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Brief Report
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A Cardiac Rehabilitation Program Increases the Acute Response of Endothelial Progenitor Cells to Maximal Exercise in Heart Failure Patients

Christos Kourek,[1] Stavros Dimopoulos,[1,2] Manal Alshamari,[1] Virginia Zouganeli,[3] Katherina Psarra,[4] Georgios Mitsiou,[1] Argyrios Ntalianis,[5] Theodoros Pittaras,[6] Serafim Nanas[1] and Eleftherios Karatzanos[1]

Purpose: The purpose of this study was to investigate the effect of a cardiac rehabilitation program on the acute response on endothelial progenitor cells and circulating endothelial cells after maximal exercise in patients with chronic heart failure of different severity.

Methods: Forty-four chronic heart failure patients were enrolled in a 36-session cardiac rehabilitation program. All patients underwent an initial maximal cardiopulmonary exercise test before and a final maximal cardiopulmonary exercise test after the cardiac rehabilitation program. The patients were divided in two groups of severity according to the median value of peak VO_2. Blood was collected at 4 time points; 2 time points at rest, and 2 time points after each cardiopulmonary exercise test. Five endothelial cellular populations were quantified by flow cytometry.

Results: Although there was a higher increase in the mobilization of subgroups of endothelial progenitor cells and circulating endothelial cells after the final cardiopulmonary exercise test compared to the initial test within each severity group ($p < 0.05$), no significant differences between severity groups were observed ($p > 0.05$).

Conclusions: A 36-session cardiac rehabilitation program had similar beneficial effects on the acute response of endothelial progenitor cells and circulating endothelial cells after maximal exercise in patients with chronic heart failure of different severity.

Key Words: Acute maximal exercise • Cardiac rehabilitation • Chronic heart failure • Endothelial progenitor cells • Exercise • Severity

INTRODUCTION

Chronic heart failure (CHF) is a multifactorial clinical syndrome with high incidence worldwide, and it is associated with a poor prognosis and poor quality of life for patients. Vascular endothelial dysfunction is strongly associated with the severity of the syndrome.[1,2] Patients with CHF usually present with low levels of endothelial progenitor cells (EPCs) and circulating endothelial cells (CECs) at rest and after exercise compared with healthy populations. Low levels of EPCs and CECs have been associated with attenuated endothelial function,[3] while EPCs have been linked to the repair mechanism of endothelial damage.[4,5] Maximal exercise has been previously proven to increase the acute mobilization of EPCs and

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[1]Clinical Ergospirometry, Exercise & Rehabilitation Laboratory, "Evaggelismos" General Hospital, School of Medicine, National and Kapodistrian University of Athens; [2]Cardiac Surgery Intensive Care Unit, "Onassis" Cardiac Surgery Center; [3]2nd Department of Internal Medicine-Propaedeutic and Diabetes Center, University General Hospital "Attikon", Medical School, National and Kapodistrian University of Athens; [4]Immunology and Histocompatibility Department, "Evaggelismos" General Hospital; [5]Heart Failure Unit, Department of Clinical Therapeutics, "Alexandra" Hospital, National and Kapodistrian University of Athens; [6]Hematology Laboratory-Blood Bank, "Aretaieion" Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece.
Corresponding author: Dr. Eleftherios Karatzanos, Clinical Ergospirometry, Exercise & Rehabilitation Laboratory, "Evaggelismos" General Hospital, School of Medicine, National and Kapodistrian University of Athens, 45-47 Ypsilantou str., 10676, Athens, Greece. Tel: +30 2132043385; E-mail: lkaratzanos@gmail.com

CECs in CHF patients,[6] with the beneficial effect being similar in patients of different severity.[7] However, the effect of exercise training on the acute response of EPCs and CECs after maximal exercise and the role of severity have not been studied. We hypothesized that an exercise training program would increase the acute response of EPCs and CECs after maximal exercise in patients with CHF of different severity. The aim of the study was to compare the acute mobilization of EPCs and CECs after a maximal cardiopulmonary exercise test (CPET) before and after a cardiac rehabilitation (CR) program in patients with CHF of different severity according to peak VO_2.

MATERIALS AND METHODS

This is a post-hoc analysis of a recently published study from our Institute (approval number: 117/3-7-2017) which assessed EPC mobilization after exercise training in patients with CHF.[8]

Forty-four CHF patients with New York Heart Association (NYHA) class ≥ II and ejection fraction (EF) ≤ 49% signed an informed consent form to enroll in a 36-session CR program. The program included high intensity interval training (HIIT) or a combined HIIT and strength training protocol, as previously described.[8] Moreover, all patients underwent an initial symptom-limited maximal CPET on a cycle ergometer (Ergoline 800; SensorMedics Corporation, Anaheim, California) before the CR program, and a final maximal CPET after the 36 sessions of the CR program.

We collected venous blood at 4 time points; 2 time points at rest before the initial and the final CPET, and 2 time points after maximal exercise after the 2 CPET. Monoclonal antibodies CD45-PerCP (BD Pharmingen, cat. no. 340665), CD34-APC (BD Pharmingen, cat. no. 340441), CD133-PE (Miltenyi Biotec, cat. no. 130-080-801) and $VEGFR_2$ (KDR)-PE (R&D Systems, cat. no. FAB 3578) defined 5 endothelial populations; 3 EPC subgroups ($CD34^+/CD45^-/CD133^+$, $CD34^+/CD45^-/CD133^+/VEGFR_2$ and $CD34^+/CD133^+/VEGFR_2$), and 2 CEC subgroups ($CD34^+/CD45^-/CD133^-$ and $CD34^+/CD45^-/CD133^-/VEGFR_2$).[9] These endothelial populations were quantified by flow cytometry and expressed as median (25^{th}-75^{th} percentiles) in cells/10^6 enucleated cells.[9]

The patients were divided in two groups of severity according to the median value of peak VO_2 (18.3 ml/kg/min). Their main medications included diuretics, beta-blockers, aldosterone antagonists, and angiotensin-converting enzyme inhibitors (Table 1). Factorial analysis of variance (ANOVA) 2 × 2 × 2 (time × intervention × severity groups) was performed using IBM SPSS version 25.

Table 1. Baseline demographic characteristics and maximal cardiopulmonary exercise testing indices of patients with chronic heart failure of different severity according to peak VO_2

Demographic characteristics	Group 1 ≤ 18.3 ml/kg/min	Group 2 > 18.3 ml/kg/min
Number of patients (N)	23	21
Gender (males/females)	17/6	18/3
Age (years)[a]	57 ± 11	54 ± 9
NYHA stage (class II/III)	17/6	17/4
Ejection fraction (%)	30 (25-40)	35 (28-38)
Medication		
Diuretics [n (%)]	19 (83%)	10 (48%)*
ACE inhibitors [n (%)]	11 (48%)	11 (52%)
beta-blockers [n (%)]	23 (100%)	20 (95%)
Aldosterone antagonists [n (%)]	17 (74%)	15 (71%)
Baseline cardiopulmonary exercise testing indices		
Peak VO_2 (ml/kg/min)[a]	15.1 ± 2.8	22.1 ± 2.3*
Predicted peak VO_2 (%)[a]	55 ± 14	74 ± 11*
Peak WR (watts)[a]	82 ± 33	122 ± 33*

ACE, angiotensin-converting-enzyme; NYHA, New York Heart Association; VO_2, oxygen uptake; WR, work rate.
Values are expressed as [a] mean ± SD, [b] median (25^{th}-75^{th} percentiles).
Difference between the 2 severity groups for demographic characteristics and CPET parameters (* $p < 0.05$).

Unadjusted differences between severity groups were analyzed, and $p < 0.05$ was defined as being statistically significant.

RESULTS

Patients with a lower peak VO_2 were more likely to receive diuretics compared to those with a higher peak VO_2 ($p < 0.05$, Table 1). No other significant differences regarding demographics, EF or medications were observed between the 2 groups. CPET indices, including peak VO_2, predicted peak VO_2, and peak work rate were significantly higher in the patients with better functional capacity status (Table 1).

The severity group with higher peak VO_2 had increased acute responses of $CD34^+/CD45^-/CD133^+/VEGFR_2$ EPCs ($p < 0.001$) and $CD34^+/CD45^-/CD133^-/VEGFR_2$ CECs ($p = 0.003$) after the final maximal CPET compared with the initial CPET before the CR program, while the severity group with lower peak VO_2 had a greater increase in the acute response of $CD34^+/CD45^-/CD133^+$ EPCs ($p = 0.004$) and $CD34^+/CD45^-/CD133^+/VEGFR_2$ EPCs ($p = 0.001$) and $CD34^+/CD45^-/CD133^-/VEGFR_2$ CECs ($p = 0.003$) after the CR program (Figure 1, Supplementary Tables 1 and 2). No significant differences in the acute responses of EPCs and CECs after maximal exercise before and after the CR program were observed between the severity groups ($p > 0.05$, Figure 1). Moreover, there were no significant correlations between the initial peak VO_2 of patients and the numeric or percentage differences of the acute responses after maximal exercise in these endothelial cellular populations ($p > 0.05$).

Finally, although the CR program increased EF in both Group 1 [from 30 (25-40) to 35 (30-45), $p = 0.01$] and Group 2 [from 35 (28-38) to 39 (30-43), $p = 0.02$], there was no significant difference between the 2 groups ($p > 0.05$).

DISCUSSION

The present study demonstrated that a CR program consisting of 36 sessions enhanced the acute responses of EPCs and CECs after maximal exercise in a similar way in patients with CHF of different severity according to peak VO_2. The beneficial effects of exercise training were similar in the patients with either lower or higher peak

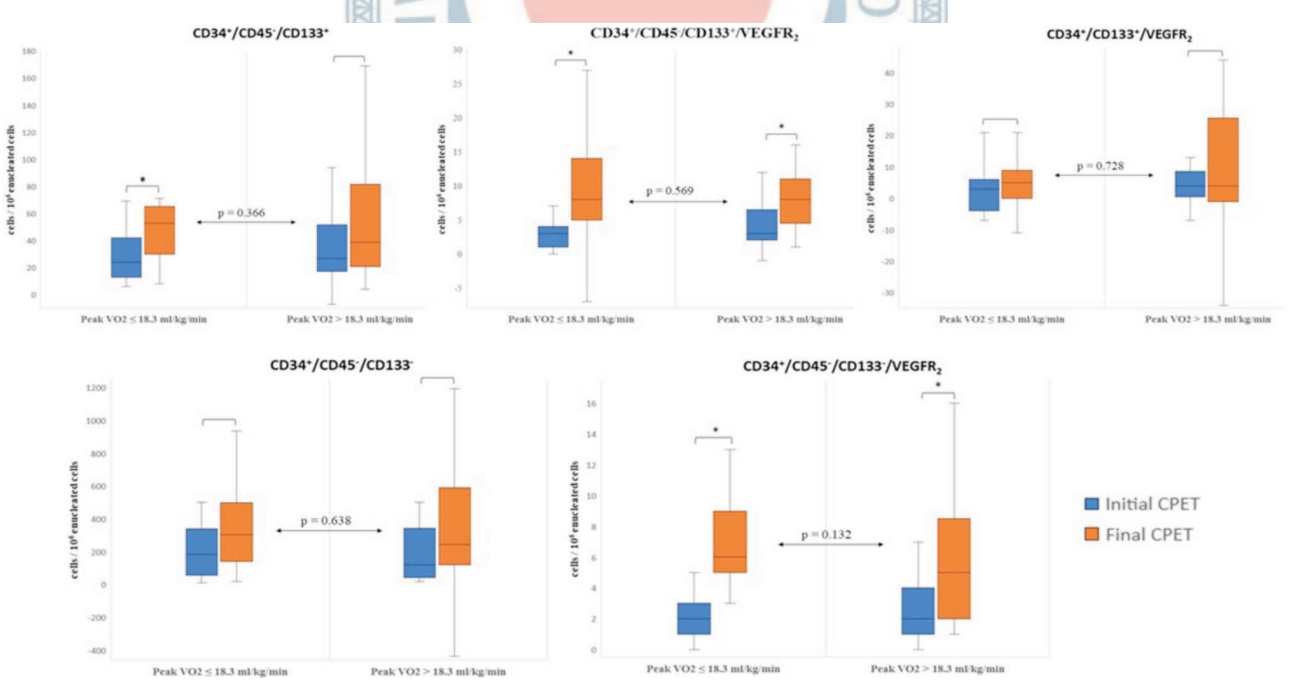

Figure 1. *Boxplots representing the acute response of the mobilization of each endothelial cellular population after the initial and the final cardiopulmonary exercise training within and between patients with chronic heart failure of high and low severity, based on peak VO_2. The asterisk (*) indicates statistically significant difference within each severity group ($p < 0.05$). No differences were observed between patients of high and low severity in each endothelial cellular population ($p > 0.05$). CPET, cardiopulmonary exercise test.*

VO_2. This is the first study to investigate the effect of a structured exercise training intervention on cellular level, and most specifically on the acute response of EPC and CEC mobilization after maximal exercise in patients with different CHF severity based on indexes of functional capacity. In our previous study,[6] we showed that maximal exercise increased the acute mobilization of these endothelial cellular populations in CHF patients. In the present study, we extend our previous findings by showing that not only maximal exercise but also exercise training seemed to be similarly effective for the acute response of EPCs, and therefore for vascular endothelial function of CHF patients of different severity. Increases in the absolute and percentage numbers of EPCs after exercise have been proven to reverse endothelial damage.[4,5]

There are some similarities between this and our previous study.[7] The patients initially underwent a symptom-limited maximal CPET as a single bout of maximal exercise before participating in a structured exercise training program in both studies, and the acute mobilization of different endothelial cellular populations was quantified after this single exercise bout. Moreover, in both studies, the patients were divided into groups of different CHF severity according to CPET indices. The main difference in the present study, and the main novelty compared to our previous study[7] and other studies, is the fact that we performed a symptom-limited maximal CPET not only before a rehabilitation program, but also after 36 sessions of regular aerobic exercise training. As a result, we could compare the effect of the rehabilitation program on the acute mobilization of EPCs and CECs after maximal exercise, and whether regular aerobic exercise acts collaboratively with regards to the acute effect of maximal exercise on microcirculation and vascular endothelial function.

Exercise can activate two different mechanisms for the mobilization of EPCs and CECs and their action on the endothelium. A mechanism related to shear stress stimulates the increase of endothelial nitric oxide and matrix metalloproteinases (MMPs). MMPs cut the bonds between EPCs and bone marrow, and facilitate their entry into the circulation.[4,10] Another mechanism is hypoxic stimulation of angiogenetic factors such as vascular endothelial growth factor, which guides EPCs and their mature form, CECs, to the point of endothelial damage.[4,10]

A study limitation is that our results cannot be generalized to all CHF populations, including patients with unstable and decompensated CHF.

Our findings indicate that the 36-session CR program had similar beneficial effects on the acute responses of EPCs and CECs after maximal exercise in patients with CHF of different severity. Further research on the potential mechanisms and in different CHF populations is required.

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DECLARATION OF CONFLICT OF INTEREST

All authors contributed equally to this work. The authors declare that there is no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Acute response of the mobilization of endothelial cellular populations after maximal exercise in patients with chronic heart failure of high severity before and after a cardiac rehabilitation program

Endothelial cellular populations[a]	Before rehabilitation		After rehabilitation		p value of the acute response between CPETs
	Before CPET	After CPET	Before CPET	After CPET	
$CD34^+/CD45^-/CD133^+$	54 (24-74)	84 (52-102)[#]	98 (76-131)	154 (102-209)[#]	0.004
$CD34^+/CD45^-/CD133^+/VEGFR_2$	2 (1-4)	5 (3-8)[#]	7 (4-9)	15 (10-20)[#]	0.001
$CD34^+/CD133^+/VEGFR_2$	13 (9-16)	13 (9-19)	22 (17-36)	27 (19-38)*	0.165
$CD34^+/CD45^-/CD133^-$	186 (131-287)	402 (204-544)[#]	431 (301-618)	738 (496-931)[#]	0.073
$CD34^+/CD45^-/CD133^-/VEGFR_2$	1 (1-3)	3 (3-5)[#]	4 (3-8)	10 (9-15)[#]	0.003

High severity group: Peak $VO_2 \leq 18.3$ ml/kg/min; CPET, cardiopulmonary exercise testing.
[a] Values are expressed as "cells/10^6 enucleated cells" in median (25^{th}-75^{th} percentiles).
Significant difference in the acute mobilization of endothelial cellular populations after a symptom-limited maximal CPET (* $p < 0.05$; [#] $p < 0.001$). CPET, cardiopulmonary exercise test.

Supplementary Table 2. Acute response of the mobilization of endothelial cellular populations after maximal exercise in patients with chronic heart failure of low severity before and after a cardiac rehabilitation program

Endothelial cellular populations[a]	Before rehabilitation		After rehabilitation		p value of the acute response between CPETs
	Before CPET	After CPET	Before CPET	After CPET	
$CD34^+/CD45^-/CD133^+$	42 (20-71)	90 (40-119)[#]	85 (50-112)	127 (95-179)[#]	0.123
$CD34^+/CD45^-/CD133^+/VEGFR_2$	2 (1-3)	5 (3-9)[#]	5 (3-7)	14 (9-17)[#]	< 0.001
$CD34^+/CD133^+/VEGFR_2$	10 (7-19)	14 (10-19)*	23 (14-54)	22 (16-73)	0.836
$CD34^+/CD45^-/CD133^-$	234 (164-259)	314 (263-637)[#]	520 (297-866)	740 (526-1194)*	0.231
$CD34^+/CD45^-/CD133^-/VEGFR_2$	1 (1-2)	4 (2-6)[#]	5 (3-8)	10 (8-12)[#]	0.003

Low severity group: Peak $VO_2 > 18.3$ ml/kg/min; CPET, cardiopulmonary exercise testing.
[a] Values are expressed as "cells/10^6 enucleated cells" in median (25^{th}-75^{th} percentiles).
Significant difference in the acute mobilization of endothelial cellular populations after a symptom-limited maximal CPET (* $p < 0.05$; [#] $p < 0.001$). CPET, cardiopulmonary exercise test.