Circulation: Heart Failure

ORIGINAL ARTICLE

Impact of Different Training Modalities on Molecular Alterations in Skeletal Muscle of Patients With Heart Failure With Preserved Ejection Fraction: A Substudy of the OptimEx Trial

Ephraim B. Winzer, MD; Antje Augstein, PhD; Antje Schauer, PhD; Stephan Mueller, MA; Tina Fischer-Schaepmann, BSc; Keita Goto, MD; Jennifer Hommel, PhD; Emeline M. van Craenenbroeck, MD; Ulrik Wisløff, PhD; Burkert Pieske, MD; Martin Halle, MD; Axel Linke, MD*; Volker Adams, PhD*

BACKGROUND: Exercise intolerance is a cardinal feature of heart failure with preserved ejection fraction and so far exercise training (ET) is the most effective treatment. Since the improvement in exercise capacity is only weakly associated with changes in diastolic function other mechanisms, like changes in the skeletal muscle, contribute to improvement in peak oxygen consumption. The aim of the present study was to analyze molecular changes in skeletal muscle of patients with heart failure with preserved ejection fraction performing different ET modalities.

METHODS: Skeletal muscle biopsies were taken at study begin and after 3 and 12 months from patients with heart failure with preserved ejection fraction randomized either into a control group (guideline based advice for ET), a high-intensity interval training group (HIIT) or a moderate continuous training group. The first 3 months of ET were supervised in-hospital followed by 9 months home-based ET. Protein and mRNA expression of atrophy-related proteins, enzyme activities of enzymes linked to energy metabolism and satellite cells (SCs) were quantified.

RESULTS: Exercise capacity improved 3 months after moderate continuous exercise training and HIIT. This beneficial effect was lost after 12 months. HIIT mainly improved markers of energy metabolism and the amount and function of SC, with minor changes in markers for muscle atrophy. Only slight changes were observed after moderate continuous exercise training. The molecular changes were no longer detectable after 12 months.

CONCLUSIONS: Despite similar improvements in exercise capacity by HIIT and moderate continuous exercise training after 3 months, only HIIT altered proteins related to energy metabolism and amount/function of SC. These effects were lost after switching from in-hospital to at-home-based ET.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT02078947.

Key Words: atrophy ■ energy metabolism ■ exercise training ■ satellite cells ■ skeletal muscle

See Editorial by Tucker and Kitzman

Heart failure with preserved ejection fraction (HFpEF) accounts for ≈50% of all heart failure cases and is a complex syndrome, with high morbidity and mortality rates.[1] The prevalence of HFpEF is still increasing, probably due to the aging population. A cardinal feature of HFpEF, which is also documented for heart failure with reduced ejection fraction (HFrEF), is exercise intolerance. Recent investigations suggested noncardiac peripheral factors as major contributors for impaired exercise capacity in HFpEF. Animal as well as human studies have shown various alterations being detectable in the skeletal muscle (SKM) of HFpEF ranging from lower SKM mass and strength,[2]

Correspondence to: Volker Adams, PhD, Heart Center Dresden, Technical University Dresden, Fetscherstrasse 76, 01307 Dresden, Germany. Email volker.adams@tu-dresden.de
*A. Linke and V. Adams contributed equally.
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WHAT IS NEW?

- Heart failure with preserved ejection fraction is associated with exercise intolerance and alterations in the peripheral skeletal muscle have been documented in human and experimental studies. Three month of exercise training, and especially high-intensity interval training, mainly improved markers of energy metabolism and the amount and function of satellite cells, with minor changes in markers for muscle atrophy. Only slight changes were observed after moderate continuous exercise training. Unfortunately, the beneficial molecular effects of high-intensity interval training were lost after switching from supervised training to home-based exercise training, probably due to a lower compliance rate.

WHAT ARE THE CLINICAL IMPLICATIONS?

- Independent from the training modality, high-intensity interval training or moderate continuous exercise training improved exercise capacity of patients with heart failure with preserved ejection fraction.
- Molecular changes were more pronounced when performing high-intensity interval training when compared with moderate continuous exercise training.
- To keep the molecular changes elicited by high-intensity interval training supervised training a high compliance rate is essential, because after switching to home-based exercise training, the molecular changes are lost.

Nonstandard Abbreviations and Acronyms

CK	creatine kinase
CS	citrate synthase
ET	exercise training
HFpEF	heart failure with preserved ejection fraction
HFrEF	heart failure with reduced ejection fraction
HIIT	high intensity interval training
HOA-DH	ß-hydroxyacyl-CoA dehydrogenase
LDH	lactate dehydrogenase
MCT	moderate continuous exercise training
PK	pyruvate kinase
SKM	skeletal muscle

muscle fiber atrophy,[3,4] fat infiltration,[5] lower capillary to fiber ratio,[3,5] reduced mitochondrial function and content,[4,6] and disturbed high energy phosphate metabolism.[7] In contrast to the wealth of proven therapeutic options for HFrEF, most efforts to improve morbidity and mortality in HFpEF have failed to date (for review see study by Wintrich et al[8]). To date, only the use of SGLT2 (sodium-glucose cotransporter 2) inhibitors reported positive effects.[9,10] In contrast, exercise training (ET), proven to be beneficial in HFrEF,[11–13] has also be shown effective in HFpEF to increase exercise capacity and improve quality of life (see meta-analysis by Fukuta et al[14]) Also, the recently published OptimEx-Clin trial[15] documented a significant increase in peak oxygen consumption (peak VO_2) after 3 months of supervised training independent of the modality chosen.[14] With respect to molecular changes in the SKM in HFpEF elicited by ET available data are limited. Two experimental studies[3,4] using different animal models to mimic HFpEF (ZSF1 and Dahl salt-sensitive rats) revealed that ET prevents mitochondrial and functional impairments[4] and attenuates glycolytic metabolism,[3] whereas data on muscular changes in patients with HFpEF are not available.

In the current study, SKM biopsies obtained from a subgroup of patients with HFpEF included into the OptimEx trial were analyzed for molecular alterations. SKM biopsies were collected at baseline, 3 and 12 months following either moderate continuous exercise training (MCT), high-intensity interval training (HIIT), or guideline-based advice for physical activity (control group).

MATERIALS AND METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Design

The present study is a substudy of the OptimEx-Clin trial (ClinicalTrials.gov Identifier: NCT02078947) investigating different training modalities of exercise to improve peak VO_2 in patients with HFpEF.[15] In total, 41 patients, 12 patients from the control group (Con), 15 patients from the MCT group, and 14 patients randomized into the HIIT group, in whom SKM biopsies were available at baseline and after completing 3 months of supervised in-hospital ET, were included in the current study. In addition, available SKM biopsies obtained from these patients after 12 months (home-based ET) were analyzed (Con: 9 patients, MCT: 8 patients, HIIT: 7 patients). For detailed description of the OptimEx study design, see Supplemental Material. The study was approved by the institutional review board of the University Leipzig and written informed consent was obtained from all participants.

Muscle Biopsies

Needle biopsies from the vastus lateralis muscle were done under local anesthesia according to standard procedures. Muscle biopsies were used for isolation of satellite cells, fixed in formalin, and subsequently embedded into paraffin, or immediately frozen in liquid nitrogen and stored at −80 °C until further processed.

Quantification of Protein Expression

Frozen muscle samples were homogenized in Relax buffer[16] containing a protease inhibitor mix (Inhibitor mix M, Serva, Heidelberg, Germany). Protein quantification by Western blot analysis was performed as recently described[16] using the specific antibodies listed in Table 1.

Table 1. List of Antibodies

Antibody against	Company	Dilution
MuRF1	abcam (ab183094)	1:1000
FBx32 (MAFBx)	abcam (ab168372)	1:1000
MG53 (Trim72)	abcam (ab154238)	1:1000
Ubiquitin (linkage-specific K48)	abcam (ab140601)	1:1000
Total OXPHOS Rodent Cocktail	abcam (ab110413)	1:250
GAPDH	HyTest (5G4)	1:30 000
Anti-mouse POD	Sigma (A9044)	1:5000
Anti-rabbit POD	Sigma (AP-187P)	1:5000

FBx32 indicates F-box only protein 32; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; K48, lysine residue 48; MAFBx, muscle atrophy F-box protein; MG53, mitsugumin-53; MuRF1, muscle ring finger protein 1; OXPHOS, oxidative phosphorylation; POD, peroxidase; and Trim72, tripartite motif-containing protein 72.

Measurement of Proteasome Activity

Aliquots of muscle samples homogenized in Relax buffer without protease inhibitors were used to assess proteasome activity as recently described.[17] For detailed information, see Supplemental Material.

Measurements of Enzyme Activities

Skeletal muscle tissue was homogenized in relaxing buffer and aliquots were used for enzyme activity measurements. Enzyme activities for PK (pyruvate kinase, EC 2.7.1.40), LDH (lactate dehydrogenase, EC 1.1.1.27), ß-HOA-DH (hydroxyacyl-CoA dehydrogenase, EC 1.1.1.35), CS (citrate synthase, EC 2.3.3.1), mitochondrial complex I and CK (creatine kinase, EC 2.7.3.2) were measured spectrophotometrically.[18–20]

RNA Isolation and Quantitative Real-Time Polymerase Chain Reaction

Total RNA was isolated from muscle biopsies using Qiazol reagent and miRNeasy Mini Kit (Qiagen, Hilden, Germany), and real-time polymerase chain reaction was performed following standard protocols. Primer sequences are listed in Table S1. For detailed information, see Supplemental Material.

Fiber Typing and Immunostaining of Pax7

Fiber typing and staining of Pax7 (paired box 7)-positive cells was performed on paraffin sections (3 µm) using primary antibodies for slow- and fast-twitch myosin and Pax7. For detailed information, see Supplemental Material.

Isolation and Culture of Skeletal Muscle Satellite Cells

Satellite cells were isolated from muscle specimens as described by Sente et al.[21] For detailed information, see Supplemental material.

Proliferative and Differentiation Capacity of Satellite Cells

Myoblast proliferation and viability were continuously monitored using the xCELLigence real-time cellular analysis system (OMNI Life Science, Bremen, Germany), according to the manufacturer's guidelines. For detailed information, see Supplemental Material.

Statistics

SPSS version 27.0 (IBM Corporation, Armonk) was used for all the analyses. Data are expressed as means±SEM or median (interquartile range). Normal distribution was tested applying the Shapiro-Wilk test. Comparisons of baseline variables between healthy individuals and HFpEF patients were tested with Student *t* test or Mann-Whitney *U* test where appropriate. Changes over time following ET training groups (pooled data of MCT and HIIT) and Con were testes with paired *t* test or Wilcoxon signed-rank test. One-way ANOVA or Kruskal-Wallis, as appropriate, followed by 2-sided post hoc test (Bonferroni multiple comparison test or Dunn multiple comparison test) was used to analyze differences among HFpEF groups. A P of <0.05 was considered statistically significant. All measurements were made by investigators blinded to the treatment group.

RESULTS

Study Population

Comparing the study population at baseline, no significant differences were detected between Con, MCT, and HIIT for body mass index, blood pressure, LVEF, NT-proBNP level, E/e´, and peak VO_2 (Table 2). Following 3 months of supervised in-hospital ET, a significant decrease in body mass index was evident in the HIIT group, and a significant improvement in peak VO_2 was detected in MCT and HIIT (Table 3). After additional 9 months of home-based ET (12 months after study begin), a decrease of body mass index was detectable in the HIIT group, whereas the significant change in peak VO_2 was no longer detectable in the MCT and HIIT group (Table 3).

Comparing the patients of the current substudy with the total OptimEx study population, a higher prevalence of arterial hypertension with elevated systolic blood pressure, consequently a higher use of blood pressure lowering medication, and a higher NT-proBNP and E/e´septal was observed. All other baseline variables were comparable between patients with and without SKM biopsy. The change in peak VO_2 after 3 and 12 months of ET did not differ in patients with and without SKM biopsy (Table S2).

Expression of Atrophy-Related Protein

After 3 months of ET, no significant improvements were observed for MuRF1 (muscle ring finger protein 1), MAFBx (muscle atrophy F-box protein), Trim72 (tripartite motif containing 72), ubiquitinylated proteins, and proteasome activity for the combined ET groups (Train; Figure 1A through 1E). Discriminating between different training modalities, MCT and HIIT, and comparing changes after 3 months with the changes in the control

Table 2. Characteristics of Patients With HFpEF at Baseline

Characteristic	Con (n=12)	MCT (n=15)	HIIT (n=14)	*P* value
Age, y	72 (69–74)	73 (75–77)	72 (67–75)	0.981*
Sex				
Female	9 (75)	9 (60)	11 (79)	0.508†
Male	3 (25)	6 (40)	3 (11)	
BMI, kg/m²	31 (25–35)	28 (27–34)	30 (26–32)	0.833*
Coronary artery disease	3 (25)	5 (33)	3 (11)	0.680†
Blood pressure systolic, mm Hg	132 (130–148)	130 (125–140)	128 (120–137)	0.143*
NYHA class				
II	7 (58)	8 (53)	11 (79)	0.337†
III	5 (42)	7 (47)	3 (21)	
NT-proBNP, pg/mL	525 (350–1055)	554 (276–1185)	337 (184–810)	0.450*
Hemoglobin, mg/dL	13.0 (12.2–13.2)	13.5 (12.1–15.6)	13.4 (12.7–14.1)	0.276‡
Creatinine, mg/dL	1.0 (0.8–1.3)	1.1 (1.0–1.3)	0.8 (0.8–1.0)	0.008*
				HIIT vs MCT
LVEF, %	62 (57–66)	58 (54–63)	61 (57–65)	0.361‡
E/e´ septal	15.1 (13.3–20.2)	19.5 (16.0–22.7)	17.3 (13.9–19.9)	0.203‡
E/e´ lateral	12.0 (8.6–12.5)	11.5 (9.1–15.2)	12.3 (10.0–15.5)	0.123*
LAVI, mL/m²	36 (28–51)	38 (27–44)	32 (28–45)	0.879*
LVMI, g/m²	110 (73–129)	90 (75–102)	104 (86–115)	0.365*
TAPSE, mm	20 (19–23)	20 (16–22)	22 (19–24)	0.136‡
RVSP max, mm Hg	26 (19–32)	26 (23–33)	26 (20–32)	0.573‡
Peak VO_2, mL/kg per min	19.0 (14.5–22.1)	17.4 (14.0–21.3)	21.1 (16.3–25.7)	0.083‡
Peak VO_2 % predicted, %	91 (76–116)	83 (67–102)	107 (86–125)	0.070‡

Data are presented as absolute (relative) frequency or median (IQR). All *P* values are 2-sided and were not corrected for multiple testing. Continuous variables were tested for normal distribution applying the Shapiro-Wilk test. BMI indicates body mass index; Con, control group; E, peak velocity blood flow from ventricular relaxation in early diastole; e´, mitral annular early diastolic velocity; HIIT, high-intensity interval training group; IQR, interquartile range; LAVI, left atrial volume index; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; MCT, moderate continuous training group; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association classification; peak VO_2, peak oxygen consumption; RVSP max, maximum right ventricular systolic pressure gradient; and TAPSE, tricuspid annular plane systolic excursion.

*Kruskal-Wallis test.

†χ^2 test.

‡One-way ANOVA with Tukey-B post hoc test.

group significant changes were observed for HIIT in MuRF1 (Figure 1A) and Trim72 (Figure 1C) expression. No impact of HIIT on changes in MAFBx expression, ubiquitinylated proteins, and proteasome activity could be detected (Figure 1B, 1D, and 1E). With respect to MCT no change in the expression of the atrophy-related markers was observed (Figure 1A through 1E). Quantifying mRNA expression by quantitative real-time polymerase chain reaction, no change in MuRF1 and Trim72 was evident after 3 months, whereas MAFBx mRNA was significantly reduced in the HIIT group (Figure S1).

Performing the same analyses after 12 months of ET, no change was evident for MuRF1, MAFBx, and Trim72 protein (Figure 1A through 1C) and mRNA expression (Figure S1A through S1C). In addition, the amount of ubiquitinylated proteins did not change in the training groups when compared with Con. Proteasome activity was significantly reduced in the training groups (Figure 1E). Full representative western blots are shown in the Figure S2 through S9.

Activity of Glycolytic and Fatty Acid Metabolism Enzymes

Measuring enzyme activities of PK and LDH after 3 and 12 months of ET revealed no changes in the training groups when compared with the control group (Figure 2A and 2B). The same was the case for ß-HOA-DH (hydroxyacyl-COA dehydrogenase), an enzyme involved in ß-oxidation (Figure 2C).

Expression and Activity of Mitochondria-Related Proteins

After 3 months of ET, a significant increase in CS and mitochondrial complex-I enzyme activity was observed (Figure 3A and 3B) with no change in CK activity (Figure 3C). Analyzing the different training modalities, MCT or HIIT, only HIIT resulted in significantly higher enzyme activity of CS and mitochondrial complex-I (Figure 3A and 3B). With respect to CK activity changes

Table 3. Changes Induced 3 and 12 Months After Randomization

	Con				MCT				HIIT			
	Begin (n=12)	3 mo (n=12)	*P* value vs B	12 mo (n=9)	Begin (n=15)	3 mo (n=15)	*P* value vs B	12 mo (n=8)	Begin (n=14)	3 mo (n=14)	*P* value vs B	12 mo (n=7)
BMI, kg/m^2	31 (25–35)	31 (25–36)	0.698*	32 (26–37)	28 (27–34)	28 (27–34)	0.110†	27 (26–28)	30 (26–32)	29 (26–31)	0.035*	28 (24–30)
Blood pres. sys., mm Hg	132 (130–148)	136 (126–144)	0.789†	133 (127–139)	130 (125–139)	133 (126–142)	0.814*	141 (127–155)	128 (120–137)	130 (110–140)	0.831*	129 (115–148)
NT-proBNP, pg/mL	525 (350–1055)	381 (241–1181)	0.929†	528 (160–2660)	354 (276–1185)	586 (209–1002)	0.427†	964 (236–1707)	337 (184–810)	198 (164–616)	0.084†	222 (143–616)
LVEF, %	58 (56–62)	63 (55–65)	0.320*	64 (56–68)	57 (53–62)	55 (54–62)	0.828*	67 (58–69)	60 (56–63)	64 (57–67)	0.161*	65 (62–67)
E/e´ septal	15.1 (13.1–20.2)	15.3 (10.8–21.9)	0.374†	15.0 (12.5–22.4)	19.5 (16.0–22.7)	17.9 (13.1–24.1)	0.696*	14.0 (10.6–21.5)	17.3 (13.9–19.9)	13.4 (11.4–16.8)	0.272†	16.6 (11.6–19.7)
E/e´ lateral	12.0 (8.6–12.5)	9.7 (8.2–12.9)	0.387*	9.5 (8.4–16.7)	11.5 (9.1–15.2)	12.1 (8.9–16.2)	0.733†	9.4 (7.7–18.7)	12.3 (10.0–15.5)	10.5 (8.7–15.9)	0.510†	11.8 (10.7–13.3)
Peak VO_2, mL/kg/per min	19.0 (14.5–22.1)	20.0 (17.3–20.9)	0.388†	19.6 (16.5–23.3)	17.4 (14.0–21.3)	19.3 (14.3–25.1)	0.002*	20.6 (16.1–27.2)	21.1 (16.3–25.7)	23.2 (17.7–28.7)	0.044*	19.6 (19.0–25.8)

Data are presented as median (IQR). All *P* values are 2-sided and were not corrected for multiple testing. Continuous variables were tested for normal distribution applying the Shapiro-Wilk test. B indicates begin; Blood pres. sys., blood pressure systolic; BMI, body mass index; Con, control group; E, peak velocity blood flow from ventricular relaxation in early diastole; e´, mitral annular early diastolic velocity; HIIT, high intensity interval training group; IQR, interquartile range; LVEF, left ventricular ejection fraction; MCT, moderate continuous training group; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and peak VO_2, peak oxygen consumption.

*Paired Student *t* test.

†Wilcoxon test.

after 3 months of ET neither MCT nor HIIT resulted in a significant change when compared with the control group (Figure 3C). After 12 months, no changes were evident for CS, mitochondrial complex I, and CK activity (Figure 3A through 3C).

The assessment of protein expression of mitochondrial complex I-V revealed a significant higher expression for complex I, II and IV after ET for 3 months (pooled analysis comparing control versus training; Figure 4A, 4B, 4D). For complex III, only a trend (*P*=0.085) was observed (Figure 4C), whereas complex V did not differ between Con and Train (Figure 4E). Discriminating between MCT and HIIT, it became evident that HIIT had a higher impact on mitochondrial complex I-IV expression than MCT. Analyzing protein expression of the mitochondrial complexes I-IV after 12 months of ET the significant changes observed after 3 months were lost (Figure 4A through 4D). For complex V, a higher protein expression was detected after 12 months in the combined training group (Train; Figure 4E). These alterations of mitochondrial complex I-V at the protein level were not detected at the mRNA level (Figure S10). Full representative western blots are shown in the Figures S11 and S12).

The greater effect of HIIT on mitochondrial complex protein expression after 3 months goes along with a significant increase in PGC-1α (peroxisome proliferator-activated receptor-γ coactivator 1α) expression when compared with Control (Figure S13A and S13B). A representative western blot is shown in Figure S14. A linear correlation was observed between the change in PGC-1α expression and the change in mitochondrial complex I-IV (complex I: r=0.49, *P*=0.015; complex II: r=0.40, *P*=0.06; complex III: r=0.51, *P*=0.013; complex IV: r=0.45, *P*=0.024). After 12 months of ET, this HIIT-mediated increase in PGC1α protein expression after 3 months was no longer detectable (Figure S13C and S13D). A representative Western blot is shown in Figure S15. No difference between Con, MCT, and HIIT was evident for the mRNA expression for PGC1α after 3 and 12 months (Figure S13E through S13H).

Impact of ET of Fiber Type

To assess if ET resulted in a shift in fiber-type composition, sections were stained with specific antibodies against fast or slow myosin heavy chain and the proportion of the respective fibers was quantified. No change in fiber type composition was observed in the Con and MCT group at 3 and 12 month (Figure S16). Only in the HIIT group, a significant reduction of slow type fibers was seen after 12 months when compared with 3 months.

Impact of ET on Satellite Cells

As reported earlier, ET has an impact on SKM satellite cells which play a crucial role in repair and remodeling of muscle in response to exercise.[22] Therefore, we assessed

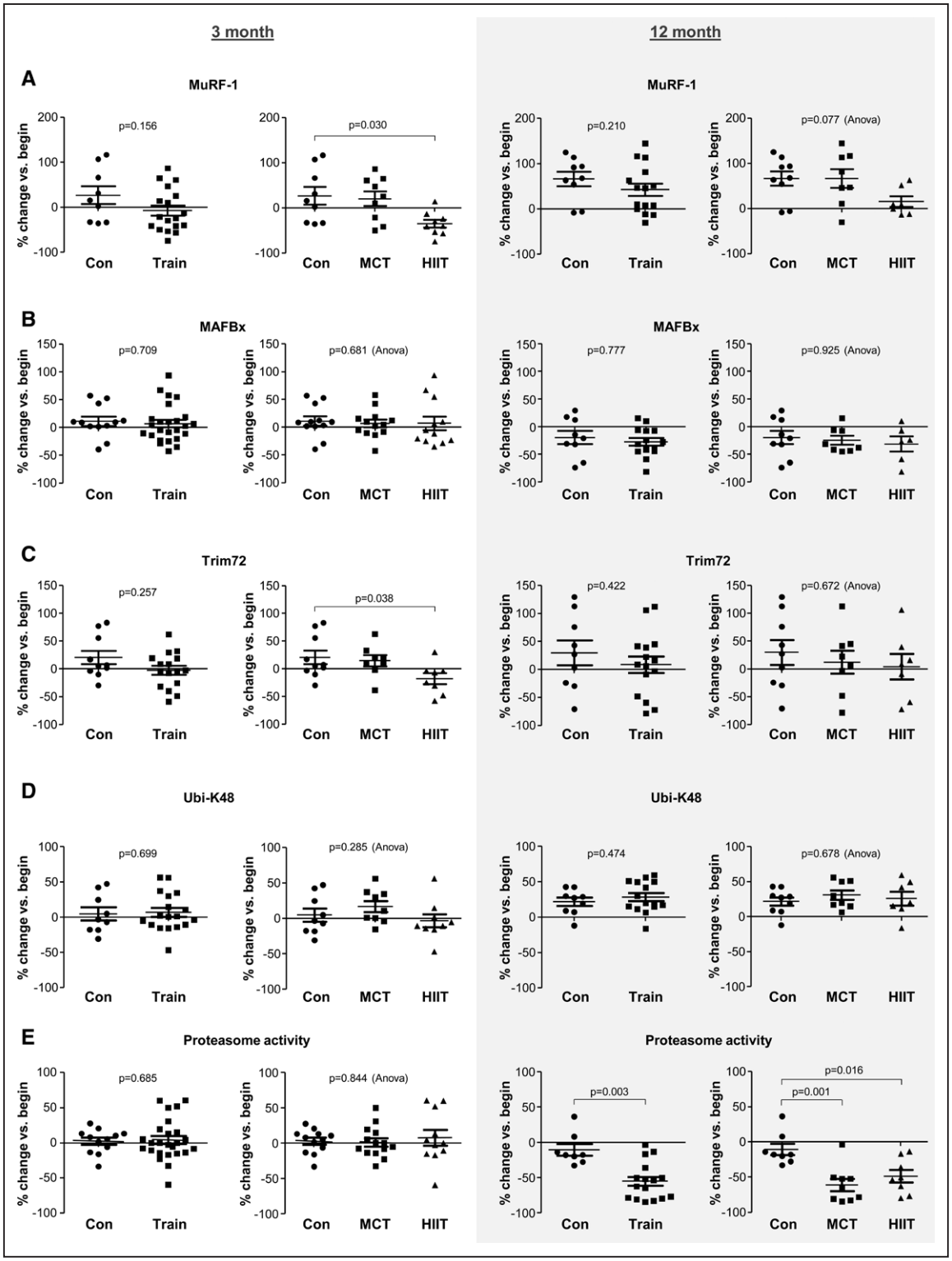

Figure 1. Changes in protein expression after 3 and 12 months of atrophy markers.
Expression of MuRF1 (muscle ring finger protein 1; **A**), MAFBx (muscle atrophy F-box protein; **B**), Trim72 (tripartite motif containing 72; **C**), ubiquitinylated proteins (**D**), and proteasome enzymatic activity (**E**) was determined in skeletal muscle tissue obtained from patients with HFpEF randomized either to the inactive control group (Con) or exercise training (Train, sum of moderate continuous training [MCT] and high-intensity interval [HIIT]). Furthermore, the exercise training group was separated into the MCT and HIIT groups, and the measurements were compared with the inactive control group. N=8 to 12 for the 3-month and N=6 to 9 for the 12-month time point. To test for statistical significance between Con and Train, an unpaired *t* test was used, whereas the Kruskal-Wallis test followed by Dunn multiple comparison test was applied for the comparison between Con, MCT, and HIIT. Trim72 indicates tripartite motif-containing protein 72; and Ubi-K48, proteins modified by polyubiquitin chains formed by Lys48 residue linkage.

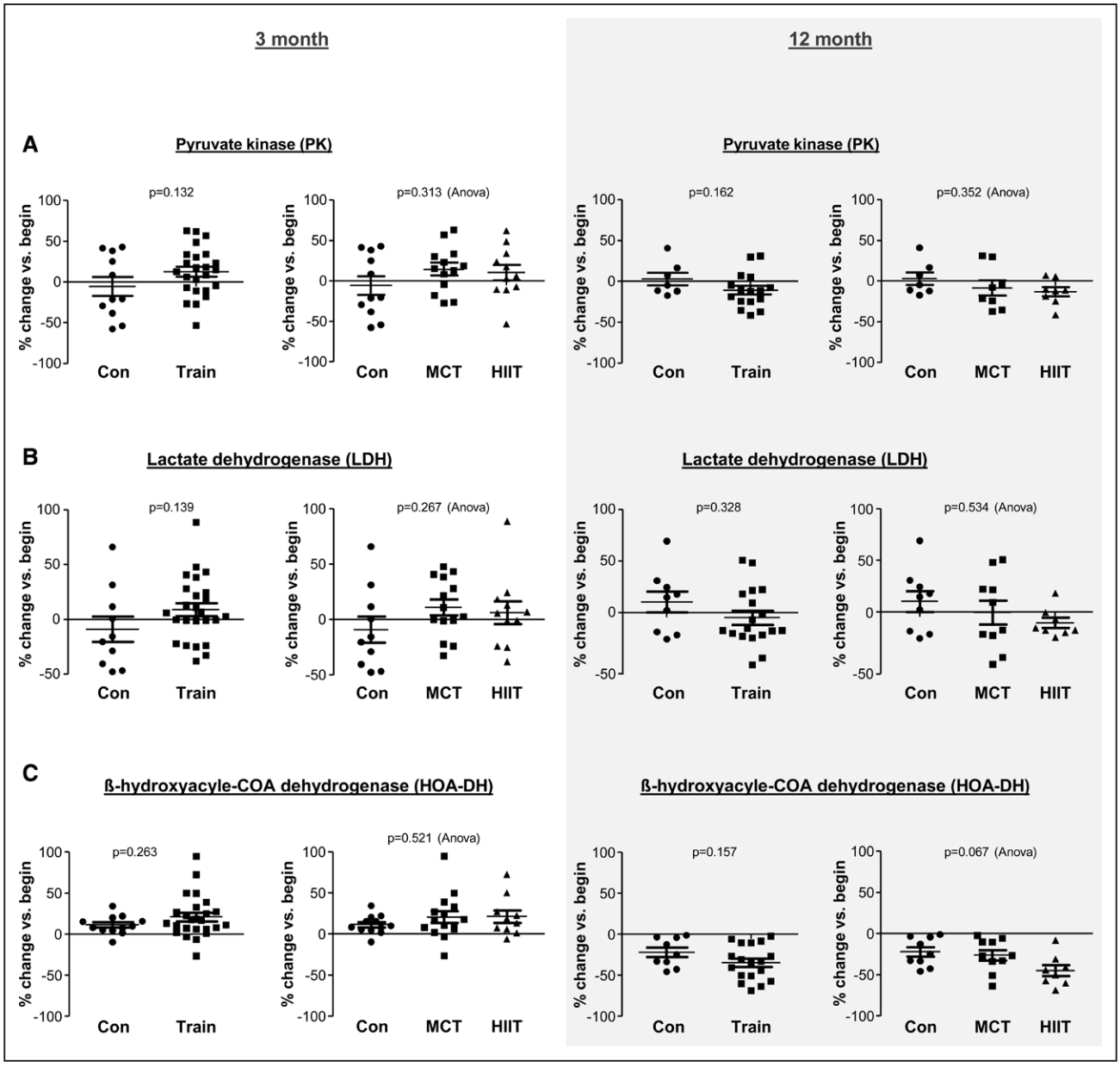

Figure 2. Changes in enzyme activities after 3 and 12 months.
PK (pyruvate kinase; **A**), LDH (lactate dehydrogenase; **B**), and HOA-DH (ß-hydroxyacyl-COA dehydrogenase; **C**) activities were determined in skeletal muscle tissue obtained from patients with HFpEF randomized to either the inactive control group (Con) or exercise training (Train, sum of moderate continuous training [MCT] and high-intensity interval [HIIT]). Furthermore, the exercise training group was separated into the MCT and HIIT groups, and the measurements were compared with the inactive control group. N=8 to 12 for the 3-month and N=6 to 9 for the 12-month time point. To test for statistical significance between Con and Train, an unpaired *t* test was used, whereas the Kruskal-Wallis test followed by Dunn multiple comparison test was applied for the comparison between Con, MCT, and HIIT.

the amount of satellite cells in muscle biopsies obtained from patients with HFpEF at baseline and after 3 and 12 months of ET by quantifying Pax7 mRNA. A significant increase in Pax7 mRNA expression was detected in the HIIT group after 3 months when compared with the control (Figure 5A). No significant change was evident in the MCT group. Analyzing Pax7 mRNA expression after 12 months no significant difference between the groups could be detected (Figure 5B). The significant difference in Pax7 mRNA expression in the HIIT group after 3 months could also be verified by quantification of Pax7 positive stained cells (Figure S17).

To explore if the higher amount of Pax7 after 3 months in the HIIT group is due to higher proliferation rate of the satellite cells, we assessed the proliferation capacity of satellite cells isolated from SKM biopsies at baseline and after 3 months. A significant increase in the proliferation capacity was seen in the HIIT group when compared

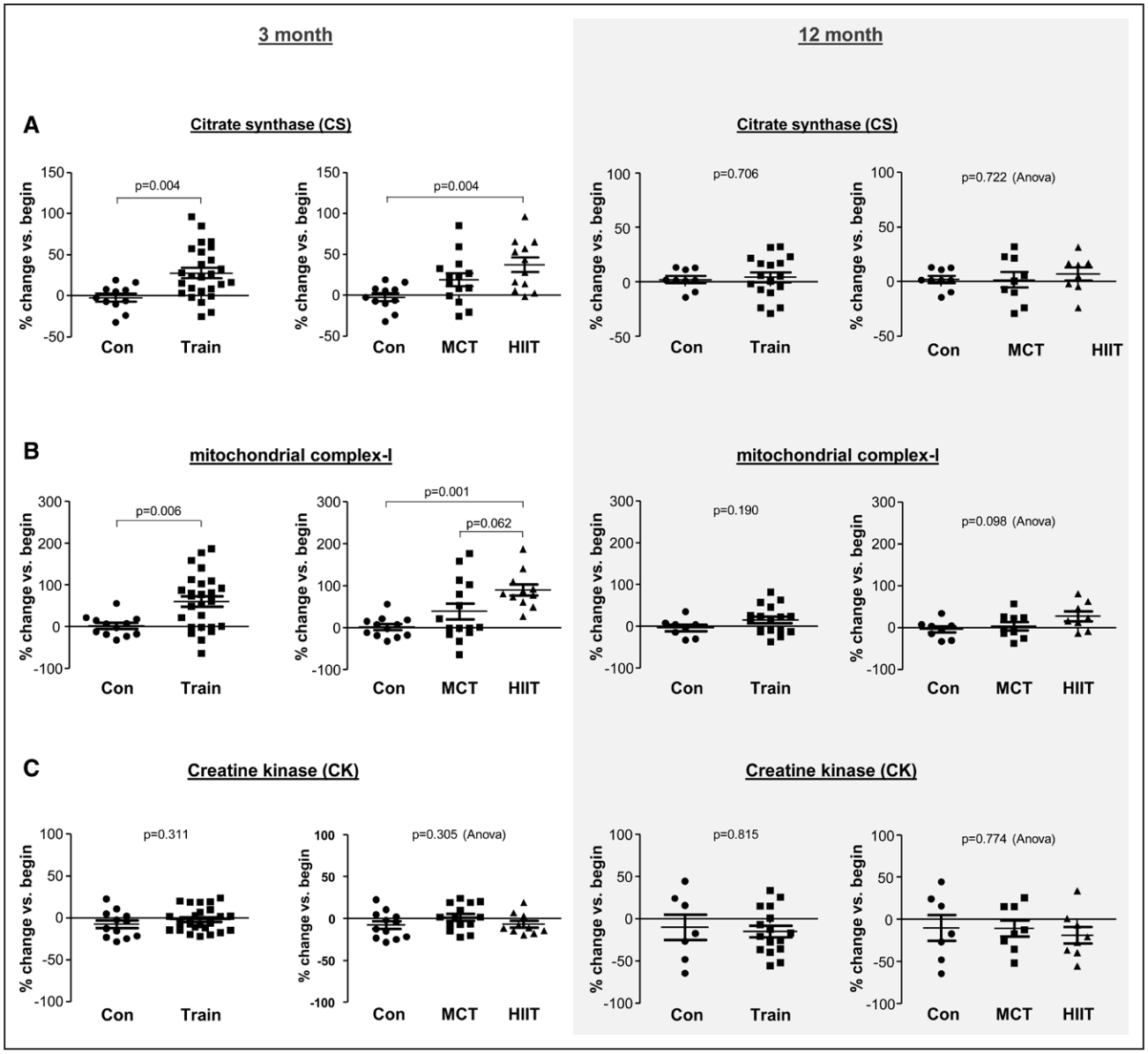

Figure 3. Changes in mitochondrial enzyme activities after 3 and 12 months.
Activity of CS (citrate synthase; **A**), mitochondrial complex-I (**B**), and CK (creatine kinase; **C**) was determined in skeletal muscle tissue obtained from patients with HFpEF randomized to either the inactive control group (Con) or exercise training (Train, sum of moderate continuous training [MCT] and high-intensity interval [HIIT]). Furthermore, the exercise training group was separated into the MCT and HIIT groups, and the measurements were compared with the inactive control group. N=8 to 12 for the 3-month and N=6 to 9 for the 12-month time point. To test for statistical significance between Con and Train, an unpaired *t* test was used, whereas the Kruskal-Wallis test followed by Dunn multiple comparison test was applied for the comparison between Con, MCT, and HIIT.

with the changes in the control group (Figure 5C). No significant change in comparison to Con was detected in the MCT group. Another important property of satellite cells is the capability to differentiate into myotubes. Investigating the differentiation capacity at baseline and after 3 months, a significant increase was evident only in the HIIT group (Figure 5D).

DISCUSSION

The lack of improvement of cardiac parameters by ET in patients with HFpEF nourished the idea that the enhancement in exercise capacity is due to peripheral adaptations.[23] Furthermore, it is presently unknown which mode of ET, MCT, or HIIT induces greater changes in the peripheral SKM of HFpEF. To investigate SKM changes elicited by ET, we analyzed in the present study molecular changes in the SKM of patients with HFpEF randomized to different training modes (HIIT, MCT, or Con). The main findings of the present study can be summarized as follows:

1. Molecular markers for muscle atrophy like MuRF1 and Trim72 were significantly reduced in SKM of patients with HFpEF after 3 months of HIIT. These

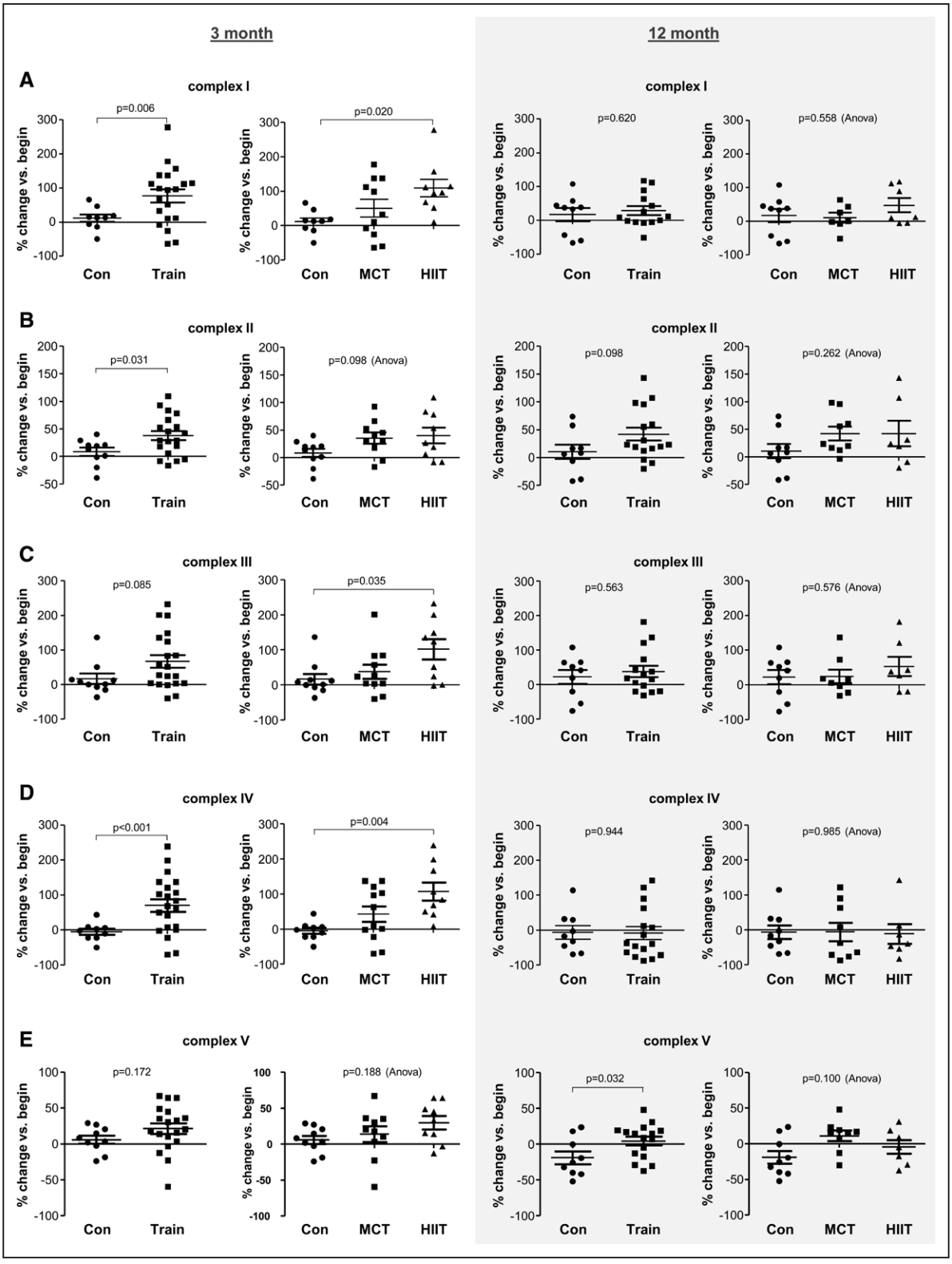

Figure 4. Changes of protein expression of mitochondrial respiration complexes after 3 and 12 months.
Expression of mitochondrial complex I (**A**), complex II (**B**), complex III (**C**), complex IV (**D**), and complex V (**E**) was determined in skeletal muscle tissue obtained from patients with HFpEF randomized to either the inactive control group (Con) or exercise training (Train, sum of moderate continuous training [MCT] and high intensity interval [HIIT]). Furthermore, the exercise training group was separated into the MCT and HIIT groups, and the measurements were compared with the inactive control group. N=8 to 12 for the 3-month and N=6 to 9 for the 12-month time point. To test for statistical significance between Con and Train, an unpaired *t* test was used, whereas the Kruskal-Wallis test followed by Dunn multiple comparison test was applied for the comparison between Con, MCT, and HIIT.

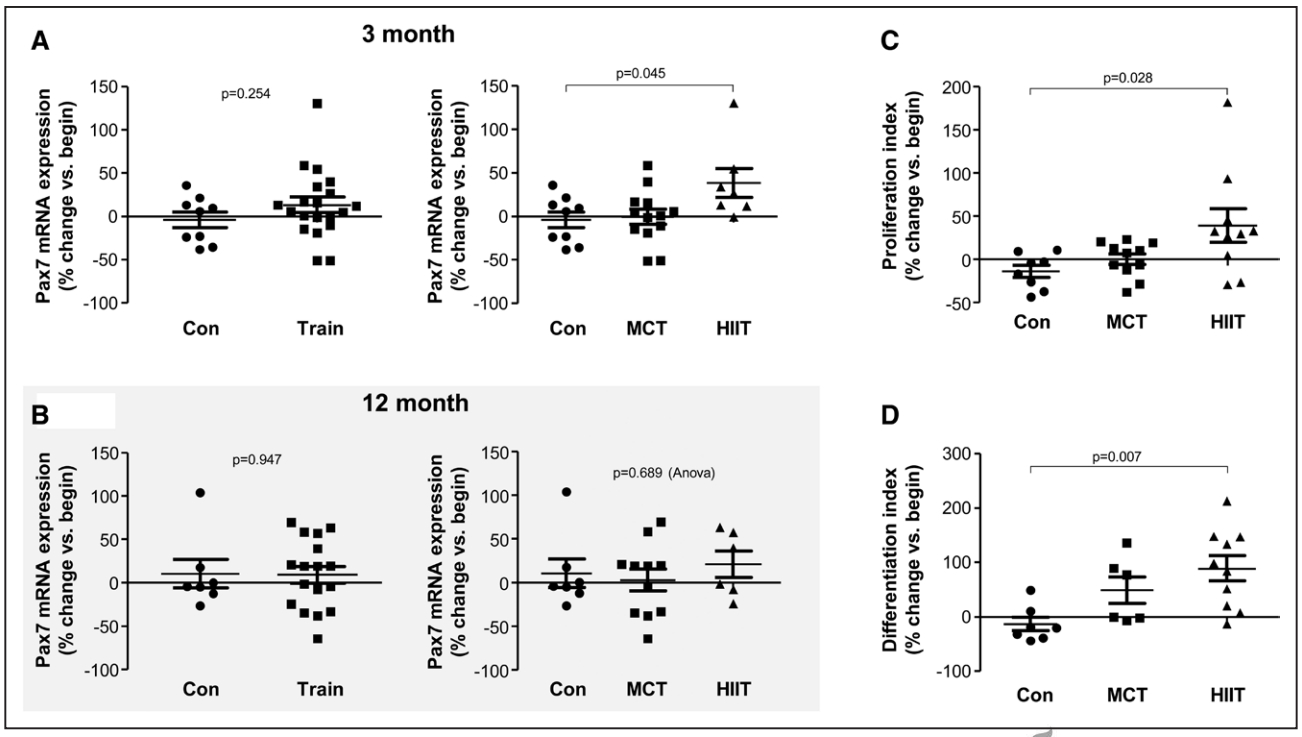

Figure 5. Changes of Pax7 (paired box 7) mRNA expression after 3 and 12 months.
Pax7 mRNA was quantified in skeletal muscle biopsies after 3 (**A**) and 12 (**B**) months obtained from patients randomized to either the inactive control group (Con) or exercise training (Train, sum of moderate continuous training [MCT] and high-intensity interval [HIIT]). Furthermore, the exercise training group was separated into the MCT and HIIT groups, and the measurements were compared with the inactive control group. Furthermore, changes in proliferation (**C**) and differentiation (**D**) of satellite cells after 3 months was quantified. N=7 to 12 for the 3-month and N=6 to 9 for the 12-month time point. To test for statistical significance between Con and Train, an unpaired *t* test was used, whereas the Kruskal-Wallis test followed by Dunn multiple comparison test was applied for the comparison between Con, MCT, and HIIT.

beneficial effect were no longer observed after 12 months. Only proteasome activity was significantly reduced by ET after 12 months.
2. Activities of mitochondrial enzymes and the expression of mitochondrial oxidative phosphorylation complexes were significantly increased by HIIT after 3 months. These effects were no longer detectable after 12 months of ET.
3. HIIT increased the amount of satellite cells probably due to an increased proliferation capacity of these cells and there differentiation capacity to generate myotubes.

Taken together, the results of the present study clearly showed that molecular changes (proteins involved in oxidative metabolism, satellite cell proliferation and differentiation) induced by 3 months of ET are more pronounced in HIIT compared with MCT. Only a minor impact of ET was detected on molecular muscle atrophy markers. These beneficial effects were lost after 12 month of ET, of which 9 months were home-based ET.

Skeletal Muscle Atrophy and Impact of ET

ET is recommended by the European Society of Cardiology and the American Heart Association to treat skeletal muscle atrophy and muscle dysfunction in heart failure.[24–26] Most studies to date analyzing ET as therapeutic intervention in heart failure for prevention/treatment of muscle atrophy were done in HFrEF.[27] Aerobic ET is associated in humans with a reduced expression of catabolic markers (MuRF1), an elevated expression of anabolic proteins (IGF-1 [insulin-like growth factor 1]) and a reduction of proteasomal activity.[28,29] In experimental models and clinical studies of HFrEF, aerobic ET resulted in a reduction of catabolic markers (MAFBx, MuRF1) and proteasomal activity and finally in increased muscle mass.[29–32] With respect to the impact of ET on muscle atrophy and molecular markers of muscle catabolism in HFpEF, only 1 experimental study is available.[4] To our knowledge, the present study is the first to study the impact of ET on molecular markers in SKM of patients with HFpEF. In contrast to the findings in HFrEF, moderate aerobic ET had no impact on the catabolic protein markers MuRF1, MAFBx, Trim72, protein ubiquitinylation, or proteasome activity in our HFpEF cohort. However, we tested not only the effects of MCT but also the much more intense HIIT. The concept of HIIT training in heart failure was first introduced by Wisløff et al[33] and in that pioneering study the authors documented a superior effect of HIIT for cardiovascular effects. With respect to HIIT and muscle mass, a clinical study by Tzanis et al[34] reported

a positive effect on skeletal myopathy with adaptive responses in anabolic pathways like IGF-1. Also, in the present study, we could document small but significant effects of HIIT on MuRF1 and Trim72 after 3 months of supervised ET. No effects were seen after 3 months for the amount of ubiquitinylated proteins and proteasome activity. The lack of an effect on the amount of ubiquintinylated proteins, despite a downregulation of MuRF1 expression by HIIT, is probably explained by the fact that the antibody used to detect ubiquitinylated proteins did not discriminate between proteins ubiquitinylated by MuRF1 or other E3 ligases. With respect to proteasome activity, an effect of ET training was seen after 12 but not after 3 months. In the current literature, the impact of ET on proteasome activity in SKM is discussed controversially. In some human or experimental studies, a reduced activity of the 26S proteasome is reported[29,35,36] whereas some reported an increase.[37,38] Nevertheless, according to the present study, it also appears that exercise intensity and duration is important for regulating proteasome activity. Besides markers for muscle catabolism, upcoming studies have to incorporate the assessment also of anabolic factors like IGF-1 and capillary density.

Proliferation and fusion of satellite cells, leading to an increase in the number of myonuclei, may also contribute to reduced muscle atrophy. This process is stimulated in adult skeletal muscle in response to increased contractile activity, as observed during ET.[39,40] Analyzing proliferation and differentiation of satellite cells in response to ET in muscle biopsies obtained from patients with HFpEF, it was evident that especially HIIT resulted in a significant increase of satellite cells and its function. That this positive effect was only seen in the HIIT group but not MCT goes along with a recently published experimental study in mice[40] documenting that the training effect on satellite cells is load-dependent, but fiber type-independent.

Taken together, we may conclude that 3 months of in-hospital ET in HFpEF, and predominantly HIIT, exerts positive effects of molecular markers of atrophy and the amount and function of satellite cells, which are cornerstones of improved muscle mass.

Oxidative Metabolism and Impact of ET

Mitochondria are the power house of the cells delivering the fuel for muscle contraction. Several adaptations to increased energy demand, due to ET, like increase in number and regulation of fusion/fission of mitochondria have been reported.[41] Analyzing SKM biopsies of patients with HFpEF, we[16] and others[6,7] have reported alterations in energy metabolism compared with healthy subjects. These changes in the metabolic pattern were also documented in animal models of HFpEF, for example, increased LDH activity,[3] decrease in CS,[4] and malate dehydrogenase,[42] and impaired mitochondrial coupling.[4] Furthermore, proteomic profiling of SKM tissue from healthy controls and patients with HFpEF revealed reduced levels of proteins related to oxidative phosphorylation.[43] Since it is documented in many experimental and clinical studies that ET is a powerful therapeutic strategy to fight exercise intolerance, it is interesting to see if ET has an impact on the metabolic changes seen in the SKM of patients with HFpEF. According to the results of the present study especially HIIT exerts positive effects on the oxidative metabolism after 3 months of in-hospital training. HIIT resulted in a significant upregulation of mitochondrial complex I activity and increased expression of mitochondrial complex proteins I-IV. This beneficial effect of HIIT was no longer detectable after 12 months, probably due to a lower compliance during the 9 months of home-based ET (see discussion below). Why does the mitochondrial metabolism only improve after 3 months of HIIT but not MCT? One reason may be the different load during training modalities—higher load during HIIT when compared with MCT. According to the results, only HIIIT significantly induced the protein expression of PGC1α, which is one of the main regulator of mitochondrial biogenesis.[44] In a recent review by Vargas-Ortiz et al,[45] it was speculated that HIIT and aerobic ET stimulated different molecular pathways leading to mitochondrial biogenesis. This idea is further supported by the observation of Wisløff et al[33] that HIIT was superior to MCT for improving skeletal muscle mitochondrial biogenesis in HRrEF. Nevertheless, more insight is needed to support this idea. Another explanation for HIIT-increased expression of mitochondrial complex proteins could be a shift in fiber type composition towards more type II fibers. Looking at the data of the present study, the opposite is the case. Performing HIIT for 12 months, a significant reduction of type II fibers was observed. Screening the current literature mixed results were reported with respect to the impact of HIIT on fiber type distribution ranging from no change[46] to downregulation of type II fibers.[47,48]

An issue completely unclear at the moment is the observation that MCT and HIIT improves exercise capacity to a similar extent, but molecular changes in the skeletal muscle were seen for most proteins investigated only by HIIT. The explanation for this discrepancy can only be speculative. Besides modulating SKM function and protein expression, ET exerts also beneficial effects on other organ systems, like the vascular system and the myocardium. At least in an animal model of HFpEF, MCT and HIIT improved endothelial function to a similar extent.[49] Since we only observed a positive impact of HIIT on skeletal muscle enzyme activities and protein expression, we have to conclude that the modulation of the skeletal muscle is not primarily responsible for the increase in exercise capacity and that other systems, like the cardiovascular system, are more important.

Difference Between In-Hospital and Home-Based ET

After 3 months of supervised in-hospital ET patients were advised to continue the ET program with continuous frequency, duration, and intensity according to their randomization group guided by telemonitoring and personal contact in case of a decline in attendance to <70% of scheduled sessions or in exercise intensity. Despite this effort adherence to exercise sessions dropped from 80% to 60% in MCT and from 76% to 56% in HIIT after 3 and 12 months.[15] The decline in treatment stimulus is probably too large to maintain or even increase treatment effects in the long run not only in MCT but also in HIIT. This major concern of insufficient compliance to ET prescriptions was seen in several other multicenter trials[50,51] and has to be addressed in future research.

Study Limitations

The present study is the first reporting on beneficial effects of HIIT on molecular parameters for muscle atrophy and mitochondrial complex expression and on the amount of satellite cells. However, the following limitations have to be considered.

First, besides describing molecular changes in SKM by different ET modalities, a detailed phenotyping including imaging or detailed tests for functional assessment and frailty of the SKM is missing. However, when we designed the study, we decided to limit the number of assessments especially with respect to secondary end points to avoid overloading with the risk of incompleteness of the data or impracticability of the study. The primary end point of the study was the impact of ET on the change in peak VO_2. Therefore, a standardized and high-quality cardiopulmonary exercise test was performed at each time point, which per se is a challenge in a randomized multicenter trial.

Second, the impact of modulating atrophy related proteins by HIIT needs to be confirmed at the histological level by measuring muscle cross-sectional area. For proper cross-sectional area quantification, the tissue has to be embedded perfectly oriented, so that sectioning plane is rectangular to the fiber. This can be achieved in muscle specimens obtained by surgery when fiber direction is known. Using muscle specimens obtained using biopsy needles, fiber orientation is unknown, and therefore a proper orientation for cutting cannot be guaranteed. The same shortcoming limits the quantification of SKM capillarization.

Third, the relevance of HIIT-induced modulation of mitochondrial complex expression needs to be validated by metabolic readouts like direct measurements of oxidative phosphorylation using a Clark electrode.

Fourth, across numerous HFpEF studies,[52,53] the average peak VO_2 (in the 14–16 mL/kg per min range) is much lower than that observed in this study population. Therefore, the population studied may not be representative of broader HFpEF populations and the results of this study may not be generalize to less-fit HFpEF patient populations.

Conclusions

The development of HFpEF is associated with molecular alterations in the peripheral skeletal muscle in proteins related to muscle atrophy and energy metabolism.[16] Performing a supervised ET with 2 different modalities (MCT, HIIT) over a time period of 3 months resulted in significant improvement in exercise capacity, with no significant difference between MCT and HIIT. On the other hand, HIIT resulted in more pronounced molecular changes, especially in proteins related to energy metabolism compared with MCT. These beneficial effects of HIIT after 3 months were lost after additional 9 months of home-based ET probably due to a lower compliance during the home-based ET.

ARTICLE INFORMATION

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Affiliations

Laboratory of Molecular and Experimental Cardiology, Technische Universität Dresden, Heart Center Dresden, Germany (E.B.W., A.A., A.S., K.G., J.H., A.L., V.A.). Department of Prevention and Sports Medicine, University Hospital Klinikum rechts der Isar, Technical University of Munich, Germany (S.M., M.H.). DZHK (German Center for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany (S.M., M.H.). Department of Internal Medicine/Cardiology, Heart Center Leipzig – University Hospital, Helios Stiftungsprofessur, Germany (T.F.-S.). Research Group Cardiovascular Diseases, GENCOR, University of Antwerp, Belgium (E.M.v.C.). Department of Cardiology, Antwerp University Hospital, Belgium (E.M.v.C.). Cardiac Exercise Research Group at Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim, Norway (U.W.). Department of Internal Medicine and Cardiology, Campus Virchow Klinikum, Charité Universitätsmedizin Berlin, Germany (B.P.). Dresden Cardiovascular Research Institute and Core Laboratories GmbH, Germany (V.A.).

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Supplemental Material

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