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Endothelial progenitor cell mobilization and changes in systematic microcirculation during exercise in patients with chronic heart failure

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ABSTRACT

Purpose: Endothelial progenitor cells (EPCs) consist a cell population that enters the bloodstream from the bone marrow during tissue hypoxia and contributes to angiogenesis. Aerobic exercise induces tissue hypoxia and stimulates the mobilization of progenitor cells facilitating the endothelial function and contributing to the treatment of cardiovascular disease. The aim of this study was to investigate the effect of an aerobic exercise protocol on EPC mobilization and microcirculation in patients with chronic heart failure (CHF) compared to healthy individuals.

Materials and Methods: Patients that participated in the study were referred from the Heart Failure Clinic of the Cardiology Department of "Evangelismos" Hospital. Blood samples were collected before exercise, immediately after exercise, and 40 minutes post-exercise to determine the number of circulating EPCs and hematopoietic progenitor cells (HPCs) using flow cytometry. Changes in microcirculation were assessed using near-infrared spectroscopy before and after exercise.

Results: Sixteen patients (n = 16) with CHF and eleven healthy individuals (n = 11) participated in the study. The continuous aerobic exercise protocol revealed significant effects ($p < 0.05$) on the mobilization of EPCs and HPCs, as well as on endothelial function, in both patients with CHF and healthy individuals.

Conclusion: A single session of aerobic exercise can increase EPCs and HPCs and improve microcirculation in patients with chronic heart failure as well as in healthy individuals. Exercise performed using a low intensity, long-duration protocol stimulates physiological mechanisms that promote progenitor cell mobilization, microcirculatory function, and vascular regeneration.

Keywords: endothelial progenitor cells, hematopoietic progenitor cells, continuous exercise, microcirculation

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INTRODUCTION

Systematic aerobic exercise has anti-atherogenic effects on the vascular system, improves autonomic nervous system function (thereby reducing the risk of arrhythmias), and decreases the risk of myocardial ischemia. Among therapeutic approaches for the management of chronic heart failure (CHF) is the implementation of exercise, both in the form of individual exercise sessions and structured cardiac rehabilitation programs (1). The endothelium exhibits endocrine and paracrine properties and regulates vascular function through vasodilation of smooth muscle cells and its anti-inflammatory actions. Endothelial dysfunction promotes both immediate and long-term effects on the functional capacity of patients

with cardiovascular diseases (2). It is associated with atherosclerosis, thrombosis, and hypertension, which constitute pathological factors contributing to the development of CHF.

Several studies have demonstrated a significant reduction in endothelium -dependent vasodilation in the peripheral arteries of patients with CHF. An important role in improving endothelial function is accomplished by endothelial progenitor cells (EPCs), which, after their release from the bone marrow, contribute to maintaining a vital layer of endothelial cells that promotes angiogenesis. In patients with cardiovascular disease, endothelial ischemia increases circulating levels of endothelial progenitor cells (EPCs) up to 1000-fold, and this increase in their number can be used as a prognostic marker for the assessment of cardiovascular risk (3). In addition, hematopoietic progenitor cells (HPCs), a cellular population related to EPCs, exhibit progenitor-like properties (4), share a common origin with EPCs—the bone marrow angioblast—support the angiogenic process, and facilitate reparative mechanisms in the vascular system.

Following the initial detection of EPCs by Asahara et al. (1997) (5), maximal cardiopulmonary exercise testing (CPET) has been used as an acute stimulus involving intense physical activity to investigate changes in EPC counts. However, although CPET contributes to the diagnosis and evaluation of underlying conditions (6), it is neither recommended nor representative of a true exercise session (7). In patients with cardiovascular disease, the short duration of the test and the inability to achieve high exercise intensity due to symptom limitations appear to be insufficient to induce an increase in EPC numbers. Therefore, it would be important to investigate the EPC response following a prolonged exercise session at different time points in order to accurately determine i) whether EPC numbers increase and ii) the duration for which they remain elevated after exercise.

In contrast, studies have shown that short-duration CPET, even as brief as 10 minutes, can improve microcirculation in healthy individuals. Nevertheless, it is likely that an exercise session of longer duration may induce more pronounced improvements in microcirculation. This was demonstrated in a study in which a 30-minute session of moderate-intensity exercise significantly improved microvascular capillary function (8). Conversely, exercise performed at very high intensity (85% VO max) or of very long duration (90 minutes) did not result in significant improvements in microcirculation in physically active men (9, 10,11). In patients with chronic heart failure (CHF), some studies have suggested that continuous exercise protocols may improve microcirculation by increasing blood flow and oxygen delivery to peripheral tissues (12). However, further studies are required to clarify the effects of exercise on microcirculation in patients with CHF.

As indicated by the aforementioned studies, the exercise stimulus provided by continuous exercise induces increased blood flow and stimulates the release of growth factors and cytokines that can mobilize EPCs from the bone marrow into the circulation. The extent of EPC mobilization appears to depend on exercise-related characteristics, such as exercise duration. However, the evidence supporting the beneficial effects of continuous exercise is primarily derived from studies in healthy individuals and studies including rehabilitation programmes, and data in patients with cardiovascular disease, including those with CHF, are lacking. Moreover, the application of CPET, which represents primarily a diagnostic clinical test rather than a structured exercise session, has yielded inconsistent results in patients with cardiovascular disease. Consequently, the available evidence regarding the effects of a specific exercise session on vascular function and on the mobilization of EPCs and HPCs remains limited. Therefore, the aim of the present study was to investigate the effects of a continuous exercise protocol on the mobilization of EPCs and HPCs and on microcirculatory function in healthy individuals and patients with chronic heart failure.

Methodology

Patients were referred to the research laboratory from the Heart Failure Clinic of the Cardiology Department of "Evangelismos" Hospital. Healthy volunteers of similar age and body mass index also participated.

Inclusion Criteria

Inclusion criteria comprised men and women aged between 18 and 75 years. Patients were required to have stable heart failure with a left ventricular ejection fraction ≤45%. They were receiving stable, optimal medical therapy and were clinically stable, with no hospitalizations during the preceding three months. Neither patients nor healthy participants had taken part in any structured exercise program during the previous six months.

Exclusion Criteria

Exclusion criteria included contraindications to cardiopulmonary exercise testing (according to the American Thoracic Society), as well as unstable angina or recent acute myocardial infarction, and pathological conditions such as cancer, liver disease, or renal disease. Additional exclusion criteria were hypertension, severe vascular disease, severe chronic obstructive pulmonary disease, peripheral arterial disease, and neuromuscular disease. Participants enrolled in the study also had no symptoms indicative of active ischemia**.**

Exercise Protocol

All participants in the study performed an exercise protocol using the continuous method. The protocol began with a 5-minute warm-up and was conducted at a constant intensity of 50% VO_{2peak}, based on the heart rate corresponding to VO_{2peak}. The mean duration of the continuous exercise sessions was 53 minutes for patients with chronic heart failure (CHF) and 62 minutes for healthy participants. The duration of exercise was based on studies investigating the effects of cardiac rehabilitation, which have shown that approximately one hour of continuous exercise promotes the mobilization of progenitor cells during both short-term (13) and long-term (14) rehabilitation programs. However, the effectiveness of continuous exercise during a single exercise session has not yet been investigated. Exercise duration was calculated so that the total workload was equal for all patients and healthy participants, respectively. Basic hemodynamic parameters were recorded three minutes before exercise, during exercise, and for five minutes following the completion of exercise.

Study Sample

The calculation of the appropriate sample size to examine differences before and at two post-exercise time points was based on an effect size of 0.48 reported in the study by Gatta et al. (13). Specifically, with an effect size of 0.48, statistical power of 0.80, and a significance level of $\alpha = 0.05$, the minimum required sample size was calculated to be 14 patients with CHF allocated to the experimental group. Taking into account an estimated dropout rate of 20% and a missing data rate of 7%, the final sample size was set at 16 patients with CHF.

Outcome measures

Participants were familiarized with cycling exercise on an electronically braked cycle ergometer and subsequently underwent maximal CPET in the upright position. They were verbally encouraged to exercise until volitional exhaustion, which was typically defined as maximal lower-limb fatigue or severe dyspnea. Work rate increments were calculated using the equation proposed by Hansen et al. (2004) (15), in order to achieve an optimal test duration of 8–12 minutes, in accordance with the American Thoracic Society guidelines. Prior to each test, the system was calibrated by passing a known volume of air through the pneumotachograph using a 3-L precision syringe. The maneuver was repeated at least three times, and the best of the three flow–volume curves was used for analysis. All metabolic measurements were recorded for two minutes at rest, throughout the incremental exer-

cise phase, and for five minutes during the post-exercise recovery period.

Gas exchange was assessed on a breath-by-breath basis using an online system, while participants breathed through a low-resistance valve with a nose clip in place, using a metabolic cart (Vmax 229; Sensormedics, Yorba Linda, CA). Heart rate was monitored using a 12-lead electrocardiographic recording system (MAX1, Model 2000; Marquette Electronics, Milwaukee, WI). Arterial blood pressure was measured every two minutes. Peak oxygen uptake (VO peak) was determined as the average value obtained during the final 20 seconds of the CPET. Maximal exercise capacity was defined as the highest work rate achieved and maintained at a pedaling cadence of no less than 60 revolutions per minute for at least 20 seconds. Throughout the exercise test, participants inhaled through a mouthpiece connected to a triple-lumen valve and pneumotachograph, while the nose was occluded with a nose clip. Breath-by-breath measurements of oxygen uptake, carbon dioxide production, and airflow were obtained using a dedicated pulmonary and metabolic testing system. Resting oxygen uptake was calculated as the mean VO during the two minutes preceding exercise onset. Peak values of oxygen uptake (VO peak), carbon dioxide production, and respiratory exchange ratio during exercise were calculated as the average of the measurements obtained during the final 20 seconds prior to exercise termination.

CPET results were analyzed and used to individually determine the appropriate exercise intensity and duration for each participant. Measurements of circulating progenitor cells were performed before exercise, immediately after exercise, and 40 minutes post-exercise. Systemic microcirculation was assessed before and after the exercise session.

Blood Sampling and Flow Cytometry

Venous blood samples were obtained from a peripheral vein using an indwelling venous catheter. Blood collection was performed at three time points: 2 minutes before exercise, immediately after exercise, and 40 minutes post-exercise. During sample processing, blood was centrifuged twice at 700 rpm for 20 minutes without braking of the centrifuge. After the second centrifugation, the upper phase of the sample was removed and discarded. The cellular pellet was resuspended, and 2.5 mL were transferred to a separate tube and kept on ice. Simultaneously, 500 µL aliquots of the samples were transferred into sample tubes, and the following monoclonal antibodies were added: 10 µL CD45-PerCP, 5 µL CD133-PE, 3 µL CD34-APC, and 5 µL VEGFR2-PE. Sample processing continued with a third centrifugation at 4 °C at 250 g. All samples were placed in specialized tubes and transferred for analysis using dedicated flow cytometry software.

Boolean gating analysis (16) was applied for data analysis using combinations of specific cell surface markers. Due to the existing inconsistency in the literature regarding the definition and nomenclature of endothelial progenitor cells (EPCs), three different antigen combinations were used for their analysis (17). EPC measurements were based on the surface expression of four antigens—CD34, CD45, CD133 (clusters of differentiation), and KDR+ (kinase insert domain receptor)—and three distinct EPC marker combinations were analyzed. KDR+ represents the extracellular domain of vascular endothelial growth factor receptor-2 (VEGFR2+). The marker combination CD34+/CD45−/CD133+, which defined the EPC_1 population, was considered a general progenitor phenotype due to the expression of early stem cell antigens, but it lacked the endothelial lineage marker VEGFR2+. Depending on the expression of the endothelial antigen and the specific combination of surface markers, the cell populations CD34+/CD133+/KDR+ and CD34+/CD45−/ KDR+ were defined as EPC_2 and EPC_3, respectively.For the assessment of angiogenic progenitor cells (APCs), the marker combination CD34+/CD45−/CD133− was used.

Measurement of Systemic Circulation

Systemic microcirculation was assessed using a vascular occlusion technique for 3 minutes, with simultaneous continuous recording by near-infrared spectroscopy (NIRS) performed 3 minutes before and 3 minutes after exercise. This technique involves placing a small sensor on the skin surface and using near-infrared light to measure tissue oxygenation and blood flow within the microcirculation (18,19).

For the evaluation of microcirculation, the main parameters assessed were: tissue oxygen saturation (StO_2, %); oxygen consumption rate (%/min), calculated from the regression slope of the first minute of StO_2 decline following vascular occlusion; oxygen reoxygenation rate, representing endothelial function (%/s), quantified by the hyperemic response at the thenar eminence after release of vascular occlusion (reflecting endothelial function); and reactive hyperemia, expressed as the percentage change in the signal during arterial occlusion and estimated from vascular reserve.

Statistical Analysis

Study data were expressed as mean ± standard deviation (SD). Normality of data distribution was assessed using the Shapiro–Wilk test. The effects of continuous exercise at the three measurement time points on the mobilization of EPCs and HPCs were evaluated using a two-way repeated-measures analysis of variance (ANOVA) with a 2 × 3 design (protocol × time). The effects of exercise on microcirculation at the two measurement time points were assessed using a two-way repeated-measures ANOVA with a 2 × 2 design (protocol × time). Post hoc multiple comparisons between groups were performed using Sidak's correction. Effect size was calculated using Cohen's *d*, defined as the difference between the means of two groups divided by the pooled standard deviation of the observations (mean difference/SD), and interpreted as small (≥0.2), medium (≥0.5), or large (≥0.8) effects. Statistical significance was accepted at $p \leq 0.05$. All statistical analyses were conducted using SPSS software, version 28 (SPSS Inc., Chicago, IL).

RESULTS

Twenty-three patients with chronic heart failure (CHF) (n = 23) were invited to participate in the study; two did not meet the inclusion criteria, and one patient withdrew prior to the initiation of the experimental procedures. Twenty non-cachectic male outpatients with stable CHF (n = 20) entered the experimental phase, of whom sixteen (n = 16; mean age 49.2 ± 10.7 years; body mass 78.9 ± 18.6 kg; height 175.4 ± 8.6 cm; body mass index [BMI] 27.2 ± 3.1 kg/m²; left ventricular ejection fraction ≤ 45%) completed the continuous exercise protocol. In addition, eleven healthy individuals (n = 11) matched for age and body mass index also participated in the study. All participants were informed about the potential risks and benefits of the study and provided written informed consent in accordance with the Declaration of Helsinki for research involving human subjects. Baseline characteristics of all participants and clinical characteristics of the patients are presented in Table 1.

Mobilization of Endothelial and Hematopoietic Progenitor Cells

Based on the combinations of surface markers used for the identification of progenitor cells, statistical analysis revealed significant effects of continuous exercise on endothelial progenitor cells (EPCs) and hematopoietic progenitor cells (HPCs) immediately after exercise and 40 minutes post-exercise ($p < 0.05$) (Figure 1) in patients with CHF. In healthy participants, EPC_1, EPC_3 and HPCs increased both immediately after exercise and 40 minutes post-exercise ($p < 0.05$), whereas no changes were observed in EPC_2 at any time point following exercise ($p > 0.05$) (Figure 2) (Table 2).

Table 1. Descriptive characteristics of patients with chronic heart failure and healthy participants

	CHF (n = 16)	Healthy (n = 11)
Anthropometry (mean ± SD)		
Age (years)	*46.4 ± 8.6*	*43.4 ± 6.8*
BMI (Kg/m2)	*26.4 ± 3.3*	*26.3 ± 3.3*
Body Mass (Kg)	*81.6 ± 17.9*	71.7 ± 7.6
Body Height (cm)	176.3 ± 8.8	169.2 ± 6.4
VO_{2peak} and KE (mean ± SD)		
VO_{2peak} (ml/kg/min)	15.3 ± 2.4	
KE%	33.2 ± 4.0	
Heart Failure Characteristics [No (%)]		
NYHA Class II	10 (64)	
NYHA Class III	6 (36)	
Non-ischemic	10 (64)	
Ischemic	6 (36)	
Arterial Hypertension	4 (38)	
Diabetes	4 (38)	
Dyslipidemia	2 (11)	
Smoking	—	
Medical Treatment [No (%)]		
B-blockers	11 (68)	
ACE inhibitors	10 (63)	
Nitrates	4 (25)	
Statins	7 (43)	
Anti-arrhythmics	8 (51)	
Anti-coagulants	3 (19)	
Anti-platelets	4 (25)	

CHF, chronic heart failure; LVEF, left ventricular ejection fraction; SD, standard deviation; ACE, angiotensin-converting enzyme; BMI, body mass index; NYHA, New Heart Association

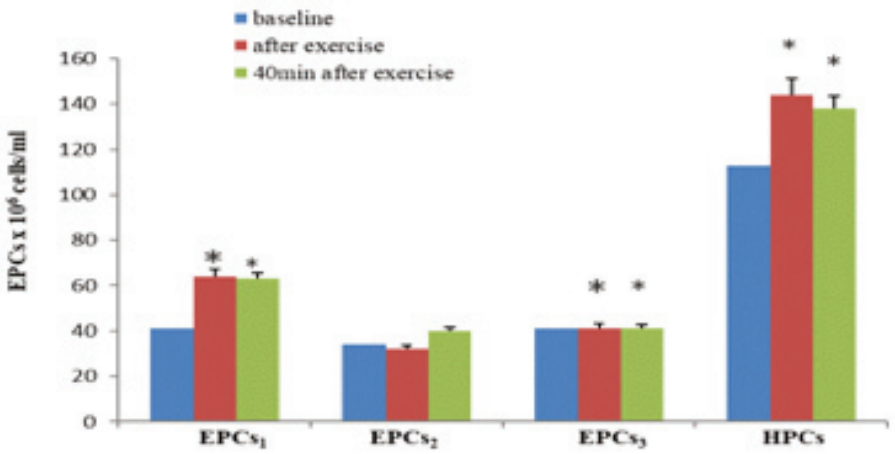

Figure 1. Mobilization of endothelial and hematopoietic progenitor cells in healthy participants EPCs: endothelial progenitor cells, HPCs: hematopoietic progenitor cells

Table 2. Mobilization of endothelial and hematopoietic progenitor cells in patients with chronic heart failure (n = 16) and healthy participants (n = 11)

Continuous exercise	Baseline	After exercise		40 min after exercise	
	Cells•10⁶ enucleated cells	Cells•10⁶ enucleated cells	Effect Size	Cells•10⁶ enucleated cells	Effect Size
Endothelial Progenitor Cells (EPCs) identified by different markers EPCs1 markers: CD34+/CD45-/CD133+					
CHF	19.7 ± 12.2	48.6 ± 34.3[b]	0.87	45.5 ± 41.8[b]	0.79
Healthy	41.5 ± 38.3	64.3.2 ± 52.8[b]	0.71	63.5 ± 51.2[b]	0.82
EPCs2 markers: CD34+/CD133+/VEGFR2+					
CHF	13.1 ± 14.2	16.7 ± 17.4	0.26	15.3 ± 16.1	0.25
Healthy	34.2 ± 26.3	32.9 ± 21.2[b]	0.33	3 9.8 ± 30.2	0.38
EPCs3 markers: CD34+/CD45-/VEGFR2+					
CHF	23.4 ± 26.7	42.3 ± 47.6	0.39	38.4 ± 31.2	0.57
Healthy	41.7 ± 25.2	41.4 ± 33.7	0.19	40.2 ± 28.1	0.23
Hematopoietic Progenitor Cells (HPCs) HPCs markers: CD34+/CD45-/CD133-					
CHF	89.4 ± 49.3	127.5 ± 61.0[a]	0.68	122.9 ± 81.3	0.49
Healthy	113.4 ± 72.6	144.9 ± 78.1	0.89	138.3 ± 86.7[a]	0.80

CHF: chronic heart failure; EPC: endothelial progenitor cells; HPC: hematopoietic progenitor cells; VEGF: vascular endothelial growth factor receptor-2ap ≤ 0.01and bp ≤ 0.05, ± SD: standard deviation

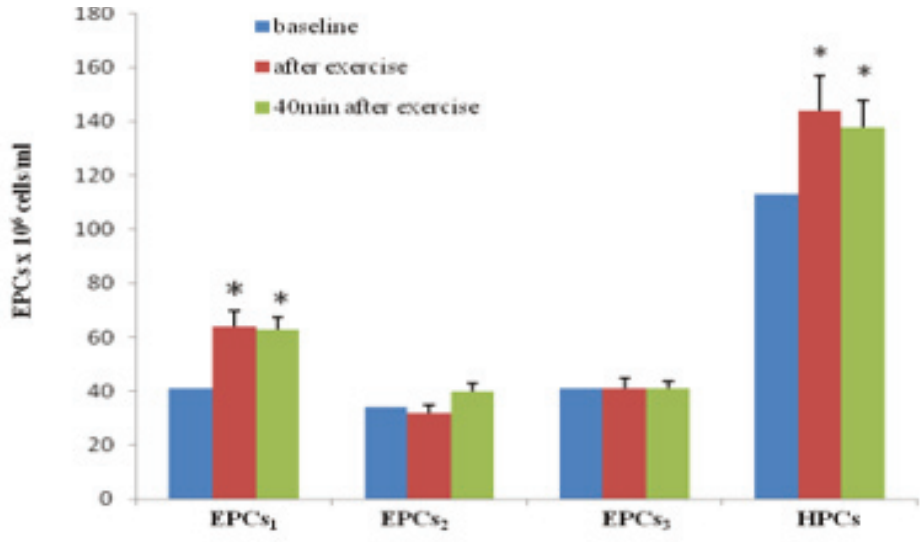

Figure 2. Mobilization of endothelial and hematopoietic progenitor cells in healthy participants EPCs: endothelial progenitor cells, HPCs: hematopoietic progenitor cells

Table 3. Changes in microcirculatory parameters monitored by near-infrared spectroscopy and a vascular occlusion test in CHF patients (n = 16) and healthy individuals (n = 11)

Continuous exercise	Baseline	After exercise	Effect size
Tissue oxygenation (StO2)			
CHF	77.7 ± 3.8	79.2 ± 4.1	0.17
Healthy individyals	81.3 ± 3.1	82.1 ± 3.7	0.14
StO2 downslope (%/minute)			
CHF	10.6 ± 2.2	12.5 ± 1.9[a]	0.69
Healthy individyals	12.3 ± 1.8	12.0 ± 1.8	0.74
StO2 upslope StO_2 (%/second)			
CHF	2.4 ± 0.7	3.1 ± 0.9[a]	0.66
Healthy individyals	3.7 ± 0.6	4.4 ± 0.6[a]	0.78
Time to baseline (seconds)			
CHF	113.2 ± 28.7	103.6 ± 21.3[b]	0.38
Healthy individyals	104.3 ± 22.6	92.4 ± 16.9	0.27

CHF, chronic heart failure; StO2, tissue oxygen saturation;
ap ≤ 0.01and bp ≤ 0.05 compared to baseline. values are expressed as mean ± SD, standard deviation

Systemic Microcirculation

Regarding the effects of continuous exercise on microcirculation, the results demonstrated significant improvements in all four systemic microcirculatory parameters in patients with CHF. Specifically, tissue oxygen saturation, oxygen consumption rate, and the reoxygenation rate following release of vascular occlusion increased significantly after exercise ($p < 0.05$). Recovery time to baseline was also significantly reduced after exercise ($p = 0.05$), concomitantly improving reactive hyperemia (Table 3). In healthy participants, improvements were observed in parameters related to endothelial function, namely the reoxygenation rate after release of vascular occlusion and reactive hyperemia. No changes were detected in tissue oxygen saturation or oxygen consumption rate.

DISCUSSION

The findings of the present study indicate that a single session of continuous, moderate-intensity, long-duration exercise promotes progenitor cell mobilization and improves systemic microcirculation in patients with CHF. In contrast, in healthy participants, although continuous exercise did not induce changes in the number of EPCs overall (with the exception of EPC_1 and HPCs immediately and 40 minutes post-exercise), it did elicit changes in systemic microcirculatory parameters that are indicative of improved endothelial function.

Recent studies have demonstrated that a continuous exercise protocol can increase both the number and activity of EPCs, highlighting the beneficial effects of exercise on endothelial function in healthy individuals. Moderate-intensity aerobic exercise (60–75% HRmax, ~50–68% VO_{2max}) performed for 30–60 minutes leads to the mobilization of circulating EPCs. However, running at a similar relative intensity (~68% VO_{2max}) but of shorter duration (10 minutes) did not induce EPC mobilization (20). In trained athletes, 240 minutes of cycling at approximately

50% VO_{2max} resulted in markedly elevated circulating EPC levels (21). It should be noted that significant mobilization was also observed following a very short exercise session (5 minutes) performed at substantially higher intensity (101 ± 5% HR_max, ~100% VO_{2max}). Conversely, two marathon race studies (~4 hours) reported conflicting results. In the first study, involving nine athletes who completed the marathon in 191 ± 26 minutes, EPC counts were duplicated (22). In contrast, a second study with a considerably larger sample (68 marathon runners) who completed the race in a mean time of 240 minutes showed no statistically significant change in EPC numbers (23). Finally, in ultradistance runners (246 km, with mean running times of 32 h 8 min and 30 h 2 min, respectively), EPC release from the bone marrow was observed as a response to increased nitric oxide bioavailability and tissue hypoxia (24).

Effect of the continuous exercise protocol

In the present study, the increase in EPC levels was associated with a concomitant improvement in endothelial reactivity. The available evidence regarding the effects of continuous exercise during interventional rehabilitation programs on EPC mobilization in patients with cardiovascular disease is limited and inconsistent. Moreover, these studies were not conducted using a single, isolated exercise bout. Continuous exercise as a stimulus for EPC mobilization has been shown to be effective in patients with myocardial ischemia, coronary artery disease, and CHF. Previous studies have reported that exercise sessions of varying duration (20–40 minutes) and intensity (50–75% VO_{2peak}), performed as part of rehabilitation programs, promote EPC mobilization in patients with CHF and are accompanied by parallel improvements in vascular function. However, the effect of a continuous exercise protocol per se in patients with cardiovascular disease, and particularly in those with CHF, had not been clearly established. In the present protocol, the prolonged exercise duration appears to have played a key role by compensating for the moderate exercise intensity, thereby providing a sufficient stimulus to activate progenitor cell mobilization mechanisms and improve endothelial function.

Previous studies have demonstrated the release of EPCs following continuous exercise. Laufs et al. (2005) (20) applied three different exercise protocols in healthy participants and showed that exercise performed at high (80% VO_{2max}) or moderate intensity (68% VO max) with a moderate duration (30 minutes) increased EPC numbers. In contrast, continuous exercise at moderate intensity (68% VO_{2max}) but of short duration (10 min) did not induce changes in EPC counts. The findings of the present study are in agreement with other reports indicating that at least 30–60 minutes of exercise are required to promote EPC mobilization and induce favorable adaptations in vascular function in patients with cardiovascular disease. In the present study, moderate exercise intensity (50% of VO-peak), which can be sustained for a prolonged period, appeared to influence the physiological mechanisms responsible for exercise-induced mobilization and activation of EPCs and HPCs. These molecular mechanisms include increased nitric oxide bioavailability and its antioxidant effects, upregulated expression of matrix metalloproteinase-9, shear stress, and the activation of cytokines and/or chemokines (25-27).

Exercise duration during continuous exercise protocols appears to be a critical factor for increasing EPC numbers in healthy individuals. Studies have shown that longer exercise duration is associated with greater increases in circulating EPCs. For example, one study reported that a 90-minute session performed at constant intensity resulted in a significantly greater increase in EPCs compared with a 30-minute cycling session (28). Similarly, another study demonstrated that a 2-hour running session led to a greater increase in EPCs than a 1-hour running session (29). In the present study, an exercise duration of approximately 1 hour did not appear to be sufficient to stimulate EPC mobilization. In healthy participants, high baseline levels of progenitor cells were observed prior to exercise, and it is possible

that the stimulus of continuous exercise at constant intensity and prolonged duration was insufficient to further increase their numbers or to activate the mechanisms responsible for EPC and HPC release into the circulation.

The prolonged duration of exercise appears to have been a key factor underlying the observed changes in systemic circulation, with improvements noted in patients with chronic heart failure (CHF), while in healthy participants, parameters of microvascular reactivity associated with endothelial function were enhanced. Our findings are consistent with previous studies that employed long-duration exercise to improve vascular function. This study supports prior evidence indicating that continuous exercise at moderate intensity represents a standard approach for promoting cardiorespiratory endurance and enhancing vascular function (30). Future studies should also investigate whether high-intensity interval training can improve cardiorespiratory fitness, promote endothelial integrity, and reverse cardiovascular risk factors (31).

Limitations

One limitation of our study was the difficulty in recruiting a larger number of patients, which is not always an easy task. Patient recruitment was limited to a single center, the Heart Failure Clinic of Evangelismos Hospital. In addition, our results primarily reflect male patients with chronic heart failure (CHF), as the majority of participants were men. Moreover, a control group was not included. However, intervention trials with control groups in patients for whom exercise constitutes a core therapeutic strategy for symptom management are ethically questionable.

Although studies employing specific markers for the identification of endothelial progenitor cells (EPCs) were included, methodological variability could not be avoided, as the optimal combination of antigens that accurately defines EPCs is still under investigation. Moreover, data obtained from a specific population cannot be automatically generalized to all populations. Therefore, consensus regarding the detection markers for EPCs is necessary.

CONCLUSION

Overall, the study shows that the duration of continuous exercise appears to be an important factor for increasing the number of EPCs and HPCs in patients with CHF. The parallel improvement in systemic microcirculatory parameters related to endothelial function indicates that exercise-induced mobilization of EPCs is an important factor for enhancing vascular function in both patients with CHF and healthy individuals.

Declarations

Funding: This work received no funding.

Conflicts of interest: The authors declare that they have no conflicts of interest.

Ethical approval: Num. 49667/179 – Date: 14/06/2018, Research Ethics Committee of Democritus University of Thrace.

Declaration of Generative AI and AI-assisted Technologies in the Writing Process: The authors declare that no generative Artificial Intelligence software (such as large language models, text-generation applications, etc.) was used in the drafting, structuring or data analysis processes of this work.

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