF) and controls] and three different HFpEF animal models [ZSF1, succinate dehydrogenase (DSS), transverse aortic constriction surgery/deoxycorticosterone acetate (TAC/DOCA)]. Values are shown as mean ± SEM expressed as *x*-fold vs. control. Statistical comparison was made between control and HFpEF of the respective model. Representative examples of Western blots are depicted on top of the figure (C, control; H, HFpEF).
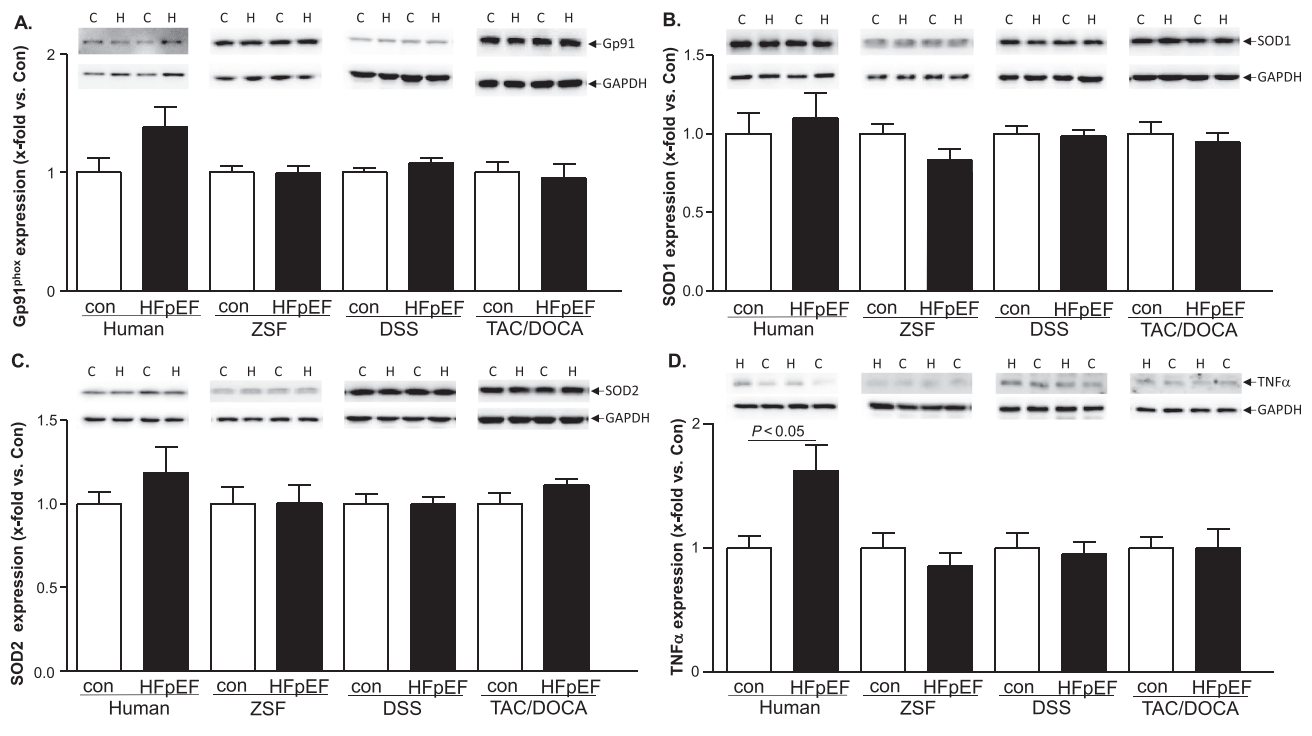
different animal models and HFpEF patients. Our results suggest that among all three animal models, the ZSF1 exhibits the best consensus with alteration observed in human samples (for summary see *Table 2*).

Heart failure with preserved ejection fraction in human subjects and animal models

The identification of authentic animal models of HFpEF is crucial to improve our understanding of underlying mechanisms towards the goal to develop new treatment strategies. For a proper comparison of alterations in the SM between human and animals, it is important to reliably verify the development of HFpEF. According to the ESC guidelines,[34] the patients included in our study were diagnosed as HFpEF due to diastolic dysfunction (assessed by elevated E/é ratio), increased levels of circulating NT-pro brain natriuretic peptide and preserved LVEF (above 60%). We analysed these parameters in the three tested animal models, which develop heart failure based on either hypertension (DSS rat and TAC/DOCA mouse), diabetes/hypertension and obesity (ZSF1 rat model). All three animal models exhibited increased E/é ratios, increased LVEDP, myocardial hypertrophy (as evident by increased heart weight), and preserved LVEF compared with

their respective controls. In addition, ZSF1-obese rats,[35] DSS rats fed with high salt diet,[32] and mice subjected to TAC and DOCA-pellet implantation[36] presented with a significantly lower exercise capacity compared with the respective control. Taken together, in all tested animal models, HFpEF was evident, and therefore, a comparability with human samples with respect to alterations in the SM was given.

Recently, an additional mouse model inducing HFpEF by feeding a high fat diet and giving L-NAME in the drinking water has been reported.[37] This model also recapitulates numerous systemic and cardiovascular features of human HFpEF, including exercise intolerance. The molecular analysis of the SM revealed no change in mRNA expression of myosin heavy chain isoforms, but an in depth molecular analysis is warranted in the future to finally conclude that in this model exercise intolerance occurred in the absence of molecular changes in the SM.

Similarities and differences between animal models and patients

Proteins involved in muscle atrophy
Skeletal muscle atrophy is a hallmark of HFpEF[14,38] and HFrEF,[39] and the ubiquitin proteasome system, as well as

Table 2 Summary of molecular alterations observed in human and animal skeletal muscle tissue

Protein	Human	ZSF1	DSS	TAC/DOCA
Anabolism				
MuRF1	↑	↑	↑	=
MAFBx	↑	=	=	=
Ubiquitination	↑	↑	=	=
LC3-I/LC3-II	↓	↓	=	=
Mitochondria				
SDH	↓	↓	=	=
MDH	↓	↓	=	=
CK	↑	=	=	=
LDH	↑	=	=	=
Porin	↑	↑	=	=
PGC1-alpha	↑	=	=	=
Structural protein				
MHC	=	=	=	=
Actin	=	=	↑	=
ROS/Inflammation				
Gp91^{phox}	=	=	=	=
SOD1	=	=	=	=
SOD2	=	=	=	=
TNF-alpha	↑	=	=	=

DSS, Dahl salt-sensitive; HFpEF, heart failure with preserved ejection fraction; LVEDP, left ventricular end-diastolic pressure; LVEF, left ventricular ejection fraction; MHC, Myosin heavy chain; ROS, reactive oxygen species; TAC/DOCA, transverse aortic constriction/deoxycorticosterone acetate.
= no change between HFpEF and control; ↑ up-regulation in HFpEF when compared with control; ↓ down-regulation in HFpEF when compared with control; grey boxes mark changes, which are similar to changes observed in human skeletal muscle.

autophagy, are the main protein degradation system activated in the SM[14]. Regarding UPS components and autophagy, our results reveal that among all three tested animal models, the ZSF1-rat acts most comparable with the human SM. In ZSF1-obese as well as in human HFpEF patients, MuRF1 is significantly upregulated, leading to an increase of ubiquitinylated proteins, which are subsequently recognized and degraded by the proteasome system. Regarding the MafBx expression, no difference was seen in all three animal models when compared with the respective controls, whereas a significant upregulation was observed in the human HFpEF samples. This is in accordance with our former study of DSS rats,[19] where no difference in MafBx expression was observed. Additionally to the observed activation of the UPS, the ratio of LC-3 I/II, a marker of autophagy, is comparable between human biopsies and SM tissue of ZSF1 obese rats. The proven down regulation of the LC3 I/II ratio in SM tissue of ZSF1 obese rats is in accordance to the findings of Bowen and colleagues.[40] In summary, with respect to proteins involved in SM atrophy, ZSF1 HFpEF rats showed the best match to changes observed in the SM of HFpEF patients (for summary see *Table 2*).

Structural proteins

In our study, for structural proteins, like MHC, actin and telethonin, no significant difference was detected between human controls and HFpEF samples. In the three animal models, only the DSS HFpEF rat showed a slightly increased expression of these proteins. For SM tissue obtained from HFpEF patients, only a shift in fibre type composition and a reduction in capillary density has been reported.[41] Until now, no reports are available regarding the expression of structural proteins for human HFpEF SM. The situation seems to be quite different in HFrEF, where a clear reduction of MHC,[42–44] and actin expression[45] have been reported. Besides reduced actin expression level, posttranslational modifications resulting in a reduced functionality have been reported.[46,47] These differences between HFpEF and HFrEF suggest that SM alterations are rather exacerbated in HFrEF, as recently postulated by Seiler and colleagues.[38]

Mitochondrial proteins

Mitochondria are central organelles in the SM as they act as important regulators of the energy supply and abnormalities can significantly contribute to impaired oxygen utilization and to exercise intolerance in HFpEF.[48] Direct assessment of mitochondrial function in a rat model of HFpEF[19] revealed an impaired coupling ratio between O_2 consumption and ATP production. In the present study, it became evident that enzymes involved in mitochondrial energy production, like SDH and MDH are significantly reduced in SM of HFpEF patients. This reduction could also be detected in the SM of ZSF1 HFpEF animals, whereas no changes were detected in the DSS rat and TAC/DOCA model. Regarding the expression of porin, we showed increased amounts in human and ZSF HFpEF samples, which is in contrast to the current literature. Molina *et al.*[49] demonstrated significantly reduced porin expression levels in muscle biopsies obtained from HFpEF patients compared with age-matched healthy controls. This discrepancy to our present study might go back to a compromised mitochondrial quality control, thereby leading to an accumulation of non-functional mitochondria (reviewed in Hammerling and Gustafsson[50]) and subsequently resulting in an increased porin expression. Furthermore, the enhanced generation of mitochondria could serve as a counter reaction of the SM to keep its important mitochondrial energy supply at a high level. At least for the human situation, this is supported by the increased expression of PGC1-α, a key transcription factor for mitochondrial biogenesis.[51] However, further investigations will be necessary for that. Taken together, mitochondrial energy metabolism seems to be impaired in SM tissue of HFpEF patients and ZSF1 obese rats, whereas no changes were observed in the other two HFpEF animal models.

Reactive oxygen species generating enzymes and inflammation

Reactive oxygen species are constantly generated in the cell by different enzymes and mostly as a by-product of mitochondrial oxidative phosphorylation.[52] In heart failure and especially in HFrEF, this homeostasis between ROS generation

and elimination is shifted towards an increased generation of ROS.[38] For HFpEF the situation is less clear, and in the present study, a trend towards increased NADPH oxidase expression was detected in the SM of HFpEF patients. The ROS load might also be increased in the SM of ZSF1 HFpEF animals because SOD1, one of the main detoxifying enzymes, was significantly reduced. We found no alterations of enzymes involved in controlling the ROS in DSS samples. This is in accordance with an earlier observation from our group, when we compared alterations in the SM of HFrEF (left anterior descending artery [LAD] ligation model) and HFpEF (DSS rat model). At least in the human HFpEF situation the increased expression of TNFα might be a trigger for increased muscle atrophy, by stimulating the expression of MuRF1, and by elevating the expression of NADPH oxidase, thereby inhibiting mitochondrial oxidative phosphorylation. In the animal models investigated in the present study, no increased TNFα expression was observed. However, further investigations have to clarify whether other inflammatory cytokines are increased in the animal models, as seen for the DSS rat model.[38]

Study limitation

Interpreting the results from the present comparison of human and animal models of HFpEF with respect to alterations of the peripheral SM we have to consider the following limitations.

First, an important limitation of the study is that the analysed animal models develop HFpEF based on one or two comorbidities like hypertension (DSS and TAC/DOCA) or metabolic syndrome (ZSF1 rat model). In the human situation, HFpEF is triggered by a variety of underlying comorbidities with different influence on SM alterations. This may be one reason why we did not found an animal model mimicking the human situation to 100% with respect to SM changes.

Second, the disease severity may differ between humans and the observed animal models, because the duration of comorbidities in patients before the onset of HFpEF might vary. Additionally, also in the animal models the time span from initiating the comorbidities until the harvest of SM tissue varies from 4 weeks (TAC/DOCA model) to 20 weeks (DSS model) and to 32 weeks (ZSF1 model). This different timing might also have an impact on the molecular alterations seen in the different models.

Third, the assessment of morphologic and functional features of mitochondria is missing in the present study. These measurements would have supported the importance of changes seen in mitochondrial enzyme activities especially in human and ZSF1 SM samples. Nevertheless, based on the current literature we have to assume that mitochondrial function is impaired in humans and animal models of HFpEF (for review refer to Adams *et al.*[14]).

Conclusions

The present study is to our knowledge the first report comparing molecular alterations in SM tissue obtained from human HFpEF patients and three different HFpEF animal models. In summary, none of the three tested animal models mimics the situation in HFpEF patients completely. Still, among the models, the ZSF1 rat (ZSF1-lean vs. ZSF1-obese) shows the highest overlap to the human condition (see *Table 2*). Therefore, when studying therapeutic interventions to treat HFpEF and especially alterations in the SM, we suggest that the ZSF1 rat is a suitable model.

Acknowledgement

Conflict of interest

Ephraim Winzer reports personal fees from Boehringer-Ingelheim, Novartis, and CVRx outside of this study.

Funding

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