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## The acute and long-term effects of a cardiac rehabilitation program on endothelial progenitor cells in chronic heart failure patients: Comparing two different exercise training protocols



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### ABSTRACT

**Background:** Vascular endothelial dysfunction is an underlying pathophysiological feature of chronic heart failure (CHF). Endothelial progenitor cells (EPCs) are also impaired. The purpose of the study was to assess the effect of a cardiac rehabilitation (CR) program on the increase of EPCs at rest and on the acute response after maximal exercise in patients with CHF and investigate whether there were differences between two exercise training protocols and patients of NYHA II and III classes.

**Methods:** Forty-four patients with stable CHF enrolled in a 36-session CR program and were randomized in one training protocol; either high-intensity interval training (HIIT) or HIIT combined with muscle strength (COM). All patients underwent maximum cardiopulmonary exercise testing (CPET) before and after the CR program and venous blood was drawn before and after each CPET. Five endothelial cellular populations, expressed as cells/10<sup>6</sup> enucleated cells, were quantified by flow cytometry.

**Results:** An increase in all endothelial cellular populations at rest was observed after the CR program ( $p < 0.01$ ). The acute response after maximum exercise increased in 4 out of 5 endothelial cellular populations after rehabilitation. Although there was increase in EPCs at rest and the acute response after rehabilitation in each exercise training group and each NYHA class, there were no differences between HIIT and COM groups or NYHA II and NYHA III classes ( $p > 0.05$ ).

**Conclusions:** A 36-session CR program increases the acute response after maximum CPET and stimulates the long-term mobilization of EPCs at rest in patients with CHF. These benefits seem to be similar between HIIT and COM exercise training protocols and between patients of different functional classes.

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## 1. Introduction

Chronic heart failure (CHF) remains a leading cause of morbidity and mortality with a significant financial and social burden [1]. Vascular endothelial dysfunction, impaired microcirculation and

increased inflammation are important underlying pathophysiological features of CHF [2,3].

During the last decades, endothelial progenitor cells (EPCs) have been used as an index of the endothelium restoration potential, therefore reflecting the vascular endothelial function [4]. Regular aerobic exercise has a beneficial impact in the function of the vascular endothelium and EPCs [5–7]. Different modalities, namely high-intensity interval and continuous ones, have been shown to enhance the stimulation of EPCs from the bone marrow and mature endothelial cells also known as circulating endothelial cells (CECs), positively influencing their number or their functional

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properties in patients with comorbidities and increased risk factors. These effects of exercise rehabilitation on EPCs stimulation have been mostly studied at rest (baseline values), before and after rehabilitation; in any case, limited data have been provided as of today [8–11]. Previous studies have also investigated the EPCs stimulation to acute exercise without exercise training before, suggesting also beneficial effects [12]. The EPCs response to acute exercise, as an effect of an exercise rehabilitation, has not been studied yet. The combination of aerobic endurance and strength training has also been shown to induce, at least comparable to aerobic training alone, beneficial effects on endothelial function [13,14]; its effect, however, on EPCs mobilization remain unknown. Moreover, limited data has suggested that exercise beneficially affects patients of different severity based on NT-proBNP levels [15]. The exercise effect on patients of different functional severity indices, eg New York Heart Association (NYHA) class, has not been studied yet.

We hypothesized that an exercise training rehabilitation program would beneficially effect EPCs mobilization in patients with CHF, either at rest or a single maximal exercise session after rehabilitation. The primary aim of the study was to assess the effect of a cardiac rehabilitation (CR) program on the EPCs mobilization both at rest and acutely. The secondary aims were to investigate whether there were differences between two exercise training protocols and between patients of different functional status, as expressed by the NYHA class.

## 2. Methods

### 2.1. Patients and study design

This randomized control trial (RCT) was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and approved by the Administration Board and the Ethics Committee of “Evangelismos General Hospital” in Athens, Greece (Approval number: 117/3–7-2017). All the patients signed an informed consent form to participate in the study. Patients were referred for assessment to the “Clinical Ergospirometry, Exercise and Rehabilitation Laboratory” of “Evangelismos General Hospital” by heart failure outpatient clinics of Athens. The diagnosis of CHF was based on personal history forms, clinical evaluation and laboratory testing of every patient.

The population of the study consisted of 44 patients with CHF who enrolled in a 36-session cardiac rehabilitation program (Supplementary Table 1 and Supplementary Fig. 1). All patients underwent a symptom limited maximal cardiopulmonary exercise testing (CPET) on an electromagnetically braked cycle ergometer before and after the rehabilitation program. Blood samples were taken before and after each CPET. Inclusion criteria for patients' participation were stable CHF at maximum tolerated medication and a reduced or mid-ranged ejection fraction ( $EF \leq 49\%$ ). The majority of patients was mainly treated with diuretics, b-blockers, aldosterone antagonists or angiotensin-converting-enzyme inhibitors. Exclusion criteria, also set in the beginning of the study, were severe valvulopathy, uncontrolled arterial hypertension, severe chronic obstructive pulmonary disease, severe peripheral angiopathy, neuromuscular diseases and contraindications for maximum CPET.

### 2.2. Cardiopulmonary exercise testing

A ramp symptom limited maximal CPET on an electromagnetically braked cycle ergometer (Ergoline 800; SensorMedics Corporation, Anaheim, California) took place before and after the rehabilitation program. CPET procedure and methodology has been

previously described in detail [16]. In short, individualized workload stage increments were calculated according to Hansen et al equations, aiming to 8–12 min test duration. The nose of the patients was clamped and they were breathing through a special mask with a low resistance valve and a known gas mixture. Breathing parameters such as oxygen uptake ( $VO_2$ ) and ventilation (VE) were measured in each breath by the software and their values were recorded at the monitor of the computer system (Vmax 229, Sensor Medics, Anaheim, California, USA). The gas exchanges of each patient were also recorded in order to calculate more specific values such as  $VO_2$  at peak exercise (peak  $VO_2$ ), predicted  $VO_2$  at peak exercise (predicted peak  $VO_2$ ) and peak work rate (WR<sub>peak</sub>). The peak values for  $VO_2$  and VE were calculated as the average of measurements made during the 20-second period before the end of exercise. Peak work rate (WR<sub>peak</sub>) was defined as the highest work rate reached and maintained at a pedaling frequency of no < 65 revolutions per minute. A 12 lead ECG system (MAX 1 system, Marquette Electronics) was attached on the patient's body in order to monitor the heart rate and the heart rhythm, a pulse oxymeter on the patient's finger demonstrated the saturation and blood pressure was measured every 2 min during CPET. The end point of the session was due to ECG abnormal rhythm at the monitor, dyspnea or leg fatigue of the patient.

### 2.3. Exercise training protocols

After their initial CPET, patients were randomly assigned by age (cut-off point: 50 years) and peak  $VO_2$  (cut-off point: 16 ml/kg/min) in either the high-intensity interval training group (HIIT Group) or HIIT combined with muscle strength training group (COM Group) and performed 36 sessions of exercise training. Any missed session was added at the end of the CR program before the final CPET.

The HIIT protocol was a modified version of Wisløff et al protocol [17]. Intensity was individually prescribed based on  $VO_2$ -workload plots of initial CPET. All patients were cycling for 7 min warm-up at 45% peak  $VO_2$  on a stationary bike (Ironman M3 Upright Cycle) followed by 3 min at 50% peak  $VO_2$ . Four 4-minute intervals at 80% peak  $VO_2$  were alternated with 3-minute repetitions at 50% peak  $VO_2$ . Workload intensity was gradually increased throughout the program to reach + 25% by the end. The total duration of the session was 31 min.

In both groups, the aerobic exercise program was similar. In the COM group, strength training followed cycle exercise and was prescribed based on one repetition maximum test (1RM). Strength training 2–3 sets, 10–12 repetitions, 60%–75% 1RM) involved knee extension, knee flexion and chest press exercises. with 1-minute rest between sets.

In the HIIT group, patients performed balance and coordination exercises after completing the aerobic training. The exercises included: narrow corridor walking, backward narrow corridor walking and side walking in both sides. The total duration of both protocols was similar.

Patients were also distributed in two different NYHA class groups (NYHA II and III) in order to investigate the benefits of regular aerobic exercise in different patients' subclasses.

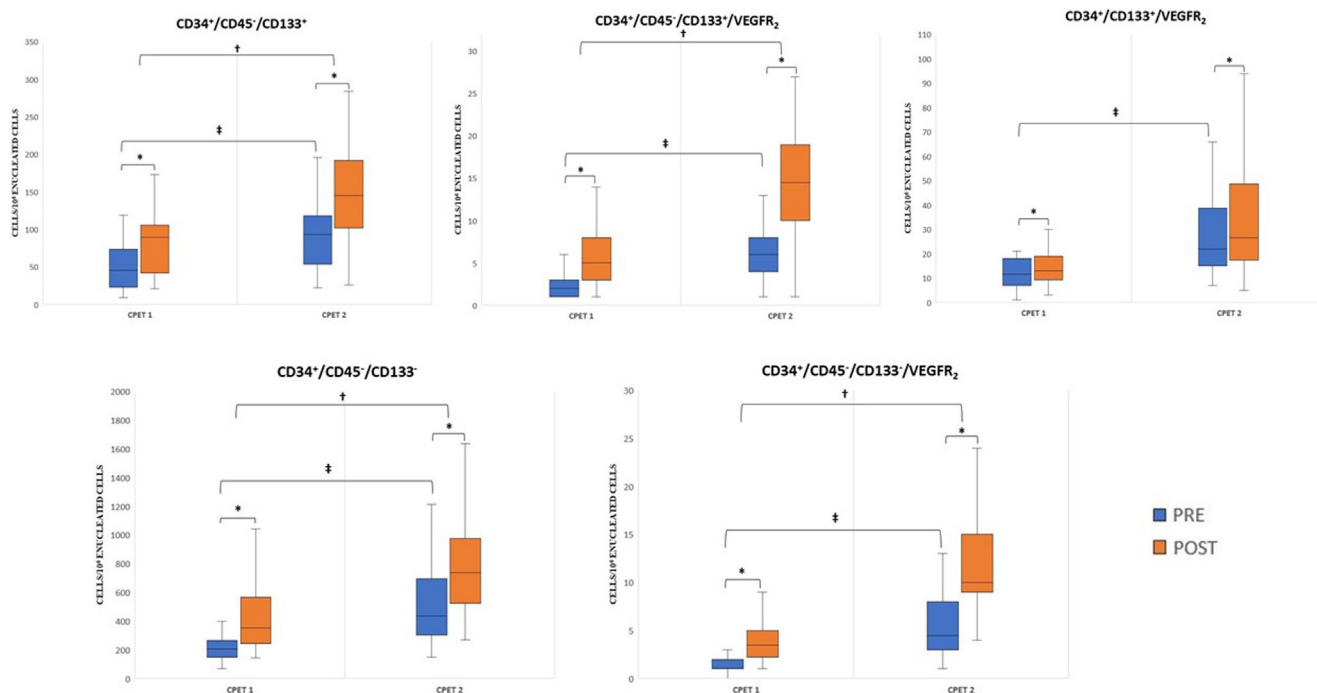
Patients of both exercise training groups (HIIT and COM) and both NYHA classes (NYHA II and NYHA III) demonstrated similar baseline demographic and CPET characteristics (Table 1). Patients were mainly treated with diuretics, angiotensin-converting-enzyme (ACE) inhibitors, b-blockers and aldosterone antagonists (Table 1). Other categories included angiotensin II receptor blockers (ARB), amiodarone, digoxin and  $Ca^{2+}$  blockers but in lower percentages.

**Table 1**

Demographic characteristics for patients with CHF enrolled in the cardiac rehabilitation program, as well as for each exercise training group and NYHA class group. Except ejection fraction and type of CHF in NYHA group comparison, not any other differences were observed between groups ( $p > 0.05$ ).

Demographic characteristics	All patients	Exercise Groups		NYHA class Groups	
		HIIT Group	COM Group	NYHA II	NYHA III
Number of patients (N)	44	21	23	34	10
Gender (Males / Females)	35 / 9	17 / 4	18 / 5	27 / 7	8 / 2
Age (years) <sup>a</sup>	56 ± 10	55 ± 11	57 ± 9	56 ± 10	55 ± 9
BMI (kg/m <sup>2</sup> ) <sup>a</sup>	28.7 ± 5.2	29.5 ± 5.7	28.0 ± 4.8	28.3 ± 5.4	29.9 ± 4.9
EF before rehabilitation (%) <sup>b</sup>	30 (28–40)	35 (30–43)	30 (25–35)	35 (30–41)	25 (19–30)*
<b>Type of CHF</b>					
Dilated cardiomyopathy [n (%)]	12 (27%)	5 (24%)	7 (30%)	7 (20%)	5 (50%)
Ischemic [n (%)]	24 (55%)	11 (52%)	13 (57%)	22 (65%)	2 (20%)
Other (valvulopathy, etc) [n (%)]	8 (18%)	5 (24%)	3 (13%)	5 (15%)	3 (30%)
<b>Medication</b>					
Diuretics [n (%)]	29 (66%)	13 (62%)	16 (70%)	20 (59%)	9 (90%)
ACE inhibitors [n (%)]	22 (50%)	11 (52%)	11 (48%)	18 (53%)	4 (40%)
ARBs [n (%)]	5 (11%)	4 (19%)	1 (4%)	3 (9%)	2 (20%)
b Blockers [n (%)]	43 (98%)	21 (100%)	22 (96%)	33 (97%)	10 (100%)
Aldosterone Antagonists [n(%)]	32 (73%)	16 (76%)	16 (70%)	27 (79%)	5 (50%)
<b>Cardiopulmonary exercise testing indexes before rehabilitation</b>					
VO <sub>2</sub> peak (ml/kg/min) <sup>a</sup>	18.4 ± 4.4	18.7 ± 5.0	18.2 ± 3.8	18.9 ± 4.1	16.8 ± 5.1
VO <sub>2</sub> peak predicted (%) <sup>a</sup>	64 ± 15	64 ± 19	65 ± 12	66 ± 14	57 ± 19
Peak WR (watts) <sup>a</sup>	101 ± 39	106 ± 43	96 ± 35	103 ± 38	95 ± 43

CHF, chronic heart failure; HIIT group, high-intensity interval training group; COM group, HIIT combined with muscle strength group; NYHA, New York Heart Association; BMI, body mass index; EF, ejection fraction; ACE, angiotensin-converting-enzyme; ARB, angiotensin II receptor blockers; VO<sub>2</sub>, oxygen uptake; WR, work rate. a Values are expressed as mean ± SD b Values are expressed as median (25th – 75th percentiles)



**Fig. 1.** Numeric mobilization of each circulating endothelial population in 44 patients with chronic heart failure at 4 time points; before and after the initial (CPET 1) and the final (CPET 2) maximal cardiopulmonary exercise testing. Asterisk (\*) indicates significant difference in the acute mobilization of circulating endothelial populations after CPET ( $p < 0.05$ ), cross (‡) indicates the increase in the acute response of circulating endothelial populations to maximum exercise after the cardiac rehabilitation program ( $p < 0.05$ ) while double cross (‡) indicates significant difference in the mobilization of circulating endothelial populations at rest ( $p < 0.05$ ).

**2.4. Epcs flow cytometry analyses**

For the identification and quantification of EPCs, blood samples were drawn from a peripheral vein of each patient twice in each CPET, once at rest and once just after maximal exercise. The same procedure was repeated twice during the rehabilitation program, once at the initial CPET before rehabilitation and once at the final CPET after rehabilitation. Venous blood was collected in K3 ethylenediaminetetraacetic acid (K3-EDTA) tubes and measured

with the use of flow cytometry within the first hour after the collection. The protocol implemented was the Duda et al. protocol [18] and 4 types of monoclonal antibodies were used; CD45-PerCP (BD Pharmingen, cat. no. 340665), CD34-APC (BD Pharmingen, cat. no. 340441), CD133-PE (Miltenyi Biotec, cat. no. 130-080-801) and VEGFR<sub>2</sub> (KDR)-PE (R&D Systems, cat. no. FAB 3578). Five different endothelial cellular populations, 3 subgroups of EPCs and 2 subgroups of circulating endothelial cells (CECs), were defined; these were CD34<sup>+</sup>/CD45<sup>+</sup>/CD133<sup>+</sup>, CD34<sup>+</sup>/CD45<sup>+</sup>/

CD133<sup>+</sup>/VEGFR<sub>2</sub>, CD34<sup>+</sup>/CD133<sup>+</sup>/VEGFR<sub>2</sub> (EPCs subgroups), CD34<sup>+</sup>/CD45<sup>-</sup>/CD133<sup>-</sup> and CD34<sup>+</sup>/CD45<sup>-</sup>/CD133<sup>-</sup>/VEGFR<sub>2</sub> (CECs subgroups). Four-color flow cytometry was performed in the Flow Cytometry Core Laboratory with BD FACSCantoII (Becton–Dickinson) flow cytometer. Each analysis on the flow cytometer included 10<sup>6</sup> events (Supplementary Fig. 2). The number of EPCs was expressed as median (25th – 75th percentiles) in absolute number of cells per 10<sup>6</sup> nucleated cells.

The upper phase (plasma) of venous blood after the centrifugation was used for the measurement of C-reactive protein (CRP) and vascular endothelial growth factor (VEGF) [18,19]. Immunoturbidimetric assay for the in vitro quantitative determination of CRP in human plasma was used (Roche/Hitachi cobas c systems, Roche Diagnostics International Ltd). Immunoturbidimetry uses the classical antigen–antibody reaction. To assess cytokine levels and VEGF, BD™ CBA Human Soluble Protein Flex Set System was used [19]. BD CBA assays provide a method of capturing a soluble analyte or set of analytes with beads of known size and fluorescence, making it possible to detect sandwich complexes (capture bead + analyte + detection reagent) using flow cytometry. Four-color flow cytometry was performed with Navios (Beckman Coulter) flow cytometer. Values of VEGF were expressed as medians (25th – 75th percentiles) in pg/ml.

### 2.5. Statistical analyses

Normality of distribution was checked with Shapiro-Wilk test. Variables are expressed as mean ± standard deviation (SD) or median (25th – 75th percentiles). Paired two sample Student *t* test analyzed differences of dependent parameters with normal distribution while Wilcoxon signed-rank test analyzed differences for nonparametric data within total sample and within exercise and NYHA class groups. Independent samples *t* test or Mann-Whitney *U* test analyzed differences between independent parameters, based on distribution of normality as appropriate. Chi-square tests were employed to check for between-group differences on categorical variables at baseline. Unadjusted differences between exercise groups and NYHA class groups were assessed with factorial analysis of variance (ANOVA) 2 × 2 × 2 (time × intervention × group). All tests were two tailed and level of statistical significance was set at 0.05. Statistical analyses were performed with IBM SPSS 25 Statistics.

### 3. Results

Patients' compliance with the rehabilitation program was over 80% in both groups. In total sample of 44 patients, values at rest (before each CPET) of all endothelial cellular populations were increased after rehabilitation indicating the effect of the CR program in their long-term mobilization (*p* < 0.001, Table 2).

As far as the acute mobilization of endothelial cellular populations after CPET is concerned, an increase was observed in all of them in both the initial and the final CPET (*p* < 0.05, Table 2). However, the absolute EPCs number of the acute response to post-CPET was significantly higher than the acute response to pre-CPET in 4 out of 5 endothelial cellular populations (Fig. 1).

All CPET indexes improved after rehabilitation. Most specifically, peak VO<sub>2</sub> (from 18.4 ± 4.4 ml/kg/min to 20.7 ± 5.7 ml/kg/min, *p* = 0.002), peak predicted VO<sub>2</sub> (from 64 ± 15% to 73 ± 22%, *p* < 0.001) and peak work rate (from 101 ± 39 W to 121 ± 45 W, *p* < 0.001) increased after the CR program. Moreover, CRP decreased [from 0.2 (0.1–0.5) mg/dL to 0.1 (0–0.2) mg/dL, *p* < 0.001] indicating an improvement in their inflammatory status while neo-angiogenetic factor VEGF increased [from 14 (13–20) pg/ml to 23 (17–37) pg/ml, *p* < 0.001]. No correlations were found

between differences in CRP and VEGF and differences in the mobilization of circulating endothelial populations at rest and after CPETs, either in absolute numbers or in percentages (*p* > 0.05).

Regarding HIIT and COM groups, baseline values of all endothelial cellular populations at rest increased in both after the CR program (*p* < 0.05, Table 3). An acute increase in the mobilization of at least 4 out of 5 endothelial cellular populations after each CPET was also observed within each group while the acute response after maximum exercise was higher after the CR program in CD34<sup>+</sup>/CD45<sup>-</sup>/CD133<sup>+</sup> EPCs, CD34<sup>+</sup>/CD45<sup>-</sup>/CD133<sup>-</sup>/VEGFR<sub>2</sub> EPCs and CD34<sup>+</sup>/CD45<sup>-</sup>/CD133<sup>-</sup>/VEGFR<sub>2</sub> CECs in both HIIT and COM groups (*p* < 0.05, Supplementary tables 1 and 2 respectively).

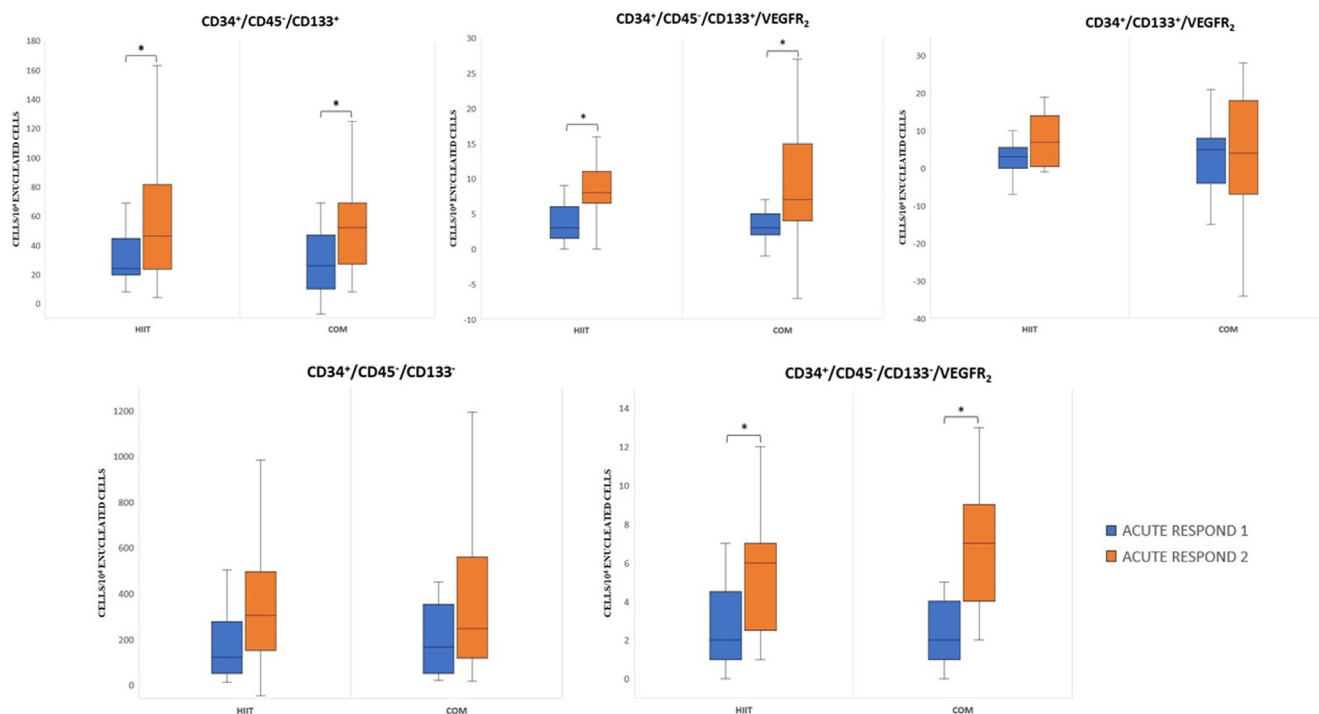
Patients in the HIIT group increased peak VO<sub>2</sub>, predicted peak VO<sub>2</sub> and peak WR while patients in COM group increased only predicted peak VO<sub>2</sub> and peak WR (*p* < 0.05, Table 3). Regarding blood sample indexes, CRP decreased and VEGF increased in both groups (*p* < 0.05, Table 3). Concerning comparison between the 2 exercise training groups, no differences were observed in the mobilization of EPCs, aerobic exercise capacity and blood markers (*p* > 0.05, Table 3, Supplementary table 3 and Fig. 2).

Baseline values of all endothelial cellular populations at rest in NYHA II and NYHA III group increased after the CR program (*p* < 0.05, Table 4). An acute increase in the mobilization of all endothelial cellular populations in NYHA II group after each CPET and in 4 out of 5 endothelial cellular populations in NYHA III group after each CPET were also observed while the acute response after maximum exercise was higher after the CR program in CD34<sup>+</sup>/CD45<sup>-</sup>/CD133<sup>+</sup>/VEGFR<sub>2</sub> EPCs and CD34<sup>+</sup>/CD45<sup>-</sup>/CD133<sup>-</sup>/VEGFR<sub>2</sub> CECs in NYHA II group and CD34<sup>+</sup>/CD45<sup>-</sup>/CD133<sup>+</sup> EPCs and CD34<sup>+</sup>/CD45<sup>-</sup>/CD133<sup>-</sup>/VEGFR<sub>2</sub> CECs in NYHA III group (*p* < 0.05, Table 4). No differences were observed in the mobilization of EPCs, CECs, aerobic exercise capacity and blood markers between the two NYHA class groups (*p* > 0.05).

### 4. Discussion

This study demonstrated that a 36-session cardiac rehabilitation program increases the acute response to maximum exercise and the long-term mobilization of EPCs at rest in patients with CHF. The comparison between 2 different exercise protocols, HIIT and COM, showed that the positive effect of the CR program on endothelial function is similar for both protocols. During the last decades, both endothelial progenitor and circulating endothelial cells have been used as an index of the endothelium restoration potential, therefore reflecting the vascular endothelial function [4].

Many studies regarding the positive effects of rehabilitation programs on inflammatory [9,20,21] and angiogenic markers [8,22] and on aerobic exercise capacity [9–11,14,21] in patients with CHF have been conducted but only a few have shown the beneficial effect of regular exercise on the vascular endothelial function in these patients, and most specifically on endothelial cellular populations [8–11]. All these studies [8–11] investigated the effect of a multi-session exercise training program, either HIIT or CT, in reversing endothelial dysfunction in CHF patients demonstrating a significant improvement in EPCs function, number or percentage at rest. Although Van Craenenbroeck et al [11] have shown a significant improvement in CD34<sup>+</sup>/CD3<sup>-</sup> cells after exercise, they did not notice a significant CD34<sup>+</sup>/KDR<sup>+</sup>/CD3<sup>-</sup> cells increase. Sandri et al [8] noticed a significant improvement in CD34<sup>+</sup>/KDR<sup>+</sup> and CD133<sup>+</sup>/KDR<sup>+</sup> cells' function and number both in younger and older patients with CHF. Eleuteri et al [9] found a significant increase in CD45<sup>dim</sup>/CD34<sup>+</sup>/KDR<sup>+</sup> cells' percentage while Mezzani et al [10] showed a significant increase in the mobilization of CD45<sup>dim</sup>/CD34<sup>+</sup>/KDR<sup>+</sup> cells both in number and percentage in patients with CHF. The main findings of these studies are in agree-



**Fig. 2.** Numeric value of acute response after maximal cardiopulmonary exercise testing of each circulating endothelial population in HIIT and COM groups at 2 time points; before (acute response 1) and after (acute response 2) the cardiac rehabilitation program. Asterisk (\*) indicates significant difference ( $p < 0.05$ ) within each exercise training group. No difference was observed between the 2 exercise training groups ( $p > 0.05$ ).

**Table 2**  
Endothelial cellular populations before and after each symptom-limited maximal cardiopulmonary exercise testing in patients with chronic heart failure.

Endothelial cellular populations <sup>a,#</sup>	Before CR program		After CR program		p value between baseline values	p value for the acute response between CPETs
	Before CPET Baseline value	After CPET	Before CPET Baseline value	After CPET		
CD34 <sup>+</sup> /CD45 <sup>-</sup> /CD133 <sup>+</sup>	46 (23–74)	90 (42–106)**	93 (54–118)	145 (102–192)**	< 0.001	0.002
CD34 <sup>+</sup> /CD45 <sup>-</sup> /CD133 <sup>+</sup> /VEGFR <sub>2</sub>	2 (1–3)	5 (3–8)**	6 (4–8)	15 (10–19)**	< 0.001	< 0.001
CD34 <sup>+</sup> /CD133 <sup>+</sup> /VEGFR <sub>2</sub>	12 (7–18)	13 (9–19)*	22 (15–39)	27 (17–49)*	< 0.001	0.441
CD34 <sup>+</sup> /CD45 <sup>-</sup> /CD133 <sup>-</sup>	208 (147–266)	352 (245–569)**	437 (302–695)	739 (525–976)**	< 0.001	0.030
CD34 <sup>+</sup> /CD45 <sup>-</sup> /CD133 <sup>-</sup> /VEGFR <sub>2</sub>	1 (1–2)	4 (2–5)**	5 (3–8)	10 (9–15)**	< 0.001	< 0.001

CR, cardiac rehabilitation; CPET, cardiopulmonary exercise testing.

<sup>a</sup> Values are presented as median (25th – 75th percentiles)

<sup>#</sup> Values are expressed as cells/10<sup>6</sup> enucleated cells

Significant difference in the acute mobilization of cellular populations before and after CPET (\* $p < 0.05$ , \*\* $p < 0.001$ )

ment with our findings as we also observed a mobilization of EPCs baseline values in all cellular populations at rest after a CR program. However, by the present study we extend previous findings showing that there is a significant mobilization in more EPCs populations and also in CECs in patients with CHF after a CR program. Potential reasons for the differences between these studies and our data may be related to the noteworthy differences in our methodology, resulting in a better discrimination of the defined endothelial cellular populations. The confusion in the proliferation between EPCs and CECs is caused by two main reasons. One is that international literature lacks of widely approved methods for the definition of EPC phenotypes and established golden standards for the quantification of endothelial cellular populations by flow cytometry [23,24]. The other reason is that there are many described, highly reproducible flow cytometric approaches, however without reaching a high agreement between them [24]. Despite these two reasons, and based on the most approved protocols so far [18,25], we used a more analytic EPCs quantification with 5 differ-

ent endothelial cellular populations using 4 monoclonal antibodies, whereas all the other studies used at most two populations of endothelial cells with less monoclonal antibodies for their determination (usually defined as CD45<sup>dim</sup>/CD34<sup>+</sup>/KDR<sup>+</sup> or CD34<sup>+</sup>/KDR<sup>+</sup> progenitor cells).

This study is the first one to investigate the EPCs acute response to maximum exercise after a CR program in patients with CHF. It was showed that a CR program enhances the mobilization of endothelial cellular populations after a single bout of maximum exercise, indicating the effect of exercise training on the acute stimulus. CHF is characterized by increased inflammatory status and endothelial dysfunction which are crucially involved in development and progression of the disease [2,3,26]. Inflammation has a key role in skeletal muscle wasting and dysfunction [27]. Impaired endothelium dependent vasodilatation, in addition to impaired cardiac function, is a main determinant of exercise intolerance in patients with CHF, limiting physical exercise capacity and deteriorating peak aerobic capacity [2,3,26,27]. Exercise has been reported

**Table 3**  
Baseline values (at rest) in variables of each exercise training group before and after a cardiac rehabilitation program.

Variables of the CR program	HIIT Group (21 patients)		COM Group (23 patients)		p value between groups
	Before CR	After CR	Before CR	After CR	
<b>Endothelial cellular populations<sup>a, #</sup></b>					
CD34 <sup>+</sup> /CD45 <sup>-</sup> /CD133 <sup>+</sup>	52 (37–73)	98 (75–125)**	41 (21–75)	89 (46–102)***	0.390
CD34 <sup>+</sup> /CD45 <sup>-</sup> /CD133 <sup>+</sup> /VEGFR <sub>2</sub>	2 (1–2)	6 (5–8)***	2 (1–4)	4 (3–8)***	0.439
CD34 <sup>+</sup> /CD133 <sup>+</sup> /VEGFR <sub>2</sub>	10 (7–16)	22 (14–39)*	14 (8–18)	24 (15–40)**	0.573
CD34 <sup>+</sup> /CD45 <sup>-</sup> /CD133 <sup>-</sup>	231 (142–279)	406 (293–690)***	202 (148–248)	520 (356–701)**	0.472
CD34 <sup>+</sup> /CD45 <sup>-</sup> /CD133 <sup>-</sup> /VEGFR <sub>2</sub>	1 (1–2)	4 (3–8)***	1 (1–3)	5 (3–8)**	0.319
<b>Cardiopulmonary exercise testing indices<sup>b</sup></b>					
Peak VO <sub>2</sub> (ml/kg/min)	18.7 ± 5.0	21.8 ± 7.1**	18.2 ± 3.8	19.8 ± 4.1	0.295
Predicted peak VO <sub>2</sub> (%)	64 ± 19	75 ± 27**	65 ± 12	71 ± 16*	0.342
Peak WR (watts)	106 ± 43	125 ± 46***	96 ± 35	116 ± 44***	0.839
<b>Blood samples indices<sup>a</sup></b>					
CRP (mg/dL)	0.2 (0.1–0.5)	0.1 (0–0.2)**	0.2 (0.1–0.5)	0.1 (0–0.3)**	0.347
VEGF (pg/ml)	14 (13–19)	22 (16–45)***	15 (12–20)	25 (18–39)***	0.473

HIIT group, high-intensity interval training group; COM group, HIIT combined with muscle strength group; CR, cardiac rehabilitation; Peak VO<sub>2</sub>, peak oxygen uptake; Predicted peak VO<sub>2</sub>, predicted peak oxygen uptake; Peak WR, peak work rate; CRP, C-reactive protein; VEGF, vascular endothelial growth factor.

a Values are presented as median (25th – 75th percentiles)

b Values are presented as mean ± SD

# Values are expressed as cells/10<sup>6</sup> enucleated cells

Significant difference in variables of patients within each exercise group at rest before and after a CR program (\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001)

**Table 4**  
Circulating endothelial populations, CRP and VEGF before and after each symptom-limited maximal cardiopulmonary exercise testing in NYHA II and NYHA III groups before and after a cardiac rehabilitation program.

Variables of the CR program	NYHA II Group (34 patients)				NYHA III Group (10 patients)				p value CPET * NYHA class	p value CR * NYHA class	p value CPET * CR * NYHA class
	Before CR program		After CR program		Before CR program		After CR program				
	Before	After CPET	Before	After CPET	Before	After CPET	Before	After CPET			
<b>Endothelial cellular populations<sup>a, #</sup></b>											
EPC <sub>1</sub>	54 (27–76)	90 (47–118)*	96 (69–119) <sup>†</sup>	147 (102–172)*	33 (18–53)	78 (33–94)*	69 (43–110) <sup>†</sup>	125 (102–261)* <sup>‡</sup>	<b>0.036</b>	0.307	0.079
EPC <sub>2</sub>	2 (1–3)	5 (3–8)*	6 (3–8) <sup>†</sup>	15 (11–19)* <sup>‡</sup>	3 (1–4)	5 (3–8)*	6 (4–9) <sup>†</sup>	12 (8–15)*	0.413	0.774	0.672
EPC <sub>3</sub>	11 (7–18)	13 (9–21)*	21 (14–41) <sup>†</sup>	24 (17–51)*	13 (9–26)	14 (10–18)	22 (20–38)	29 (23–52)	0.906	0.299	<b>0.028</b>
CEC <sub>1</sub>	228 (150–274)	411 (269–591) <sup>†</sup>	436 (309–622) <sup>†</sup>	722 (516–924)*	174 (126–273)	232 (186–315)*	584 (281–867) <sup>†</sup>	926 (664–1647)	0.338	<b>0.011</b>	0.111
CEC <sub>2</sub>	1 (1–2)	3 (3–5)*	4 (3–8) <sup>†</sup>	10 (9–13)* <sup>‡</sup>	1 (1–2)	5 (2–8)*	6 (3–8) <sup>†</sup>	15 (9–20)* <sup>‡</sup>	0.229	0.178	0.563
<b>Indices from blood samples<sup>a</sup></b>											
CRP (mg/dL)	0.2 (0.1–0.4)	0.2 (0.1–0.4)	0.1 (0–0.2) <sup>†</sup>	0.1 (0.1–0.3)*	0.4 (0.2–0.6)	0.3 (0.2–0.6)	0.1 (0.1–0.3) <sup>†</sup>	0.3 (0.1–0.3)	0.948	0.553	0.836
VEGF (pg/ml)	14 (13–18)	21 (18–27)*	22 (17–32) <sup>†</sup>	32 (22–47)	17 (12–36)	21 (15–29)	26 (18–70) <sup>†</sup>	22 (18–35)	0.185	0.448	0.430

CR, cardiac rehabilitation; CPET, cardiopulmonary exercise testing; NYHA, New York Heart Association; EPC<sub>1</sub>, CD34<sup>+</sup>/CD45<sup>-</sup>/CD133<sup>+</sup>; EPC<sub>2</sub>, CD34<sup>+</sup>/CD45<sup>-</sup>/CD133<sup>+</sup>/VEGFR<sub>2</sub>; EPC<sub>3</sub>, CD34<sup>+</sup>/CD133<sup>+</sup>/VEGFR<sub>2</sub>; CEC<sub>1</sub>, CD34<sup>+</sup>/CD45<sup>-</sup>/CD133<sup>-</sup>; CEC<sub>2</sub>, CD34<sup>+</sup>/CD45<sup>-</sup>/CD133<sup>-</sup>/VEGFR<sub>2</sub>; CRP, C-reactive protein; VEGF, vascular endothelial growth factor.

a Values are presented as median (25th – 75th percentiles)

# Values are expressed as cells/10<sup>6</sup> enucleated cells

\* Significant difference in variables before and after CPET within each NYHA class group (p < 0.05)

† Significant difference in variables between baseline values at rest before and after CR within each NYHA class group (p < 0.05)

‡ Significant difference in the acute response of EPCs and CECs between the initial and the final CPET within each NYHA class group (p < 0.05)

to increase blood flow and shear stress, therefore increasing endothelial nitric oxide synthase (eNOS) activity and nitric oxide (NO) production and reducing inflammation [5]. Targeting endothelial dysfunction could be a breakthrough therapy as endothelial function is recognized as a crucial component underlying HF. The proposed hypothesis is that regular exercise, organized as a training program, has an anti-inflammatory effect induced by acute multiple sessions, which in turn leads to protection against chronic inflammatory conditions, especially by reducing the levels of CRP [28]. High levels of CRP are known to directly inhibit EPCs differentiation, survival, and function via an effect of CRP to reduce EPCs eNOS expression [29]. Levels of CRP of our patients were low,

and as result, EPCs kinetics or survival was not affected avoiding thus a bias in our methodology.

An additional new insight of this study is the comparison of the effect between 2 different exercise training protocols on EPCs and CECs. HIIT is known to have a beneficial impact on the vascular endothelium function and enhance the stimulation of EPCs from the bone marrow by positively influencing the EPCs number and their functional properties [30]. However, it was unknown whether the addition of muscle strength training would enhance effects. In this study, mobilization of endothelial cellular populations was similarly increased in both exercise groups.

Finally, it is the first study to compare EPCs in patients of different functional status, as expressed by NYHA class. Exercise is shown to have beneficial effect in CHF patients in general but the effect in each subgroup of different functional status was unknown. Only one single study compared the effect of maximal exercise on EPCs in CHF patients of different functional status based on NT-proBNP levels but no increase was found within each subgroup [15]. In our study, we found increase of EPCs levels after a rehabilitation program within each subgroup; the beneficial effect of exercise training was similar between these groups.

Concerning the potential mechanisms of the mobilization of EPCs, shear stress and ischemic/hypoxic stimulus could be suggested as triggering factors for their release after exercise. Shear stress seems to upregulate the activity of endothelial nitric oxide synthase, increase the production of nitric oxide (NO) and activate channels leading to a transient increase in intracellular  $Ca^{2+}$  [31,32]. Moreover, exercise has the potential to induce hypoxic stimuli resulting to up-regulation of angiogenic factors, such as stromal cell-derived factor 1 (SDF-1) and vascular endothelial growth factor (VEGF) mediating in this way processes to promote proliferative and migratory capacities of circulating EPCs [32,33]. These endothelial functions contribute to the amplified number and activity of circulating EPCs and they could play a role in signaling to the cell that it is under shear and eliciting a response [31–33].

Recent studies have investigated the effect of exercise training in inflammatory markers such as CRP and angiogenic factor VEGF [8,9,20–22]. Most of them did not show significant difference in CRP [9,20] after a rehabilitation program in patients with CHF. Our study demonstrated a beneficial effect of regular aerobic exercise in the inflammatory process of HF by reducing CRP in these patients after a CR program. Only de Meirelles et al [21] come in agreement with our findings regarding CRP. Studies which examined VEGF [8,22] demonstrated increased levels of this factor after exercise, a fact which we also found in our study. In our study, the increase in VEGF levels after rehabilitation did not correlate with the increase in EPCs levels indicating thus that the relation between them may be more qualitative than quantitative. Thus, it seems that there is no linear relationship between the mobilization of EPCs and the increase of VEGF and the effect of other factors is also important in the mechanisms of EPCs.

A study limitation is related to the sample size. Between-group comparisons were, in some instances, underpowered to reach definite conclusions. In addition, data on the statin therapy of patients and cardiovascular risk factors such as tobacco smoke, dyslipidemia, diabetes and hypertension were not included; however EPCs, CRP and VEGF values at baseline were similar between groups, likely suggesting absence of differences also in these factors.

In conclusion, a 36-session cardiac rehabilitation program increases the acute response to maximum exercise and stimulates the long-term mobilization of endothelial progenitor cells at rest, promotes neo-angiogenesis and improves functional indexes in patients with chronic heart failure. These benefits seem to be similar between high-intensity interval aerobic training and combined aerobic and strength training, as well as between patients of different functional status expressed by the NYHA class. Further investigation is needed to better understand function and role of different endothelial cellular populations.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix

<b>CEC</b>	Circulating Endothelial Cells
<b>CHF</b>	Chronic Heart Failure
<b>COM</b>	High-intensity interval training combined with muscle strength training
<b>CPET</b>	Cardiopulmonary Exercise Testing
<b>CR</b>	Cardiac rehabilitation
<b>CRP</b>	C-reactive protein
<b>ECG</b>	Electrocardiogram
<b>EDTA</b>	Ethylenediaminetetraacetic Acid
<b>EF</b>	Ejection Fraction
<b>EPC</b>	Endothelial Progenitor Cells
<b>HIIT</b>	High-intensity interval training
<b>NO</b>	Nitric Oxide
<b>NYHA</b>	New York Heart Association
<b>Peak VO<sub>2</sub></b>	Peak oxygen uptake
<b>Peak WR</b>	Peak work rate
<b>Predicted peak VO<sub>2</sub></b>	Predicted peak oxygen uptake
<b>SD</b>	Standard deviation
<b>SDF-1</b>	Stromal Cell-Derived Factor 1
<b>VEGF</b>	Vascular Endothelial Growth Factor
<b>VO<sub>2</sub></b>	Oxygen Uptake

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijcha.2020.100702>.

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