International Journal of Cardiology 274 (2019) 214–220

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

High-intensity interval training enhances mitochondrial bioenergetics of platelets in patients with heart failure

Cheng-Hsien Chou [a], Tieh-Cheng Fu [b,1], Hsing-Hua Tsai [a], Chih-Chin Hsu [b], Chao-Hung Wang [c], Jong-Shyan Wang [a,b,*]

[a] Healthy Aging Research Center, Graduate Institute of Rehabilitation Science, Medical College, Chang Gung University, Tao-Yuan, Taiwan
[b] Department of Physical Medicine and Rehabilitation, Chang Gung Memorial Hospital, Keelung, Taiwan
[c] Division of Cardiology, Department of Internal Medicine, Chang Gung Memorial Hospital, Keelung, Taiwan

ARTICLE INFO

Article history:
Received 28 January 2018
Received in revised form 25 June 2018
Accepted 20 July 2018
Available online 27 July 2018

Keywords:
Heart failure
Exercise
Platelet
Mitochondria

ABSTRACT

Objective: Exercise improves cardiopulmonary fitness and reduces the risk of vascular thrombosis in patients with cardiovascular diseases. In platelets, mitochondria carry out cellular bioenergetics and thrombogenesis. This study aimed to elucidate the effect of high-intensity interval training (HIIT) on systemic aerobic capacity and platelet mitochondrial bioenergetics in patients with heart failure (HF).

Methods: Thirty-four randomly selected HF patients engaged in HIIT (3-min intervals at 40% and 80% of VO_{2peak}, n = 17) for 30 min/day, 3 days/week for 12 weeks, or to a control group that received general healthcare (GHC; n = 17). Systemic aerobic capacity (i.e., peak O_2 consumption, VO_{2peak}) and platelet mitochondrial O_2 consumption rate (OCR) in the HF patients were measured through automatic gas analysis and high-resolution respirometry, respectively.

Results: The HIIT group exhibited higher VO_{2peak} and O_2 uptake efficiency slope and lower V_E-VCO_2 slope after 12-week intervention, compared to those of the GHC group. Moreover, the HIIT regimen increased the maximal and reserve OCR capacities, enhanced the Complex I- and II-mediated OCRs, and elevated the bioenergetic health index in platelet mitochondria; however, these effects were not observed with the GHC regimen. Additionally, the VO_{2peak} levels were positively correlated with the maximal and reserve OCR capacities and Complex I- and II-mediated OCRs in platelet mitochondria.

Conclusion: Platelet mitochondrial function is an ideal bioenergetic indicator in patients with HF. HIIT for 12 weeks elevates platelet mitochondrial OCRs via increasing Complex I and II activities. Moreover, systemic aerobic capacity is positively associated with platelet mitochondrial OCRs in HF patients.

1. Introduction

Elevated inflammatory cytokines, coagulation factors, and platelet reactivity have been reported as thrombogenic indices in patients with heart failure (HF) [1–3]. Circulating platelets can detect and respond to systemic metabolic and inflammatory stressors; furthermore, they serve as functional biomarkers in pathophysiology of cardiovascular diseases [4, 5]. Despite containing relatively fewer small mitochondria than other cell types, platelets have a higher ATP turnover rate and are metabolically more active than mammalian muscles [6]. Hence, monitoring of platelet mitochondrial characteristics in blood is useful to assess the overall bioenergetic health of an individual [7, 8]. Additionally, platelet mitochondria are directly involved in the cellular redox balance, activation, and apoptosis, thereby modulating thrombogenesis [4, 9]. Chacko et al. have indicated that the bioenergetics health index (BHI) of platelets has the potential to be a novel biomarker for assessing with both prognostic and diagnostic values of the individuals' health [7]. Accordingly, the BHI in platelets may be an ideal indicator of the bioenergetic or thrombogenic status of HF patients.

Regular exercise improves cardiopulmonary fitness and aerobic capacity and is associated with reductions in the risk of major vascular thrombotic events [10, 11]. Moreover, high-intensity exercise elicits greater metabolic and cardiovascular adaptations than low- and moderate-intensity exercise [12]. However physical exercise both enhances and suppresses platelet reactivity, depending on the type and intensity of exercise [11]. Our recent study on healthy sedentary men revealed that the high-intensity interval training (HIIT) effectively ameliorates hypoxia-induced mitochondrial dysfunction in platelets by reducing cellular oxidative stress, which may reduce the risk of thrombosis due to hypoxic stress [13]. To our knowledge, the relationship

* Corresponding author at: Graduate Institute of Rehabilitation Science, Chang Gung University, 259 Wen-Hwa 1st Road, Kwei-Shan, Tao-Yuan 333, Taiwan.
E-mail address: s5492@mail.cgu.edu.tw (J.-S. Wang).
[1] Equal contribution as the first author.

https://doi.org/10.1016/j.ijcard.2018.07.104
0167-5273/

between HIIT-induced changes in systemic aerobic capacity and platelet mitochondrial bioenergetics in HF patients remained unclear. Therefore, we hypothesize that HIIT influences systemic aerobic capacity along with platelet mitochondrial function in HF patients.

The present study aimed to establish an effective exercise regimen for improving cardiopulmonary fitness, and to enhance platelet mitochondrial function in patients with HF. Effects of 12-week HIIT (3-min intervals at 40% and 80% of VO_{2peak}) on systemic aerobic capacity and oxidative phosphorylation (OXPHOS) in platelet mitochondria were evaluated in HF patients.

2. Materials and methods

2.1. Subjects

Thirty-four individuals diagnosed with HF at the Department of Cardiology, Chang Gung Memorial Hospital, Keelung, Taiwan, were enrolled randomly from August 2016 to July 2017 in this study. HF was diagnosed if the patients had a left-ventricular ejection fraction (LVEF) ≦40% (HF with reduced EF) and belonged to New York Heart Association functional classes II to III despite receiving optimal treatment for at least 12 months in accordance with the American Heart Association/American College of Cardiology guidelines, or HF with preserved EF, i.e., LVEF >40% with episodes of acute pulmonary edema after excluding other non-cardiogenic etiologies. Exclusion criteria were the presence of atrial fibrillation/flutter, second/third-degree heart block, or anemia (hemoglobin concentration ≦12 g/dL in men and ≦11 g/dL in women), history of potentially lethal ventricular arrhythmias, recent unstable angina, myocardial infarction or coronary revascularization (<4 weeks), uncontrolled diabetes mellitus, severe chronic obstructive pulmonary disease, or symptomatic cerebrovascular disease within 12 months, collagen vascular disease, alcohol or drug abuse during the previous 12 months or significant renal or hepatic disease. The computer-generated random numbers were used for simple randomization of subjects. Subjects were randomly divided into HIIT ($n = 17$) and general healthcare (GHC, $n = 17$) groups. This study was performed in accordance with the tenets of the Declaration of Helsinki and approved by the Institutional Review Board of Chang Gung Memorial Hospital, Taiwan. All subjects provided informed consent after the experimental procedures were explained.

2.2. Graded exercise test

The participants performed the graded exercise test on a bicycle ergometer (Ergoselect 150P, Germany) by one rehabilitation physician who was blinded to the HIIT or GHC subjects to assess their cardiopulmonary parameters 2 days before and 2 days after 12-week intervention. Moreover, the data collector was isolated from the data analytic specialist. Each participant was instructed to fast for at least 8 h and to refrain from exercise for at least 24 h before the test. All participants arrived at the testing center at 9:00 A.M. to eliminate diurnal effects. The test comprised 2 min of unloaded pedaling, followed by a progressive increase in work-rate of 10 W/min until exhaustion (progressive exercise to peak oxygen consumption, VO_{2peak}). Minute ventilation (V_E), oxygen consumption (VO_2), and carbon dioxide production (VCO_2) were measured in a breath-by-breath basis, using a computer-based system (Master Screen CPX, Cardinal-health Germany). Heart rate (HR) was determined from the R-R interval on a 12-lead electrocardiogram, mean arterial pressure (MAP) was measured using an automatic blood pressure system (Tango, SunTech Medical, UK), and arterial O_2 saturation was monitored through finger pulse-oximetry (model 9500, Nonin Onyx, Plymouth, MN, USA) [14, 15]. VO_{2peak} was defined by the following criteria: VO_2 increased by <2 mL/kg/min over at least 2 min, HR ≥ 85% of its predicted upper threshold, respiratory exchange ratio ≥ 1.15, or some other symptom/sign limitations in accordance with the guidelines of the American College of Sports Medicine for exercise testing [16]. In this investigation, although only 67.7% of subjects achieved RER ≥ 1.15, all subjects showed VO_2 increased by <2 mL/kg/min over at least 2 min, HR ≥ 85% of its predicted upper threshold, or symptom/sign of myocardial ischemia or dysrhythmia at the end of exercise.

Ventilation and VCO_2 responses, acquired from the initiation of exercise to the peak values, were used to calculate the V_E-VCO_2 slope, using least-squares linear regression ($y = m \cdot x + b$, m = slope) [17]. O_2 uptake efficiency slope (OUES) is an estimation of the efficiency of ventilation with respect to VO_2 [18, 19]. OUES was derived from the slope of a natural logarithm plot of V_E vs. VO_2, with greater slopes indicating higher ventilatory efficiency [18, 19].

2.3. Training protocols

HIIT patients performed supervised hospital-based training on a bicycle ergometer (Ergoselect 150P, Germany), completing three weekly sessions for 12 weeks, whereas GHC patients only engaged in general home-based health care, as instructed by their rehabilitation physicians [14, 15]. The HIIT group warmed up for 3 min at 30% of VO_{2peak} [≈30% heart rate reserve (HRR); $\approx 30\% \cdot (HR_{peak} - HR_{rest}) + HR_{rest}$] before exercise five 3-min intervals at 80% of VO_{2peak} (≈80% HRR). Each interval was followed by 3-min exercise at 40% of VO_{2peak} (≈40% HRR). The exercise session was terminated by a 3-min cool-down at 30% of VO_{2peak}. An HR monitor (Tango, SunTech Medical, UK) was used for all patients to calibrate the assigned intensity of exercise. The Borg 6-to-20 scale was used to assess the rate of perceived exertion during and after each exercise session. The work-rate of the bicycle ergometer was adjusted continuously to ensure that exercise intensity matched the target HR throughout the training period. Patients were instructed to immediately stop exercise training if they experienced chest pain or other cardiac symptoms/signs. The compliance rates with HIIT and GHC patients were 88.2% and 88.2%, respectively.

2.4. Echocardiography

All subjects underwent the echocardiography examination twice by an experienced cardiologist who was blinded to the HIIT or GHC intervention using a transthoracic ultrasound system (GE, Vivid E9, Norway). The first test was performed within one week before exercise training and the second test was performed within one week after the completion of the total intervention. The subjects arrived at the testing center at 9:00 AM to eliminate any possible circadian effect. The subjects maintained regular breathing patterns, and images were not captured during a breath-hold at end-expiration. M-mode images were used to determine the LV wall and cavity dimensions at end-systole and end-diastole. Measurements of LV mass and the short fraction index were automatically derived using standard equations. LV ejection fraction (LVEF) was determined using the modified Simpson's method from the apical 4-chamber views. Before the intervention, 3 patients in the HIIT group and 4 patients in the GHC group belonged to HF with preserved EF. This study used one dedicated investigator to perform the collection of echocardiographic data. Moreover, the data collector was isolated from the data analytic specialist.

2.5. Platelet isolation

Before the graded exercise test at the beginning of the present study and 12 weeks later in various groups, 20 ml of blood was sampled from each subject's antecubital vein within 1 min by venipuncture (20-gauge needle). Blood samples (20 mL) were collected in polypropylene tubes containing sodium citrate (3.8 g/dL, 1–9 vol. blood). Platelet rich plasma (PRP) was prepared through centrifugation at 300 ×*g* for 10 min at approximately 20 °C. Platelets were sedimented through centrifugation of the PRP at 1500 ×*g* for 10 min at approximately 20 °C and then washed once with phosphate buffered saline containing ethylenediaminetetraacetic acid (final concentration, 4 mM) (Sigma) to inhibit platelet activation [13]. The number of platelets was adjusted to 2×10^8 cells/mL with RPMI medium (Sigma). Blood analysis was repeated twice to ensure reproducibility of the results. All platelet fractions were analyzed within 2 h after cell purification. Blood cells were enumerated using a Sysmax SF-3000 cell counter (GMI, Inc., Ramsey, MN, USA).

2.6. Mitochondrial respiration and bioenergetics health index (BHI) in intact platelets

Mitochondrial O_2 consumption of platelets (2×10^8 cells/mL) in RPMI 1460 medium was measured using high-resolution respirometry (Oroboros O2K) [13]. Mitochondrial respiration coupled with ATP production (ATP-linked OCR) was measured through reductions in O_2 consumption after the addition of oligomycin (0.2 μg/mL), an ATP synthase inhibitor. The residual mitochondrial respiration represents a proton leak that uncouples OXPHOS from the electron transport system (ETS). Total O_2 consumption of platelets was measured at baseline and after adding the uncoupling agent carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP; 2 μM) to induce maximal O_2 consumption. The difference between the basal and maximal respiration is referred to as the reserve capacity of OCR. Non-mitochondrial respiration (non-mito OCR) was quantified by inhibiting mitochondrial respiration through the treatment with rotenone (a mitochondrial Complex I inhibitor, 1 μM) and antimycin A (a mitochondrial Complex III inhibitor, 1 μM) (Fig. 1A and B) [7, 13].

BHI was calculated from the results of coupling control to quantify platelet mitochondrial function, using the following equations (7,13):

$$\text{Log}\left[(\text{ATP}-\text{linked OCR}) \times (\text{Reserved capacity of OCR})/(\text{Proton leak}) \times (\text{Non}-\text{mito OCR})\right]$$

$$\text{ATP}-\text{linked OCR} = \text{Routine state} - \text{Leak state}$$

$$\text{Reserved capacity} = \text{ETS state} - \text{Routine state}$$

$$\text{Non}-\text{mito OCR} = \text{Residual } O_2 \text{ consumption (ROX) state}$$

$$\text{Proton leak} = \text{Leak state} - \text{ROX state}$$

2.7. Mitochondrial respiration in permeablized platelets

A substrate, uncoupler, inhibitor titration (SUIT) protocol was used to permeabilize platelets and analyze the aerobic capacity of platelet mitochondria (Fig. 1C and D). Through multiple substrate titration, electron flow from fatty acid oxidation (FAO) and mitochondrial Complex I and II were well controlled, and the mitochondrial OCR at each state was measured using a high-resolution respirometer (Oroboros O2K) [13, 20].

Intact platelets (2×10^8 cells/mL) were incubated at 37 °C in the O2K chamber in mitochondria respiratory medium MiR05 (EGTA, 0.5 mM; $MgCl_2 \cdot 6H_2O$, 3 mM; lactobionic acid, 60 mM; taurine, 20 mM; KH_2PO_4, 10 mM; HEPES, 20 mM; D-sucrose, 110 mM; bovine serum albumin, 1 g/L; pH = 7.1). Data were acquired after the oxygen flux stabilized. O_2

consumption in this state was the routine respiration from endogenous substrates in cells. Further, the plasma membrane was permeabilized with a digitonin (0.1 mg) titration after a concomitant addition of malate (2 mM) and palmitoyl-DL-carnitine-HCl (20 μM). Owing to the absence of adenylate in the chamber, respiration in this state resulted from mitochondrial proton leakage (LEAK). O_2 consumption through FAO was evaluated through addition of 1 mM ADP (Calbiochem). Oxidative phosphorylation capacity of mitochondrial Complex I and II were acquired through the addition of pyruvate (5 mM) and glutamate (10 mM), both being sources of NADH, and succinate (10 mM), an $FADH_2$ source. A cytochrome *c* (10 μM) assay was performed to determine whether the outer mitochondrial membrane was intact. In this study, there were not significant change in the OCRs of the permeabilized platelets after the Cytochrome C titration, reflecting by the intact of out mitochondrial membrane integrity (data no shown). The maximal convergent capacity of the ETS was subsequently obtained through FCCP titration (0.5 μM/steps). Finally, inhibitors of Complex I (0.1 μM rotenone) and III (2.5 μM antimycin A) were continually added to suppress O_2 consumption of platelet mitochondria (Fig. 1C and D). All reagents were purchased from Sigma-Aldrich (St Louis, MO, USA), unless stated otherwise. This study used a single investigator performed the SUIT protocol, and the data collector was isolated from the data analytic specialist. Moreover, either data collector or analytic specialist was blinded to the HIIT or GHC group.

The mitochondrial respiratory rates in permeablized platelets were calculating from the results of the SUIT protocol, using the following equations (13,20):

Basal = Malate + Palmitoyl−DL−carnitine

LEAK = Malate + Palmitoyl−DL−carnitine + Digitonin

FAO = Malate + Palmitoyl−DL−carnitine + Digitonin + ADP

FAO + CI = Malate + Palmitoyl−DL−carnitine + Digitonin + ADP + Pyruvate + Glutamate

FAO + CI + CII = Malate + Palmitoyl−DL−carnitine + Digitonin + ADP + Pyruvate + Glutamate + Succinate

$ETS_{FAO+CI+CII}$ = Malate + Palmitoyl−DL−carnitine + Digitonin + ADP + Pyruvate + Glutamate + Succinate + FCCP

ETS_{CII} = Malate + Palmitoyl−DL−carnitine + Digitonin + ADP + Pyruvate + Glutamate + Succinate + FCCP + Rotenone

ROX = Malate + Palmitoyl−DL−carnitine + Digitonin + ADP + Pyruvate + Glutamate + Succinate + FCCP + Rotenone + Antimycin A

2.8. Plasma biomarkers

An additional 5-mL blood sample was obtained from all subjects, placed in a cold centrifuge tube containing EDTA (final concentration, 4 mM), and immediately centrifuged at 3000 *g* for 10 min at 4 °C. The plasma samples were then stored at −80 °C until assay. Plasma brain natriuretic peptide (BNP) (USCN Life Science Inc., Burlington, NC) and myeloperoxidase (MPO) (Immunology Consultants Labaory, Newberg, OR) concentrations were quantified by commercially available ELISA kids.

2.9. Health-related quality of life

Generic and disease-specific qualities of life (QoL) in the HF population were measured using the Short Form-36 Health Survey questionnaire (SF-36) and Minnesota Living with Heart Failure questionnaire (MLHFQ), respectively [21]. MLHFQ is developed as a self-assessment measure of therapeutic response to interventions for HF, whereas SF-36 is a generic measure and can help differentiate QoL issues related to co-morbidities from those related to HF [21].

2.10. Statistical analysis

All variables are expressed as mean ± standard deviation (SD), and were analyzed using the statistical software package StatView. Experimental results were analyzed using 2 (groups) × 2 (time sample points; i.e., pre- and post-interventions) repeated-measures ANOVA with Bonferroni's post hoc test to compare cardiopulmonary fitness and platelet mitochondrial OXPHOS at the beginning of this study and after 12 weeks in the HIIT and GHC groups. Pearson correlation analysis was used to determine the association between aerobic capacity and platelet mitochondrial OXPHOS in HF patients. Differences with $P < 0.05$ were considered statistically significant.

3. Results

3.1. Cardiopulmonary fitness and hematologic parameters

At the start of the study, anthropometric and clinical parameters or functional capacities of both HIIT and GHC groups did not differ significantly (Table 1). After 12 weeks of the interventions, the HIIT group displayed significantly higher peak work-rate, V_{Epeak}, VO_{2peak}, and VCO_{2peak} than the GHC group (Table 2, $P < 0.05$). Moreover, the HIIT group exhibited significantly higher OUES (Table 2, $P < 0.05$) and lower V_E-VCO_2 slope (Table 2, $P < 0.05$) than the GHC group. Regarding

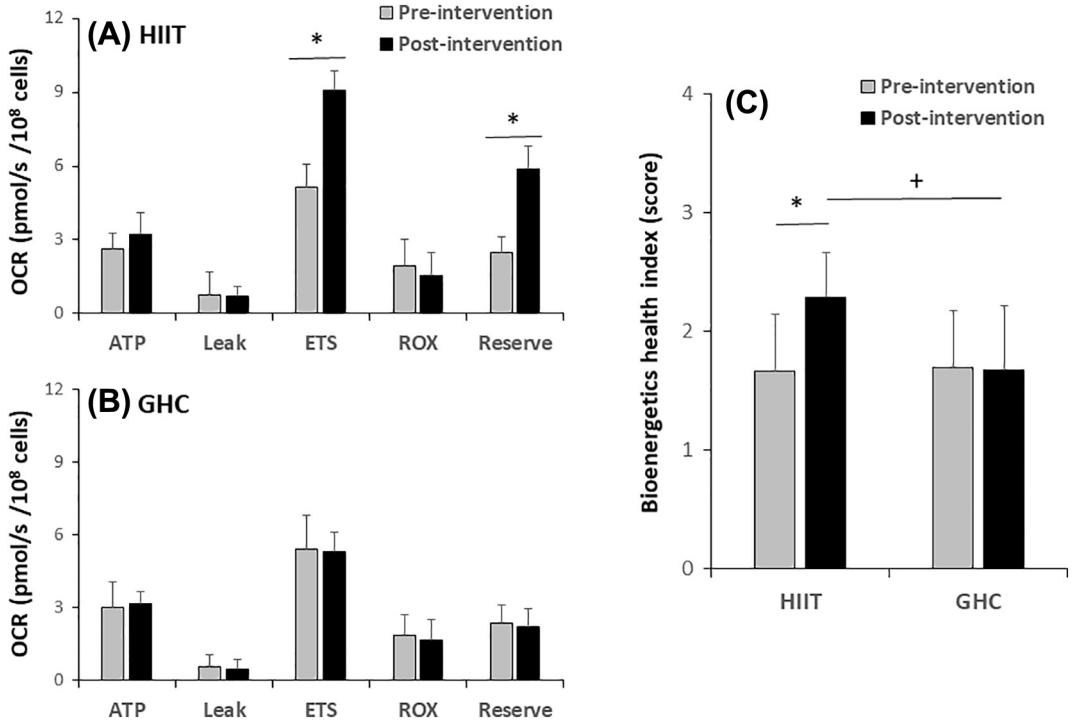

Fig. 1. Effects of high-intensity interval training (HIIT) on mitochondrial O_2 consumption rate (OCR) and bioenergetics health index of the intact platelets in patients with heart failure. GHC, general health care; ATP, ATP-linked OCR; Leak, proton leak; OCR; RESERVE, the reserve capacity of OCR; ROX, residual O_2 consumption. *$P < 0.05$, Pre-intervention vs. Post-intervention; +$P < 0.05$, HIIT vs. GHC. Values were mean ± SD.

myocardial contractility, the HIIT regimen also resulted in significantly elevated left-ventricular ejection fraction (LVEF) from 36.1% to 48.9%, (Table 1, $P < 0.05$); however, this change was not observed after the GHC regimen (Table 1).

Additionally, hematologic parameters (i.e., erythrocyte, hemoglobin, hematocrit, leukocyte, and platelet) did not change significantly after 12-week interventions in the HIIT and GHC groups (data not shown).

3.2. Mitochondrial respiration of platelets

Supplemental Fig. 1A–D show the analysis of OCRs in the intact (1A and 1B) and permeabilized (1C and 1D) platelets using a high-resolution respirometry (Oroboros O2K). HIIT for 12 weeks significantly increased the capacities for the ETS (Fig. 1A, $P < 0.05$) and reserve of OCR (Fig. 1A, $P < 0.05$) in the intact platelets. Additionally, the HIT regimen also significantly elevated the mitochondrial BHI value in platelets (Fig. 1C, $P < 0.05$). Furthermore, HIIT also enhanced the FAO + CI-, FAO + CI + CII-, ETS $_{FAO+CI+CII}$-, and ETS $_{CII}$-mediated OCRs (Fig. 2A, $P < 0.05$) in the permeabilized platelets. However, there were no significant changes in the ETS and reserve capacities of OCR (Fig. 1B) and the BHI value (Fig. 1C) in the intact platelets, as well as, the mitochondrial OXPHOS (Fig. 2B) levels in the permeabilized platelets after 12-week GHC.

3.3. Correlation between aerobic capacity and platelet mitochondrial bioenergetics

Pearson correlation coefficients of VO_{2max} and main variables of mitochondrial bioenergetics in the intact and permeabilized platelets were presented in Supplemental Figs. 2 and 3, respectively. In the variables of intact platelets, the VO_{2max} levels were positively correlated with the capacities for the ETS (Supplemental Fig. 2B, $r = 0.787$, $P < 0.001$) and reserve (Supplemental Fig. 2C, $r = 0.821$, $P < 0.001$) of OCR. In the variables of permeabilized platelets, the VO_{2max} levels were directly related to the OCRs mediated by FAO (Supplemental Fig. 3B, $r = 0.325$, $P = 0.0120$), FAO + CI (Supplemental Fig. 3C, $r = 0.387$, $P = 0.003$), FAO + CI + CII (Supplemental Fig. 3D, $r = 0.719$, $P < 0.001$), ETS $_{FAO+CI+CII}$ (Supplemental Fig. 3E, $r = 0.781$, $P < 0.001$), and ETS $_{CII}$ (Supplemental Fig. 3F, $r = 0.791$, $P < 0.001$).

This study further explored correlations between changes of aerobic capacity and mitochondrial bioenergetics of intact and permeabilized platelets induced by HIIT and GHC in HF patients (Supplemental Table 1). The analyzed results showed that changes of VO_{2peak} were positively correlated with changes of ETS ($r = 0.756$, $P \le 0.001$) and reserve capacity ($r = 0.727$, $P \le 0.001$) of OCR in intact platelets and changes of the OCRs mediated by FAO ± CI ($r = 0.372$, $P = 0.043$), FAO ± CI ± CII ($r = 0.699$, $P \le 0.001$), ETS $_{FAO\pm CI\pm CII}$ ($r = 0.722$, $P \le 0.001$), and ETS $_{CII}$ ($r = 0.712$, $P \le 0.001$) following 12 week-HIIT or GHC intervention.

3.4. Health-related QoL and biomarkers

HIIT substantially reduced MLHFQ score from 35.1 to 22.2 (Table 1, $P < 0.05$). Moreover, the exercise regimen also significantly increased the subclass scores of the physical (Table 1, 45.2 to 53.5, $P < 0.05$) and mental (Table 1, 44.5 to 52.1, $P < 0.05$) dimensions in SF-36, respectively. However, GHC remained unchanged the scores of MLHFQ and SF-36 physical/mental components (Table 1).

Furthermore, HIIT considerably reduced plasma levels of BNP (Table 1, 402 to 218 pg/mL, $P < 0.05$) and MPO (Table 1, 57.5 to 35.3 ng/mL, $P < 0.05$), and these biomarkers were unchanged following GHC (Table 1).

4. Discussion

This investigation clearly exhibits that 12-week HIIT improves systemic aerobic capacity and ventilatory efficiency in patients with HF. To our knowledge, this study is the first to demonstrate that the HIIT regimen effectively enhances mitochondrial OXPHOS capacity of platelets through increasing Complex I and II activities, which response is associated with improved aerobic capacity by HIIT in HF patients.

4.1. Cardiopulmonary fitness and systemic aerobic capacity

Reduced exercise capacity decreases the ability of HF patients to independently perform activities of daily living, thereby further worsening their quality of life [22–24]. In this study, 12-week HIIT increased VO_{2peak} and OUES and decreased V_E-VCO_2 slope, accompanied by increased LVEF in HF patients. The ventilatory parameters obtained from the graded exercise test may convey information regarding HF prognosis [17]. The VO_{2peak} and OUES are indicators of aerobic capacity and efficiency, respectively [17–19]. V_E-VCO_2 slope is a powerful predictor of survival in chronic HF patients [17]. Our previous studies reported that HIIT was a more effective modality than traditional endurance training for improving functional capacity in HF patients [14, 15]. Moreover, these indices of ventilatory efficiency modulated by exercise training were correlated with exercise-induced central and peripheral hemodynamic changes [14]. Additionally, an early study using an

Table 1
Demographic and clinical characteristics in patients with heart failure.

		HIIT		GHC	
		Pre	Post	Pre	Post
Anthropometrics/clinical characteristics					
Gender	n (M/F)	17 (12/5)	15 (11/4)	17 (12/5)	15 (11/4)
Age	year	60.9 ± 0.5	61.2 ± 0.9	59.7 ± 5.3	59.2 ± 4.7
Height	cm	163.9 ± 8.7	163.5 ± 8.3	166.2 ± 9.7	166.5 ± 8.5
Weight	kg	70.2 ± 12.8	70.1 ± 12.6	69.6 ± 11.0	70.2 ± 11.3
Heart rate	bpm	78 ± 15	76 ± 13	79 ± 16	80 ± 14
Systolic BP	mmHg	131 ± 14	128. ± 12	132 ± 14	134 ± 15
Diastolic BP	mmHg	78 ± 6	75 ± 7	79 ± 6	80 ± 7
Etiology (primary cause)					
Ischemic heart disease	n (%)	10 (59)	9 (60)	9 (56)	9 (60)
Hypertension	n (%)	4 (24)	3 (20)	3 (19)	3 (20)
Cardiomyopathy	n (%)	3 (17)	3 (20)	4 (25)	3 (20)
Heart failure duration	year	5.4 ± 1.7	5.6 ± 1.9	5.7 ± 2.3	5.5 ± 2.1
Functional capacity	MET	4.52 ± 1.13	7.06 ± 0.92*+	4.54 ± 1.00	4.53 ± 0.98
LVEF	%	36.1 ± 5.2	48.9 ± 6.5*+	34.7 ± 5.1	36.5 ± 6.4
Biomarkers					
BNP	pg/mL	402 ± 44	218 ± 52*	427 ± 45	436 ± 62+
MPO	ng/mL	57.5 ± 6.4	35.3 ± 5.3*	60.3 ± 5.7	57.6 ± 6.2+
Medications					
Digoxin	n (%)	3 (18)	3 (20)	4 (25)	3 (20)
β-blocker	n (%)	16 (94)	14 (93)	15 (94)	14 (93)
ACE/ARB	n (%)	16 (94)	14 (93)	14 (88)	13 (87)
Ca^{2+}channel blocker	n (%)	8 (47)	8 (53)	8 (50)	8 (53)
Diuretics	n (%)	9 (53)	8 (53)	7 (44)	6 (40)
Health-related quality of life					
Short Form-36 Health Survey questionnaire					
Physical	score	45.2 ± 5.1	53.5 ± 5.0*	46.5 ± 5.2	47.4 ± 6.6+
Mental	score	44.5 ± 4.5	52.1 ± 4.4*	46.3 ± 6.3	45.1 ± 6.7+
Minnesota Living with Heart Failure questionnaire					
	score	35.1 ± 6.1	22.2 ± 5.0*	36.3 ± 5.4	35.5 ± 4.8+

HIIT, high-intensity interval training; GHC, general healthcare; Pre, pre-intervention; Post, post-intervention; M, male; F, female; BP, blood pressure; MET, metabolic equivalences; LVEF, left ventricular ejection fraction; BNP, brain natriuretic peptide; MPO, myeloperoxidase; ACE/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker. Values are mean ± SD. * $P < 0.05$, Pre vs. Post; + $P < 0.05$, HIIT vs. GHC.

Table 2
The effect of high-intensity interval training on cardiopulmonary responses to exercise in patients with heart failure.

		HIIT		GHC	
		Pre	Post	Pre	Post
Exercise performance					
Work-rate	watt	85 ± 18	121 ± 27*+	92 ± 15	87 ± 17
Circulatory responses					
HR	bpm	137 ± 7	142 ± 6	138 ± 6	136 ± 8
Systolic BP	mm Hg	132 ± 16	133 ± 11	136 ± 12	135 ± 13
Diastolic BP	mm Hg	78 ± 9	81 ± 10	80 ± 11	79 ± 9
MAP	mm Hg	96 ± 10	98 ± 9	99 ± 11	97 ± 012
Respiratory responses					
V_E	L/min	43.9 ± 9.2	60.3 ± 9.4*+	45.1 ± 8.9	47.1 ± 9.9
VO_2	mL/min/kg	15.8 ± 4.0	24.7 ± 3.2*+	15.9 ± 3.5	16.0 ± 3.4
VCO_2	mL/min/kg	17.1 ± 5.8	26.5 ± 5.5*+	17.4 ± 5.3	18.2 ± 5.5
RER	ratio	1.05 ± 0.24	1.08 ± 0.25	1.09 ± 0.23	1.13 ± 0.35
OUES	unit	566 ± 74	876 ± 92*+	622 ± 89	645 ± 82
V_E-VCO_2 slope	unit	34.8 ± 5.1	31.2 ± 4.3*+	35.2 ± 5.6	34.6 ± 5.9

Values are mean ± SD. HIIT, high-intensity interval training; GHC, general healthcare; Pre, pre-intervention; Post, post-intervention; HR, heart rate; BP, blood pressure; MAP, mean arterial pressure; V_E, minute ventilation; VO_2, O_2 consumption; VCO_2, CO_2 production; RER, respiratory exchange ratio; OUES, oxygen uptake efficiency slope. * $P < 0.05$, Pre vs. Post; + $P < 0.05$, HIIT vs. GHC.

animal model of post-infarction HF reported that HIIT rescued impaired contractility, attenuated hypertrophy, and downregulated atrial natriuretic peptide in cardiac myocytes [25]. In summary, HIIT may effectively improve ventilatory and hemodynamic efficiencies, thereby enhancing systemic aerobic capacity in HF patients.

4.2. Mitochondrial bioenergetics in circulating platelets

Progression to HF is probably associated with a gradual decline in bioenergetic reserve capacity owing to the inability of endogenous homeostatic mechanisms to compensate for the insufficient energy supply [26, 27]. Moreover, mitochondrial dysfunction in HF may result in systemic inflammation that facilitates susceptibility to energy-based pathologies associated with oxidative stress [28]. Additionally, atherosclerosis and thrombosis are reported to be associated with deterioration of platelet mitochondrial function [4]. Hence, it is plausible that platelet mitochondrial bioenergetics is a marker for metabolic stress and thrombogenesis in HF progression.

Recently, our investigation demonstrated that acute hypoxic exercise suppressed platelet BHI value in healthy sedentary males [13]. However, the HIIT intervention significantly elevated the BHI value in platelets at rest and attenuated the suppression of BHI caused by acute hypoxic exercise. In the present study, HIIT also elevated platelet mitochondrial BHI at rest, accompanied by increases in VO_{2peak} and LVEF in HF patients. Accordingly, platelet mitochondrial BHI could serve as a dynamic biomarker for assessing aerobic fitness and cardiac contractility of HF patients.

Mitochondria are highly sensitive to oxidative stress and respond dynamically to changes in their microenvironment [28, 29]. Mitochondrial-derived reactive oxygen species (ROS) are prominent early drivers of ischemia-reperfusion injury, and have been considered the consequence of the interaction of dysfunctional respiratory chain with O_2 during reperfusion [30]. Previous studies have revealed that exercise preconditioning improved myocardial tolerance to ischemia by activating mitochondrial ATP-sensitive K^+ channel [24, 31]. Moreover, the protective effects of exercise training on cardiovascular systems are associated with up-regulating expression of antioxidant enzymes and stress-related proteins [24, 31]. This investigation further demonstrated that HIIT significantly decreased plasma MPO and BNP levels in patients with HF. Accordingly, we posit that these HIIT-induced adaptations protect against oxidative stress/inflammation associated with mitochondrial dysfunctional processes in the HF patients.

A recent study reported that ischemia results in accumulation of intracellular succinate, thus leading to elevated mitochondrial ROS production [30]. Elevation of oxidative stress may also induce succinate accumulation by decreasing SDH activity [32]. Recently, our study including sedentary healthy men reported that HIIT significantly enhanced platelet SDH activity and Complex II-related respiration following hypoxic stress [13]. In the present study, 12-week HIIT effectively enhanced platelet mitochondrial OXPHOS capacity by increasing Complex II activity in HF patients. Therefore, HIIT-induced elevation in SDH activity and Complex II respiration in platelets may rapidly eliminate succinate, thereby further reducing ROS production from platelet mitochondria in HF patients.

Previous studies have reported that long-term exercise upregulates muscular mtDNA genes by upregulating Tfam in skeletal muscles [33]. Short-term exercise promotes transcriptional or post-translational regulation of peroxisome proliferator-activated receptor gamma coactivator-1α in skeletal muscles [33]. However, platelets are anucleate cytoplasmic fragments derived from megakaryocytes, thereby possibly lacking of mitochondrial biogenesis [4]. Our recent study also revealed no significant change in the Complex IV/II ratio of platelets after HIIT in healthy sedentary men [13]. Accordingly, the present results suggest that HIIT-induced platelet metabolic adaptation may be associated with improved ETS efficiency, rather than modulated biogenesis of platelet mitochondria in HF patients.

4.3. Health-related quality of life

Beside an increase in aerobic capacity, this present study demonstrated that HIIT significantly decreased the score of MLHFQ and increased the scores of the SF-36 physical and mental dimensions. These findings imply that HIIT simultaneously improves generic and disease-specific QoL in patients with HF. Hence, this exercise regimen effectively enhances the ability of patients to cope with the physical demands of daily activity and subsequently improving psychosocial status in HF patients. Furthermore, the better health-related QoL might exhibit less potential for mortality in HF patients and simultaneously reduce the financial burden in their health care system [34].

5. Limitations

Our small sample size in each group is a major limitation. However, results regarding platelet mitochondrial bioenergetics have high values of statistical power ranging from 0.876 to 1.000. Additionally, this study mainly focused on the effects of exercise on platelet mitochondrial functions rather than platelet reactivity (adhesion and aggregation) and platelet-mediated coagulation [thrombin generation (TG)]. Our previous studies have investigated the effect of exercise training on platelet adhesiveness and aggregation and their underling mechanisms in patients with cardiovascular diseases [11]. Our recent study also reported that HIIT markedly suppressed hypoxia-induced oxidative damage of platelet mitochondria and consequent attenuation of platelet-mediated TG caused by HE in healthy sedentary men [13]. However, the role of platelet mitochondrial function on exercise-mediated platelet reactivity and coagulation in HF patients warrants further investigation.

6. Conclusions

Currently, treatment for HF patients is limited to interference with neurohormonal activation and reduction of myocardial O_2 consumption [35]. However, the alleviation of mitochondrial dysfunction to improve bioenergetic efficiency seems to be an important strategy for the HF therapy in future. In the present study, 12-week HIIT improved exercise performance through increased

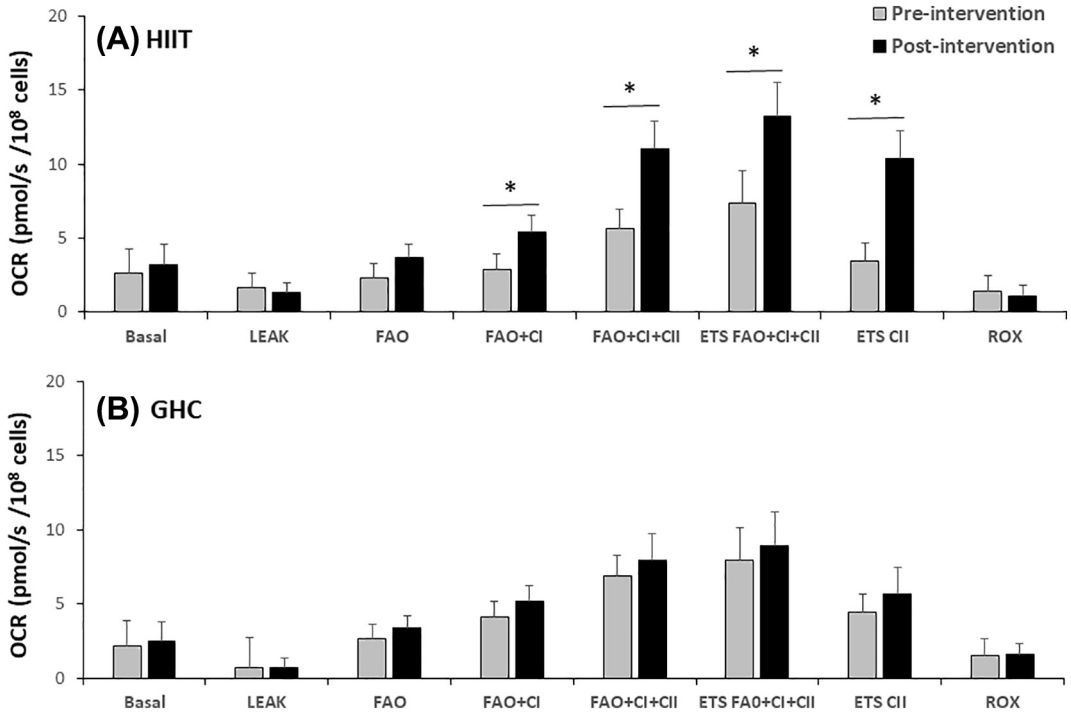

Fig. 2. Effects of high-intensity interval training (HIIT) on mitochondrial O_2 consumption rate (OCR) of the permeabilized platelets in patients with heart failure. GHC, general health care. FAO, fatty acid oxidation; FAO + CI, fatty acid oxidation + Complex I; FAO + CI + CII, fatty acid oxidation + Complex I + Complex II; ETS $_{FAO+CI+CII,}$ adding FCCP (carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone) after fatty acid oxidation + Complex I + Complex II; ETS_{CII}, adding FCCP after Complex II; ETS, electron transport system; $^{*}P < 0.05$, Pre-intervention vs. Post-intervention; Values were mean ± SD.

ventilatory efficiency and myocardial contractility in HF patients. Simultaneously, the exercise regimen also enhanced mitochondrial OXPHOS capacity by improved efficiency of mitochondrial ETS in platelets. Moreover, systemic aerobic capacity was positively correlated with the capacities for the maximal and reserve OCRs of platelet mitochondria in HF patients. These experimental findings may facilitate the identification of effective exercise training regimens to increase systemic aerobic capacity and improve the efficiency for platelet mitochondrial bioenergetics in patients with HF.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2018.07.104.

Author contributions

Jong-Shyan Wang was involved in conception and design of research; Cheng-Hsien Chou and Tieh-Cheng Fu performed experiments; Jong-Shyan Wang, Cheng-Hsien Chou and Tieh-Cheng Fu analyzed data, interpreted results of experiments, prepared the Figures, and drafted the paper; Jong-Shyan Wang, Tieh-Cheng Fu, and Hsing-Hua Tsai edited and revised the paper; Jong-Shyan Wang, Cheng-Hsien Chou, Tieh-Cheng Fu, and Hsing-Hua Tsai approved the final version of paper.

Funding sources

This work was supported by the National Science Council of Taiwan (grant number NSC 105-2314-B-182-013-MY3), Chang Gung Medical Research Program (grant number CMRPD 2C0162), and Healthy Aging Research Center, Chang Gung University (grant number EMRPD1A0841).

Acknowledgments

The authors would like to thank the volunteers for their enthusiastic participation.

Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

References

[1] A. Blum, H. Miller, Pathophysiological role of cytokines in congestive heart failure, Annu. Rev. Med. 52 (2001) 15–27.
[2] R. Marcucci, A.M. Gori, F. Giannotti, et al., Markers of hypercoagulability and inflammation predict mortality in patients with heart failure, J. Thromb. Haemost. 4 (2006) 1017–1022.
[3] E. Shantsila, G.Y.H. Lip, The endothelium and thrombotic risk in heart failure, Thromb. Haemost. 102 (2009) 185–187.
[4] S. Zharikov, S. Shiva, Platelet mitochondrial function: from regulation of thrombosis to biomarker of disease, Biochem. Soc. Trans. 41 (2013) 118–123.
[5] Y.W. Chen, Y.C. Chen, T.C. Fu, C.H. Wang, S.C. Huang, J.S. Wang, Anemic comorbidity reduces capacity of endogenous thrombin generation and is associated with consumptive coagulopathy in patients with heart failure, Int. J. Cardiol. 168 (2013) 4965–4967.
[6] J.W. Akkerman, Regulation of carbohydrate metabolism in platelets. A review, Thromb. Haemost. 39 (1978) 712–724.
[7] B.K. Chacko, P.A. Kramer, S. Ravi, et al., The bioenergetic health index: a new concept in mitochondrial translational research, Clin. Sci. 127 (2014) 367–373.
[8] P.A. Kramer, B.K. Chacko, S. Ravi, et al., Bioenergetics and the oxidative burst: protocols for the isolation and evaluation of human leukocytes and platelets, J. Vis. Exp. 85 (2014), e51301.
[9] S.I. Obydennyy, A.N. Sveshnikova, F.I. Ataullakhanov, M.A. Panteleev, Dynamics of calcium spiking, mitochondrial collapse and phosphatidylserine exposure in platelet subpopulations during activation, J. Thromb. Haemost. 14 (2016) 1867–1881.
[10] K.W. Lee, G.Y. Lip, Acute versus habitual exercise, thrombogenesis and exercise intensity, Thromb. Haemost. 91 (2004) 416–419.
[11] J.S. Wang, Exercise prescription and thrombogenesis, J. Biomed. Sci. 13 (2006) 753–761.
[12] Y.K. Kesaniemi, E. Danforth Jr., M.D. Jensen, P.G. Kopelman, P. Lefèbvre, B.A. Reeder, Dose-response issues concerning physical activity and health: an evidence-based symposium, Med. Sci. Sports Exerc. 33 (2001) S351–S358.
[13] L.H. Wu, S.C. Chang, T.C. Fu, C.H. Huang, J.S. Wang, High-intensity interval training improves mitochondrial function and suppresses thrombin generation in platelets undergoing hypoxic stress, Sci. Rep. 7 (2017), e4191.
[14] T.C. Fu, C.H. Wang, P.S. Lin, et al., Aerobic interval training improves oxygen uptake efficiency by enhancing cerebral and muscular hemodynamics in patients with heart failure, Int. J. Cardiol. 167 (2013) 41–50.

[15] J.S. Wang, T.C. Fu, H.Y. Lien, et al., Effect of aerobic interval training on erythrocyte rheological and hemodynamic functions in heart failure patients with anemia, Int. J. Cardiol. 168 (2013) 1243–1250.
[16] American College of Sports Medicine, General principle of exercise prescription, in: W.R. Thompson, N.F. Gordon, L.S. Pescatello (Eds.), ACSM's Guidelines for Exercise Testing and Prescription, Lippincott Williams & Wilkins, Philadelphia 2010, pp. 152–182.
[17] R. Arena, M. Guazzi, J. Myers, Ventilatory abnormalities during exercise in heart failure: a mini review, Curr. Respir. Med. Rev. 3 (2007) 179–187.
[18] M. Hollenberg, I.B. Tager, Oxygen uptake efficiency slope: an index of exercise performance and cardiopulmonary reserve requiring only submaximal exercise, J. Am. Coll. Cardiol. 36 (2000) 194–201.
[19] C. Van Laethem, N. Van De Veire, G. De Backer, et al., Response of the oxygen uptake efficiency slope to exercise training in patients with chronic heart failure, Eur. J. Heart Fail. 9 (2007) 625–629.
[20] H.H. Tsai, S.C. Chang, C.H. Chou, T.P. Weng, C.C. Hsu, J.S. Wang, Exercise training alleviates hypoxia-induced mitochondrial dysfunction in the lymphocytes of sedentary males, Sci. Rep. 6 (2016), e35170. .
[21] N.V. Sneed, S. Paul, Y. Michel, A. Vanbakel, G. Hendrix, Evaluation of 3 quality of life measurement tools in patients with chronic heart failure, Heart Lung 30 (2001) 332–340.
[22] C.M. O'Connor, D.J. Whellan, K.L. Lee, et al., Efficacy and safety of exercise training in patients with chronic heart failure: HF-ACTION randomized controlled trial, JAMA 301 (2009) 1439–1450.
[23] S.A. Hunt, W.T. Abraham, M.H. Chin, et al., 2009 focused update incorporated into the ACC/AHA 2005 guidelines for the diagnosis and Management of Heart Failure in adults: a report of the American College of Cardiology Foundation/American Heart Association task force on practice guidelines: developed in collaboration with the International Society for Heart and Lung Transplantation, Circulation 119 (2009) e391–e479.
[24] E. Crimi, L.J. Ignarro, F. Cacciatore, C. Napoli, Mechanisms by which exercise training benefits patients with heart failure, Nat. Rev. Cardiol. 6 (2009) 292–300.
[25] U. Wisløff, J.P. Loennechen, S. Currie, G.L. Smith, Ø. Ellingsen, Aerobic exercise reduces cardiomyocyte hypertrophy and increases contractility, Ca^{2+} sensitivity and SERCA-2 in rat after myocardial infarction, Cardiovasc. Res. 54 (2002) 162–174.
[26] H. Lemieux, S. Semsroth, H. Antretter, D. Höfer, E. Gnaiger, Mitochondrial respiratory control and early defects of oxidative phosphorylation in the failing human heart, Int. J. Biochem. Cell Biol. 43 (2011) 1729–1738.
[27] R. Ventura-Clapier, A. Garnier, V. Veksler, F. Joubert, Bioenergetics of the failing heart, Biochim. Biophys. Acta 2011 (1813) 1360–1372.
[28] H. Tsutsui, Oxidative stress in heart failure: the role of mitochondria, Intern. Med. 40 (2001) 1177–1182.
[29] J.W. Walters, D. Amos, K. Ray, N. Santanam, Mitochondrial redox status as a target for cardiovascular disease, Curr. Opin. Pharmacol. 27 (2016) 50–55.
[30] E.T. Chouchani, V.R. Pell, E. Gaude, et al., Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS, Nature 515 (2014) 431–435.
[31] S. Gielen, G. Schuler, V. Adams, Cardiovascular effects of exercise training: molecular mechanisms, Circulation 122 (2010) 1221–1238.
[32] E. Mills, L.A. O'Neill, Succinate: a metabolic signal in inflammation, Trends Cell Biol. 24 (2014) 313–320.
[33] H.N. Carter, C.C. Chen, D.A. Hood, Mitochondria, muscle health, and exercise with advancing age, Physiology (Bethesda) 30 (2015) 208–223.
[34] M.C. Zuluaga, P. Guallar-Castillón, E. López-García, et al., Generic and disease-specific quality of life as a predictor of long-term mortality in heart failure, Eur. J. Heart Fail. 12 (2010) 1372–1378.
[35] D.A. Brown, J.B. Perry, M.E. Allen, et al., Expert consensus document: mitochondrial function as a therapeutic target in heart failure, Nat. Rev. Cardiol. 14 (2017) 238–250.