



Exercise in the prevention of cardiovascular diseases

Acute and chronic effects of exercise on the circulating levels of endothelial progenitor cells and vascular regeneration potential

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To my lovely family

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Resumo

As células progenitoras endoteliais (CPEs) desempenham um papel crucial na reparação vascular e angiogénese, enquanto as células endoteliais circulantes (CECs) indicam dano endotelial. O desequilíbrio entre CPEs e CECs representa o potencial de regeneração vascular, muitas vezes comprometido em indivíduos com fatores de risco cardiovasculares (FRCVs) e doenças cardiovasculares (DCVs). Existem poucos dados sobre o impacto do exercício no equilíbrio entre CPEs e CECs nestas populações. Esta tese avalia os efeitos agudos e crónicos do exercício nos níveis circulantes de CPEs e CECs de indivíduos com FRCVs e DCVs. Especificamente, objetivou-se investigar: 1) o impacto de duas sessões de exercício com durações diferentes (30 minutos versus 45 minutos) nos níveis circulantes de CPEs e CECs de adultos com FRCVs; 2) os efeitos do exercício máximo nos níveis de CPEs e CECs em pacientes com insuficiência cardíaca (IC); e 3) os efeitos de um programa de 18 semanas de treino multicomponente nos níveis de CPEs e CECs em indivíduos com FRCVs. Amostras de sangue foram colhidas para quantificar as CPEs e as CECs por citometria de fluxo. Os resultados revelaram: aumento de $1,8 \pm 1,7\%$ nas CPEs e diminuição nas CECs após a sessão de exercício de 30 minutos nos adultos com FRCVs. Não houve alterações nas CPEs e CECs após a sessão de exercício de 45 minutos. Em pacientes com IC, os níveis de CPEs aumentaram, enquanto nenhuma alteração foi observada nas CECs após o exercício máximo. Um programa de exercício multicomponente (combinação de exercício aeróbio, fortalecimento muscular, flexibilidade e equilíbrio), supervisionado (36 sessões), com frequência de duas sessões/semana (60 minutos/sessão), aumentou o nível de CPEs e não alterou as CECs. Concluindo, tanto as sessões de exercícios isolados quanto o exercício físico multicomponente demonstraram aumentar os níveis circulantes de CPEs sem aumentar o dano endotelial, indicado pela contagem de CECs. Portanto, o exercício representa uma ferramenta útil na prevenção de DCVs através do aumento do potencial do reparo vascular.

PALAVRAS-CHAVE: MEDICINA PREVENTIVA, DOENÇA CARDIOVASCULAR, EXERCÍCIO, CÉLULAS PROGENITORAS ENDOTELIAIS, CITOMETRIA DE FLUXO

Abstract

Endothelial progenitor cells (EPCs) play a pivotal role in vascular repair and angiogenesis, whereas circulating endothelial cells (CECs) serve as indicators of endothelial damage. The imbalance between the levels of EPCs and CECs represents the vascular regeneration potential, often compromised in individuals with cardiovascular risk factors (CVRFs) and cardiovascular diseases (CVDs). Limited data exist on the impact of exercise on the balance between EPCs and CECs in these populations. This thesis aims to evaluate the acute and chronic effects of exercise on the circulating levels of EPCs and CECs in individuals with CVRFs and CVDs. Specifically, the objectives are: 1) Investigate the impact of two exercise sessions with different durations (30 minutes versus 45 minutes) on circulating levels of EPCs and CECs in individuals with CVRFs; 2) Examine the effects of maximal exercise on the circulating levels of EPCs and CECs in patients with heart failure (HF); and 3) Evaluate the effects of 18 weeks of multicomponent exercise training on circulating levels of EPCs and CECs in individuals with CVRFs. Blood samples were collected to quantify EPCs and CECs using flow cytometry. The main findings revealed an increase of $1.8 \pm 1.7\%$ in the levels of EPCs and a decrease in the CEC count after the 30-minute exercise session in participants with CVRFs. No significant changes in EPCs and CECs were observed after the 45-minute exercise session. In patients with HF, the levels of EPCs increased with no significant change in CECs after maximal exercise. A supervised exercise training program comprising 36 exercise sessions, held twice weekly (each session lasting 60 minutes), and consisting of multicomponent exercises (i.e., a combination of aerobic, muscle strengthening, flexibility, and balance exercises) resulted in increased levels of EPCs and no change in CECs levels. In conclusion, both single exercise sessions and multicomponent exercise training demonstrated the capacity to increase the circulating levels of EPCs without a concurrent increase in endothelial damage, as indicated by the CECs count. Therefore, exercise stands as a useful tool to prevent CVDs by enhancing vascular repair potential.

KEYWORDS: PREVENTIVE MEDICINE, CARDIOVASCULAR DISEASE, EXERCISE, ENDOTHELIAL PROGENITOR CELLS, FLOW CYTOMETRY

List of Abbreviations

CAD, coronary artery disease
CECs, circulating endothelial cells
CVDs, cardiovascular diseases
CVRFs, cardiovascular risk factors
ECS, endothelial progenitor cell capturing stent
eNOS, endothelial nitric oxide synthase
EPCs, endothelial progenitor cells
EPO, erythropoietin
FITT, frequency, intensity, time, and type
HF, heart failure
HFmrEF, heart failure with mildly reduced ejection fraction
HFpEF, heart failure with preserved ejection fraction
HFrEF, heart failure with reduced ejection fraction
HIIT, high-intensity interval training
HR_{max}, maximum heart rate
HRR, heart rate reserve
MICON, moderate-intensity continuous
NT-proBNP, N-terminal pro-B-type natriuretic peptide
SDF-1 α , stromal cell-derived factor-1 α
PAD, peripheral arterial disease
PPPM, Predictive, Preventive, Personalized Medicine
VEGF, vascular endothelial growth factor
VO_{2 peak/max}, peak/max oxygen uptake

Chapter I

Introduction and Aims

Introduction

Cardiovascular risk factors (CVRFs) are linked to the development of cardiovascular diseases (CVDs) (Magnussen et al., 2023), which stand as the leading cause of death worldwide (Taylor et al., 2023). The high prevalence of CVDs imposes a burden of over ~200 billion euros annually on the European Union economy (Timmis et al., 2022).

CVDs are mechanistically associated with impaired endothelial integrity (Poredos et al., 2021; Tsigkou et al., 2023), and can be exacerbated by CVRFs (Benincasa et al., 2022; Marzoug, 2023). Damaged and dysfunctional endothelium is considered the first step of atherosclerosis (Poredos et al., 2021), consequently increasing the risk for cardiovascular disorders, such as coronary artery disease (CAD) (Björkegren & Lusis, 2022) and heart failure (HF) (Zuchi et al., 2020). Given the clinical significance of endothelial health, different assessment methods (both invasive and non-invasive) have been used in clinical settings. Among these methods, assessing specific molecules in peripheral blood may provide a more precise description of an individual's endothelial health and present diagnostic potential that could support clinical decisions (Burger & Touyz, 2012; Poredos et al., 2021).

A healthy endothelium maintains homeostasis through a continuous dynamic process of activating proliferation and repair mechanisms to resist endothelial damage (Huang et al., 2022; Mazini et al., 2020; Sabatier et al., 2009). Conversely, an imbalance between an inadequate endogenous endothelial repair capacity, increased levels of cellular apoptosis and endothelial dysfunction can exacerbate the risk of developing or progressing CVDs (Qiu et al., 2018; Sabatier et al., 2009).

The interplay between regenerative endothelial responses and degenerative processes can reflect an endothelial clinical phenotype, defining an individual's "vascular competence". This competence is regarded as an indicator of endothelial integrity and vascular balance. Assessment of this balance can be conducted in peripheral blood, measuring factors such as circulating levels of endothelial progenitor cells (EPCs) and circulating endothelial cells (CECs) (Sabatier et al., 2009; Sabatier et al., 2011). Accordingly, vascular competence

represents a promising tool for the management of the prevention and treatment of CVDs (Sabatier et al., 2009).

EPCs play a paramount role in endothelial repair and vascular angiogenesis. In response to endothelial damage, mobilized EPCs move towards the site of endothelial injury, differentiate into mature endothelial cells, and repair damaged endothelium (Asahara et al., 1997; Real et al., 2008; Yan et al., 2022). Therefore, the concentration of EPCs serves as a predictor of endogenous vascular repair potential, exhibiting an inverse relationship with clinical events and cardiovascular mortality (Burger & Touyz, 2012; Rigato et al., 2016). Lower levels of EPCs are observed in individuals with CVRFs (Maiorino et al., 2015; Maroun-Eid et al., 2015) and CVDs (Fadini et al., 2006; Vasa et al., 2001), including HF (Kakzanov et al., 2021). The presence of established CVD may further reduce the levels of EPCs in individuals with CVRFs (Fadini et al., 2006), and this phenomenon tends to worsen with increasing age (Scheubel et al., 2003). CECs are mature endothelial cells released by the first endothelial layer following a pathological insult or endothelial injury, serving as a circulating indicator of endothelial damage or vascular stress. An increased count of CECs is associated with a heightened risk of mortality in patients with acute coronary syndromes (Burger & Touyz, 2012) and has been identified as a diagnostic biomarker for diabetic nephropathy and HF (Farinacci et al., 2019). Patients with CVRFs (Boos et al., 2007; McClung et al., 2005) and CVDs (Lee et al., 2005), including those with HF (Martínez-Sales et al., 2011), exhibit higher levels of CECs.

Together, these data may suggest a diminished vascular competence in individuals with CVRFs and patients with CVDs at rest, which could potentially elevate the risk of developing or progressing CVDs in these populations. Therefore, clinical tools aimed at enhancing vascular competence by improving the potential for vascular repair may have a significant impact on clinical preventive medicine. Exercise training is widely recommended for the prevention and treatment of CVDs (Bull et al., 2020; Pelliccia et al., 2021). The vasculature demonstrates a robust adaptive response to exercise training in both healthy individuals and patients with CVDs, irrespective of age (Königstein et al., 2023). The beneficial vascular adaptations resulting from exercise training (chronic effects) are the outcome of cumulative stimuli from repeated single exercise

sessions (acute effects) (Whyte & Laughlin, 2010; Zanchi et al., 2009). However, vascular responses following a single exercise session and those observed after an exercise training program may exhibit specific patterns (Königstein et al., 2023), that must be considered for an optimal personalized exercise training prescription. The type (Ferentinos et al., 2022a), duration, and intensity of exercise (Mitsiou et al., 2022) seems to influence the exercise-induced mobilization of EPCs. Extensive research has examined the effects of aerobic exercise on EPC levels (Mitsiou et al., 2022), however the impact of other types of exercise remains less studied. While multicomponent exercise is strongly recommended for older adults (Bull et al., 2020), there is limited data available on the acute or chronic effects of multicomponent exercise on the levels of EPCs and CECs in individuals with CVRFs (Ferentinos et al., 2022b; Mitsiou et al., 2022). Moreover, conflicting data exist regarding the effect of a maximal exercise session on the levels of EPCs in patients with HF (Ferentinos et al., 2022a).

Currently, promising innovative research has been developed on the use of EPCs as an adjunctive source in preventive and regenerative medicine. For instance, by testing stents with specific molecules capable of mobilizing the EPCs to improve re-endothelialization and reduce the risk of in-stent restenosis (Kou et al., 2020) and by using EPC transplantation to heal ischemic tissue (Yan et al., 2023). Alternatively, the identification of an optimal personalized exercise training prescription focusing on improving the levels of EPCs and assessing endothelial damage (evaluated by CECs) is a promising, low-cost non-pharmacological tool in preventive medicine. Certainly, these are the initial steps on a promising pathway regarding stem cells and exercise, which may lead to new evidence with a positive impact on the prevention and treatment of CVDs in the near future.

Aims

The general objective of the present thesis was to evaluate the acute and chronic effects of exercise on the endothelial repair capacity, determined by the quantification of EPCs, and endothelial damage, assessed by the quantification of the CECs, in primary and secondary prevention of CVDs. Considering this purpose, three studies were designed with the following specific objectives:

a) To investigate the effect of single multicomponent exercise sessions of different durations (30 minutes versus 45 minutes) on the circulating levels of EPCs and CECs among adults with CVRFs (**Study 1**).

b) To evaluate the impact of a maximal exercise bout on the levels of EPCs and CECs among patients with HF (**Study 2**).

c) To determine the effect of a multicomponent exercise training program on the circulating levels of EPCs and CECs in adults with CVRFs (**Study 3**).

Thesis Outline

This thesis comprises five chapters.

Chapter I (Introduction and Aims): Presents an initial introduction with the main motivations for the development of the present thesis, the description of the content of each chapter that composes this thesis, and the list of contributions, with the scientific research originated from this research.

Chapter II (Background): Introduces the current literature's insights and emerging scientific queries concerning the impact of acute and chronic exercise on the circulating levels of EPCs and CECs among adults with CVRFs and patients with CVDs. This chapter includes a systematic review with meta-analysis analysing the effectiveness of exercise training in increasing the number of circulating EPCs in patients with CVDs.

Chapter III (Original studies): Contains the three original studies included in this research.

Study 1 aims to accomplishes the specific objective a)

Study 2 aims to accomplishes the specific objective b)

Study 3 aims to accomplishes the specific objective c).

Chapter IV (General Discussion): Includes a discussion of the main results and the strengths and limitations of this thesis.

Chapter V (Conclusions and Future Perspectives): Highlights the main conclusions and future perspectives in this field.

List of Contributions

The scientific outcomes arising from the current thesis are listed below.

Articles/manuscripts published

1. **Cavalcante, S. L.**, Lopes, S., Bohn, L., Cavero-Redondo, I., Álvarez-Bueno, C., Viamonte, S., Santos, M., Oliveira, J., & Ribeiro, F. (2019). Effects of exercise on endothelial progenitor cells in patients with cardiovascular disease: A systematic review and meta-analysis of randomized controlled trials. *Rev Port Cardiol (Engl Ed)*, 38(11), 817-827. doi:10.1016/j.repc.2019.02.016
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4. **Cavalcante, S.**, Teixeira, M., Gouveia, M., Duarte, A., Ferreira, M., Simões, M. I., Conceição, M., Costa, M., Ribeiro, I. P., Gonçalves, A. C., Oliveira, J., & Ribeiro, F. (2023). Endothelial progenitor cell response to a multicomponent exercise training program in adults with cardiovascular risk factors. *Ger J Exerc Sport Res*, 53(2), 225-231. doi:10.1007/s12662-023-00882-x

Abstracts in peer-reviewed journals

1. **Lima S.**, Gouveia M., Ribeiro I. P., Gonçalves A. C., Venâncio J. P., Santos M., Viamonte S., Oliveira J., Ribeiro F. (2019). Effects of maximal exercise on the circulating number of endothelial progenitor cells in patients with heart failure. Europrevent 2019. European Society of Cardiology (ESC web site/Europrevent 2019).

<https://esc365.escardio.org/presentation/190749?query=Effects%20of%20maximal%20exercise%20on%20the%20circulating%20number%20of%20endothelial%20progenitor%20cells%20in%20patients%20with%20heart%20failure>.

2. Cavalcante, S. L., Teixeira M., Duarte A., Ferreira M., Simões M. I., Conceição M., Costa M., Ribeiro I. P., Gonçalves A. C., Oliveira J., Ribeiro, F. (2020). Acute effects of multicomponent exercise sessions with different durations on markers of endothelial repair potential and vascular health. European College of Sport Science: Book of Abstracts of the 25th Annual Congress of the European College of Sport Science – 28th - 30th October 2020. Edited by Dela, F., Müller, E., Tsolakidis, E. ISBN 978-3-9818414-3-5

Oral and poster presentations

1. Lima S., Gouveia M., Ribeiro I.P., Gonçalves A.C., Venâncio J.P., Santos M., Viamonte S., Oliveira J., Ribeiro F. (2019). Effects of maximal exercise on the circulating number of endothelial progenitor cells in patients with heart failure. EuroPrevent 2019, April 11 to 13, 2019, Lisbon, Portugal.

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Poster presentation.

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4. Cavalcante S. L., Teixeira M., Duarte A., Ferreira M., Simões M. I., Conceição M., Costa M., Ribeiro I. P., Gonçalves A. C., Oliveira J., Ribeiro F. (2020). An exercise training program increases the endothelial repair/regeneration of adults with cardiovascular risk factors. Reunião do Grupo de Estudo de Fisiopatologia do Esforço e Reabilitação Cardíaca (GEFERC) – Avanços em saúde, February 28 to 29, 2020, no Hotel Axis Vermar, Póvoa de Varzim, Portugal.

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Oral presentation.

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Chapter II

Background

Cardiovascular Diseases And Heart Failure

In 2020, CVDs accounted for approximately 19 million deaths worldwide (Tsao et al., 2022). Among CVDs, HF affects over 60 million individuals worldwide, constituting approximately 2 to 4% of the general adult population (Groenewegen et al., 2020). This prevalence is anticipated to rise due to an aging population and improved survival rates (Groenewegen et al., 2020; Lindgren & Börjesson, 2021). HF is defined as a complex syndrome characterized by structural and/or functional alterations of the cardiac muscle, increased levels of natriuretic peptide, and potential pulmonary or systemic congestion (Bozkurt et al., 2021; Savarese et al., 2022). HF can be classified based on ejection fraction into preserved (HFpEF), mildly reduced (HFmrEF), and reduced ejection fraction (HFrEF) (McDonagh et al., 2021).

Cardiovascular Risk Factors And Endothelial Integrity

CVRFs such as hypertension, diabetes mellitus, and obesity contribute significantly to CVD development (Timmis et al., 2022) and predict the incidence and severity of HF (Bui et al., 2011). These risk factors notably impact endothelial structure and function (Sabatier et al., 2009), elevating the risk of developing of cardiovascular disorders (Sun et al., 2019), including HF (Zuchi et al., 2020). For instance, hypertension induces pathological vascular network remodelling, increasing vasoconstriction and decreasing vasodilation pathways, ultimately leading to sustained elevated blood pressure, hemodynamic overload, vascular maladaptation, and endothelial dysfunction (Song et al., 2022). Similarly, diabetes mellitus impacts oxidative stress, inflammation, endothelial nitric oxide synthase (eNOS) activity, and nitric oxide bioavailability, thereby leading to endothelial dysfunction, thus contributing to microvascular disorders (e.g., nephropathy and neuropathy) and macrovascular disorders (e.g., CVDs and cardiovascular events) (Bays et al., 2022; Poredos et al., 2021; Visseren et al., 2021). Obesity is associated with higher risk of developing other CVRFs, such as hypertension and diabetes, and CVDs, including HF (Bays et al., 2022; Francula-Zaninovic & Nola, 2018). Its impact on endothelial function is associated with increased oxidative stress, insulin resistance, and inflammation (Kajikawa & Higashi, 2022).

In general, CVRFs overload endothelial defences, inhibiting protective endothelial substances and inducing endothelial damage, commonly resulting in endothelial dysfunction (Poredos et al., 2021).

Mechanisms Of Cardiovascular Risk Factors Impact On Endothelium Integrity

There are several mechanisms that potentiate endothelial dysfunction and damage in the presence of CVRFs. Most CVRFs induce vascular inflammatory responses by activating endothelial cells, stimulating chemokines, cytokines, and adhesion molecules production, which interact with leukocytes and platelets (Libby et al., 2002; Poredos et al., 2021). Another important mechanism is related to oxidative stress. Increased oxidative stress due to CVRFs promotes endothelial damage by disrupting the balance between nitric oxide and oxidative stress, contributing to cardiovascular complications (Poredos et al., 2021). Furthermore, the intimate relationship between oxidative stress and inflammation exacerbates endothelial dysfunction, increasing the risk of atheroma formation and cardiovascular disorders. Acute phase reactants like C-reactive protein impact nitric oxide bioavailability, elevate oxidative stress, and directly induce endothelial dysfunction (Karch et al., 2014; Li et al., 2004; Poredos et al., 2021). Additionally, inflammatory cytokines stimulate endothelial cells and leukocytes to express adhesion molecules, aggravating endothelial disturbance (Blake & Ridker, 2001), which leads to increased adhesion of monocytes to the endothelium (Ross, 1999) and subsequent atheroma plaque development (Poredos et al., 2021; Ross, 1999). The progression of atherosclerotic process and atheroma plaque development may lead to CAD (Björkegren & Lusis, 2022), a major cause of HF (Zuchi et al., 2020).

Heart Failure And Endothelial Integrity

HF has been linked to reduced endothelial integrity and functionality, which relates to HF development and progression (Marti et al., 2012; Zuchi et al., 2020). Endothelial dysfunction holds prognostic value in HF, as it is independently associated with hospitalizations (Zuchi et al., 2020), cardiac transplantation, and mortality (Fujisue et al., 2015; Zuchi et al., 2020).

Impaired endothelial function results in maladaptation of skeletal muscle response in HF, decreased blood perfusion capacity, and compromised oxygen

distribution during episodes of increased oxygen demand (e.g., exercise), leading to episodes of fatigue and decreased cardiorespiratory fitness (Carbone et al., 2020; Zuchi et al., 2020). Notably, the impaired endothelial integrity/function represents a potential therapeutic target in HF management (Ambrosino et al., 2021).

Due to the importance of endothelial integrity on CVDs outcomes, crescent effort have been dedicated to understand endothelial integrity in CVDs emphasize balancing endothelial damage and repair capacity by quantifying circulating endothelial damage indicators (e.g., endothelial microparticles, CECs) and endothelial repair and angiogenic potential (e.g., EPCs) (Budzyń et al., 2019b; Lopes et al., 2022; Sabatier et al., 2009; Zahran et al., 2019).

Endothelial Progenitor Cells

EPCs were first described in 1997 by Asahara et al. (Asahara et al., 1997) as potential progenitors or angioblasts capable of differentiating into endothelial cells in *in vitro* and integrating into sites of active angiogenesis in animal models of ischemia. Although lacking a standardized definition, EPCs are generally characterized as a population of progenitor cells displaying an endothelial phenotype, endowed with self-renewal capability and the capacity for angiogenesis (Medina et al., 2017; Yan et al., 2022). Due to their role on vascular repair in ischemic tissues and their capacity expanding the collateral vessel growth to ischemic tissues, EPCs have being reported as a potential strategy for therapeutics related to cardiovascular disorders (Bianconi et al., 2018; Yan et al., 2022).

EPCs play pivotal roles in repairing damaged endothelium and facilitating angiogenesis (Huang et al., 2022; Yan et al., 2022). Their involvement in repair occurs through both directly and indirectly pathways. Directly, EPCs can differentiate and proliferate into mature endothelial cells, which in turn are integrated into the damaged vessel (Bianconi et al., 2018; Real et al., 2008; Yan et al., 2022). Indirectly, EPCs release exosomes, paramount in mediating cell-to-cell communication and endothelial repair, as well as angiogenic factors like vascular endothelial growth factor (VEGF), hepatocyte growth factor, granulocyte colony–stimulating factor, and granulocyte-macrophage colony–stimulating factor (Rehman et al., 2003; Yan et al., 2022).

Reduced levels of EPCs are observed in adults with CVRFs, namely hypertension (Maroun-Eid et al., 2015), diabetes mellitus (Maiorino et al., 2015) and obesity (MacEneaney et al., 2009), when compared to healthy adults. EPCs levels are inversely associated with cumulative cardiovascular risk and positively correlated with endothelial function in men with CVRFs and no history of CVDs (Hill et al., 2003). In patients with HF, the number and functionality of EPCs are even lower than in adults with CVRFs alone (Kakzanov et al., 2021). Reduced levels of EPCs in patients with HF are related to increased hazard ratios for all-cause mortality and cardiovascular death, independently of N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels (Djohan et al., 2018; Koller et al., 2016; Samman Tahhan et al., 2017). Moreover, reduced levels of EPCs were related to a 2-fold increased risk of cardiovascular events and death (Rigato et al., 2016), and predicted severe endothelial dysfunction, independently of CVRFs (Werner et al., 2007).

The clinical implications and therapeutic potential of EPCs seem logical as stem cells, particularly circulating EPCs, hold promise for both explored prevention and treatment of cardiovascular disorders (Brixius et al., 2006; Qiu et al., 2018; Salybekov et al., 2022). For instance, clinical studies explore their role in preventive and therapeutic medicine, such as autologous bone marrow-mononuclear cell implantation to enhance angiogenesis in limb ischemia patients. The implantation of autologous bone marrow-mononuclear cells improved the angiogenesis process in patients with limb ischemia, which was described as result from the bone marrow cells capacity to supply EPCs and also release cytokines and angiogenic factors (e.g., VEGF) (Tateishi-Yuyama et al., 2002). Innovative techniques like the “endothelial progenitor cell capturing stent” (ECS) aim to mobilize EPCs using specific biomaterials to expedite reendothelization and decrease the risk of intra-stent restenosis (Kou et al., 2020). In addition to the use of manipulated bioactive molecules, others stimulus like some medications (Min et al., 2004; Spadaccio et al., 2010), and physical exercise (Kourek et al., 2021; Ribeiro et al., 2013) may increase the mobilization of EPCs from bone marrow to circulation.

Regarding assessment methods, EPCs can be evaluated using flow cytometry or *in vitro* cell isolation methods (Medina et al., 2017). Flow cytometry quantifies EPCs using surface markers. Although a consensus on surface marker

combinations is lacking, the CD133, CD34, VEGFR2 combination is widely used and accepted to identify EPCs. Additional surface markers such as CD177, vWF, CD31 or CD144 are also employed, albeit with lower specificity (Burger & Touyz, 2012). Initially EPCs were characterized by expressing CD34, KDR, and VE-cadherin (also known as “CD144”) surface markers, which showed consistent endothelial cell characteristics when plated on fibronectin-coated plates (Asahara et al., 1997; Salybekov et al., 2022). However, using only CD34⁺KDR⁺ and CD133⁺KDR⁺ as surface markers may not represent all the characteristics of EPCs, impacting cell quantification. In this regard, CD45^{dim}CD34⁺KDR⁺ surface markers have shown increased reliability, and were, thus, suggested for the quantification of EPCs (Schmidt-Lucke et al., 2010). Although most studies use surface markers for direct EPC identification and isolation from peripheral blood, an agreed-upon ideal set of surface markers to identify cells as EPCs remains elusive (Patel et al., 2016).

Circulating Endothelial Cells

CECs were first described in the 1970s by Bouvier and Hladovec using light microscopy (Bouvier, 1970; Erdbruegger et al., 2010; Hladovec et al., 1978). These cells are endothelial cells detached from the endothelial layer and circulate peripherally following a pathological insult or endothelial injury (Boos et al., 2006; Burger & Touyz, 2012).

The concentration of CECs in peripheral blood serves as an indicator of endothelial damage (Erdbruegger et al., 2010) and was inversely correlated with endothelial function (Chong et al., 2004). In addition, CECs level is an independent predictor of death, a major cardiovascular endpoint at 30 days and 1 year in acute coronary syndrome (Lee et al., 2005), and function as a diagnostic biomarker for diabetic nephropathy and HFpEF (Farinacci et al., 2018).

The levels of CECs in peripheral blood are typically low in healthy people (Burger & Touyz, 2012) but increase in patients with CVDs (Chong et al., 2004; Farinacci et al., 2018), including HF (Chong et al., 2006; Martínez-Sales et al., 2011), and adults with CVRFs such as hypertension (Boos et al., 2007), diabetes mellitus (Burger & Touyz, 2012; McClung et al., 2005) and obesity (Soltero et al., 2021).

Like EPCs, CECs can be identified through surface antigens that indicate their endothelial origins including CD31, CD62e, CD54, CD105, CD106, CD141, CD146 and tissue factor (Burger & Touyz, 2012). Various surface markers have been suggested for CECs identification (Farinacci et al., 2018; Kraan et al., 2012; Sabatier et al., 2009). Notably, the absence of CD133 can serve as a criterion for differentiating CECs from EPCs (Burger & Touyz, 2012; Erdbruegger et al., 2010). Once the relevant surface markers are identified, CECs can be isolated from peripheral blood by immunomagnetic isolation or flow cytometry (Burger & Touyz, 2012; Erdbruegger et al., 2010). Flow cytometry, allowing the simultaneous use of different surface markers, helps avoid false-positive results when quantifying CECs (Erdbruegger et al., 2010).

Vascular Competence

Vascular competence denotes an individual's endogenous endothelial repair capacity against endothelial damage, often estimated non-invasively by circulating biomolecular indicators, such as the levels of EPCs and CECs in peripheral blood (Sabatier et al., 2009). Reduced levels of EPCs and increased CECs are indicative of reduced endogenous vascular repair capacity, ultimately leading to impaired endothelial integrity (Boos et al., 2006; Budzyń et al., 2019b; Sabatier et al., 2009). Evaluating EPCs and CECs holds promise as an indicator reflecting endothelial integrity (Budzyń et al., 2019b; Kou et al., 2020). Recent studies assessed the balance between vascular endothelium regeneration and damage through the levels of EPCs and CECs in patients with CVRFs (Budzyń et al., 2019a; Budzyń et al., 2019b; Kou et al., 2020; Zahran et al., 2019), and CVDs, namely HF (Farinacci et al., 2018; Lopes et al., 2022). Patients with hypertension had a reduced repair capacity (as represented by the EPCs/CECs ratio) compared to healthy controls (Budzyń et al., 2019a; Budzyń et al., 2019b; Kou et al., 2020). In addition, a CECs cut-off value of 60 cells/4 mL and EPCs/CECs ratio below 2.72 were moderately strong predictors for endothelial disorders among patients with hypertension (Budzyń et al., 2019b).

Exercise Training: Acute And Chronic Effects

The "acute exercise" refers to a single exercise session, while "chronic exercise" (i.e., exercise training) involves the accumulation of single sessions of exercise over a period (long or short) (Sellami et al., 2018). The cumulative

exposure to acute exercise, which embodies exercise training, leads to well-known cardiovascular functional enhancements. These improvements include increased maximal oxygen consumption, maximal cardiac output, and enhanced blood flow to cater to the demands of skeletal and cardiac muscles (Whyte & Laughlin, 2010). Long-term improvements in the cardiovascular system are likely a consequence of cumulative responses triggered by acute exercise at the molecular level (Whyte & Laughlin, 2010).

Therefore, exercise training is widely recommended for the prevention and treatment of CVDs (Bull et al., 2020; Pelliccia et al., 2021). There is a growing interest in tailoring exercise training prescriptions based on specific exercise characteristics—such as mode, intensity, and duration—to enhance endothelial repair capacity via the modulation of circulating levels of EPCs. However, there is limited data available concerning vascular competence in this context.

Acute Effects Of Exercise

Regarding the acute effects of exercise, it has been shown that a single exercise session can acutely enhance circulating EPCs in both healthy individuals and those with clinical conditions (Kourek et al., 2021; Ribeiro et al., 2017; Volaklis et al., 2013). Previous data suggest that specific characteristics of an exercise session may lead to different endothelial and vascular responses. For instance, different exercise modes elicit distinct vascular responses (Thijssen et al., 2009a; Thijssen et al., 2009b), which should be considered when developing personalized exercise prescriptions. In a previous systematic review, Ferentinos et al. (2022a) identified four studies that examined the acute effects of exercise in patients with metabolic diseases such as diabetes and metabolic syndrome (Lutz et al., 2016; Rocha et al., 2015; Wacławovsky et al., 2016; West et al., 2015). A blunted capacity to mobilize EPCs was observed in patients with type 1 diabetes mellitus compared to individuals without diabetes mellitus after an aerobic exercise session lasting 45 min at 70% of peak/maximal oxygen uptake ($VO_{2\text{ max}}$). In this study, no change was observed in the levels of CECs after the single exercise session in patients with type 1 diabetes or non-diabetics (West et al., 2015). In accordance, patients with type 1 diabetes did not exhibit changes in EPC levels immediately after an aerobic exercise session (cycling) lasting 40 min at moderate intensity or after a resistance exercise session, while

healthy individuals showed a decrease in EPC levels after the aerobic exercise session and an increase after the resistance exercise session (Waclawovsky et al., 2016). Also, patients with impaired glucose tolerance and those with type 2 diabetes did not exhibit changes in the levels of EPCs after 30 min of aerobic exercise, while an increase of 23% in the levels of EPCs was observed in patients with normal glucose tolerance (Lutz et al., 2016). The exercise prescription variables influencing the mobilization of EPCs to circulation are a matter of debate. While some authors suggested that both the modality of exercise and the clinical condition may influence the mobilization of EPCs to peripheral blood (Ferentinos et al., 2022a), others (Mitsiou et al., 2022) suggested that the mobilization of EPCs is primarily influenced by the volume and intensity of exercise, irrespective of the mode. Thus, it was suggested that in specific cases of CVD in which patients may not be able to exercise at high intensities, adjusting the exercise duration might serve as an alternative approach to increase EPCs mobilization into peripheral blood (Mitsiou et al., 2022). Although, it is highlighted that the ideal exercise duration and intensity to mobilize EPCs in both healthy individuals and those with health conditions are still to be fully elucidated in the literature (Mitsiou et al., 2022). Additionally, scarce data exist on the acute effects of multicomponent exercises, both in healthy individuals and patients with CVDs.

To date, only one study compared the effects of different durations of a single aerobic exercise session (Laufs et al., 2005). To the best of our knowledge, there is no available data regarding the effects of varying durations of a single multicomponent exercise session on the vascular competence of adults with CVRFs. Over the years, the discussion about the impact of a single maximal or strenuous exercise session on the mobilization of circulating EPCs in patients with HF has been gaining momentum. Despite a recent systematic review (Ferentinos et al., 2022a) identifying five studies addressing this topic (Gevaert et al., 2019; Kourek et al., 2020; Van Craenenbroeck et al., 2011; Van Craenenbroeck et al., 2009; Van Craenenbroeck et al., 2010), limited data are available on the acute effects of maximal exercise on vascular competence (e.g., quantification of circulating EPCs and CECs) among HF patients.

Furthermore, regarding the effects of a single maximal exercise session on the circulating levels of EPCs in patients with HF, the available data have shown controversial results irrespective of the HF phenotype (i.e., HFpEF,

HFrEF, or HFmrEF). For instance, patients with HFrEF and HFmrEF showed increased levels of EPCs immediately after maximal exercise performed on a cycle ergometer, regardless of HF severity (Kourek et al., 2020). On the other hand, patients with HFpEF demonstrated reduced levels of EPCs at rest compared to the healthy group and remained unchanged after 10 min of the cardiopulmonary exercise test (CPET) (Gevaert et al., 2019). Accordingly, while patients with HFrEF presented similar EPC levels to the healthy group at rest, these patients did not exhibit changes in EPC levels over time after maximal exercise (i.e., at 10 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h, and 48 h post-maximal exercise) (Van Craenenbroeck et al., 2011).

Table 1 presents the results of previous studies that investigated the effects of single exercise sessions on the levels of circulating EPCs in adults free from cardiac disease.

Table 1. Studies that evaluated the effects of single exercise sessions (acute effects) in adults free from cardiac disease

Study	Participants	Exercise session characteristics	EPCs phenotype	Levels of EPCs
<i>Studies assessing MICON exercise</i>				
Chang et al. (2015)	EG: 5 healthy men (29.8 yrs; 26–36 yrs)	Modality: Aerobic (treadmill); Intensity: HR > 140 bpm; Duration: 30 min	KDR ⁺ /CD11b ⁻ /CD34 ⁺ /AC133 ⁺ (% PBMNCs)	10 min post-exercise: No change 24h post-exercise: ↑ EPCs
Cubbon et al. (2010)	EG 1 (White European): 15 healthy men (28.2 ± 1.3 yrs) EG 2 (South Asian): 15 healthy men (30.0 ± 1.3 yrs)	Modality: Aerobic (cycle ergometer); Intensity: 80% of individual lactate threshold; Duration: 30 min	CD34 ⁺ /KDR ⁺ ; CD133 ⁺ /CD34 ⁺ /KDR ⁺ (% Lymphocytes)	EG 1 Vs. EG 2: ↓ EPCs mobilization (all evaluated) after exercise in EG 2
Lansford et al. (2016)	EG 1: 16 healthy men (24.5 ± 0.8 yrs) EG 2: 10 healthy women (22.40 ± 0.52 yrs)	Modality: Aerobic (cycle ergometer); Intensity: 60–70% VO ₂ max; Duration: EG 1: 43.6 ± 1.5 min; EG 2: 62.7 ± 1.9 min	CD34 ⁺ /KDR ⁺ (PBMNCs/50,000 events)	EG 1: No change EG 2: No change
Laufs et al. (2005)	EG: 25 healthy men (28.4 ± 6.5 yrs)	Modality: 1: Aerobic (Intensive running on 400 m track); Intensity: 100% velocity of the IAT; Duration: 30 min Modality: 2: Aerobic (Moderate running on 400 m track); Intensity: 80% velocity of the IAT; Duration: 30 min	CD34 ⁺ /KDR ⁺ (Cells/10 ⁵ events)	Modality 1: ↑ EPCs Modality 2: ↑ EPCs

		Modality: 3: Aerobic (Moderate short duration running on 400 m track); Intensity: 80% velocity of the IAT; Duration: 10 min		Modality 3: No change
Lockard et al. (2010)	EG 1: 12 healthy highly active men (62.0 ± 1.6 yrs) EG 2: 11 healthy less active men (65.0 ± 1.5 yrs)	Modality: Aerobic (treadmill); Intensity: 75 ± 5% VO _{2 max} ; Duration: 30 min	CD34 ⁺ /KDR ⁺ (Cells/10 ⁵ events)	EG 1: No change EG 2: No change
Lutz et al. (2016)	EG 1: 17 patients with type 2 diabetes mellitus (60 ± 2 yrs; 53% men) EG 2: 10 patients with impaired glucose tolerance (64 ± 2 yrs; 50% men) EG 3: 18 participants with normal glucose tolerance (60 ± 2 yrs; 39% men)	Modality: Aerobic (treadmill); Intensity: 60% VO _{2 max} ; Duration: 30 min	CD34 ⁺ /KDR ⁺ (Cells/10 ⁶ events)	30 min post-exercise: EG 1 and EG 2: No change; EG 3: ↑EPCs (by 23%)
Rocha et al. (2015)	EG 1: 15 participants with early MetS (37.0 ± 2.0 yrs; 80% men) EG 2: 9 healthy participants (33.0 ± 3 yrs; 67% men)	Modality: Aerobic (cycle ergometer); Intensity: 80% of ventilatory threshold; Duration: 40 min	CD34 ⁺ /KDR ⁺ and CD133 ⁺ /CD34 ⁺ /VEGFR2 ⁺ (%)	EG 1 Vs. EG 2: ↓EPCs after exercise in EG 1 compared to EG2.
West et al. (2015)	EG 1: 10 men with type 1 diabetes mellitus (27 ± 2 yrs) EG 2: 9 healthy men (27 ± 1 yrs)	Modality: Aerobic (treadmill); Intensity: 70% VO _{2 max} ; Duration: 45 min	CD34 ⁺ /KDR ⁺ /CD45 ^{dim} (Cells/100 Leucocytes)	15 h post-exercise: EG 1; No change; EG 2: ↑EPCs 60 min post-exercise: EG 1: No change; EG 2: ↑EPCs
Waclawovsky et al. (2016)	EG 1: 14 men with type 1 diabetes mellitus (30.3 ± 1.6 yrs) EG 2: 5 healthy men (26.8 ± 2.3 yrs)	Modality 1: Aerobic (30 min cycling at 60% VO _{2 peak}) Modality 2: Resistance exercise (40 min lower limb resistance exercise: 4 exercises × 4 sets × 12 reps at 60% 1RM and 90 s rest between sets)	CD34 ⁺ /KDR ⁺ /CD45 ^{dim} (Log scale)	After resistance exercise: EG 1: No change; EG 2: ↑EPCs After aerobic exercise: EG 1: No change; EG 2: ↓EPCs
Niemi et al. (2017)	EG: 7 healthy men (25.3 ± 2.4 yrs)	Modality: Aerobic (treadmill); Intensity: 70% VO _{2 peak} ; Duration: 60 min	CD45 ⁻ /CD34 ⁺ /CD31 ⁺ (Cells/mL)	No change
Ross et al. (2018)	EG 1: 8 healthy young men (23 ± 2 yrs) EG 2: 9 healthy old men (65 ± 3 yrs)	Modality: Aerobic (cycle ergometer); Intensity: 70% VO _{2 max} ; Duration: 30 min	CD34 ⁺ /KDR ⁺ /CD45 ^{dim} (Cells/mL)	EG 1: ↑EPCs EG 2: ↑EPCs EG 1 Vs. EG 2: No significant difference

Strömberg et al. (2017)	EG: 10 healthy men (25 yrs; 20 to 31 yrs)	Modality: Aerobic (cycle ergometer); Intensity: 50% $\text{VO}_{2\text{ max}}$ in 20 first min and 65% $\text{VO}_{2\text{ max}}$ for the next 40 min; Duration: 60 min	CD34 ⁺ /KDR ⁺ /CD45 ^{dim} (Cells/mL)	No change
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Studies assessing maximal exercise

Bonsignore et al. (2010)	EG: 8 healthy men (43.4 ± 10.9 yrs)	Modality: Aerobic (1500 m field test); Intensity: 101 ± 5% predicted HR_{max} ; Duration: 5 min 35 s ± 35 s	CD34 ⁺ /KDR ⁺ (Cells/mL)	↑ EPCs (~three-fold increase)
Shill et al. (2016)	EG 1: 11 healthy men (24.8 ± 1.6 yrs) EG 2: 11 healthy women (23.7 ± 1.6 yrs)	Modality: Aerobic ($\text{VO}_{2\text{ max}}$ test; treadmill); Intensity: Maximal; Duration: 6-12 min	CD34 ⁺ /KDR ⁺ (PBMNCs/100,000 events)	EG 1: ↑ EPCs EG2: ↑ EPCs
Thijssen et al. (2006)	EG 1: 8 healthy sedentary young men (19-28 yrs) EG 2: 8 healthy endurance men (18-28 yrs) EG 3: 8 healthy sedentary old men (67-76 yrs)	Modality: Aerobic ($\text{VO}_{2\text{ max}}$ test; treadmill); Intensity: Maximal	CD34 ⁺ /KDR ⁺ (Cells/mL)	EG 1: No change EG 2: No change EG 3: No change
Van Craenenbroeck et al. (2008)	EG 1: 11 healthy (23.9 ± 1.4 yrs; 55% men) EG 2: 14 healthy (36.2 ± 9.3 yrs; 64% men)	Modality: Aerobic (CPET; cycle ergometer); Intensity: Maximal	CD34 ⁺ /KDR ⁺ (Cells/mL)	EG 1: ↑ EPCs (by 76%) EG 2: ↑ EPCs (by 69%)
Yang et al. (2007)	EG: 16 healthy men (25.1 ± 2.7 yrs)	Modality: Aerobic (modified Bruce treadmill protocol; 5.5 km.h ⁻¹ , 14% grade); Intensity: Maximal; Duration: 9.6 ± 2.2 min	CD34 ⁺ /KDR ⁺ (% PBMNCs)	↑ EPCs

Studies assessing prolonged endurance exercise

Adams et al. (2008)	EG: 68 healthy men (57 ± 6 yrs)	Modality: Aerobic (Marathon race); Intensity: Not reported; Duration: 4 h, 11 min ± 4 min	CD34 ⁺ /KDR ⁺ (Cells/mL)	No change
Bonsignore et al. (2010)	EG: 9 healthy men (43.6 ± 11.6 yrs)	Modality: Aerobic (Marathon race); Intensity: Not reported; Duration: 191 ± 26 min	CD34 ⁺ /KDR ⁺ (Cells/mL)	↑ EPCs (~two-fold increase)
Möbius-Winkler et al. (2009)	EG: 18 healthy men (32.4 ± 2.3 yrs)	Modality: Aerobic (cycle ergometer); Intensity: 70% of the IAT; Duration: 240 min	CD34 ⁺ /KDR ⁺ ; CD133 ⁺ /KDR ⁺ (Cells/mL)	210 min exercise: ↑CD34 ⁺ /KDR ⁺ ; ↑CD133 ⁺ /KDR ⁺ 240 min exercise: ↑CD34 ⁺ /KDR ⁺ ; ↑CD133 ⁺ /KDR ⁺ ↑CD133 ⁺ /KDR ⁺ at 30, 60 and 120 min post-exercise

<i>Studies assessing resistance exercise</i>				
Krüger et al. (2015)	EG 1: 12 healthy men (26.4 ± 1.3 yrs)	EG 1: Modality: Aerobic (Concentric endurance cycling); Intensity: 80% VO _{2 max} ; Duration: 43 ± 5 min.	CD34 ⁺ /KDR ⁺ /CD45 ⁻ (Cells/mL)	EG 1: Post-exercise: No change; 3 h post-exercise: ↑EPCs; 24 h post-exercise: No change
	EG 2: 12 healthy men (25.9 ± 4.7 yrs)	EG 2: Modality: Resistance exercise (i.e., bench press, latissimus pull down, seated rows, shoulder press, bicep curls and leg curls); Intensity: 75% 1RM; Duration: 90 ± 5 min		EG 2: Post-exercise: No change; 3 h post-exercise: ↑EPCs; 24 h post-exercise: ↑EPCs; 48 h post-exercise: No change
	EG 3: 12 healthy men (25.5 ± 4.3 yrs)	EG 3: Modality: Aerobic (Eccentric downhill; treadmill running); Intensity: 80% VO _{2 max} ; Duration: 51 ± 6 min.		EG 3: Post-exercise: ↑EPCs; 3 h post-exercise: ↑EPCs; 24 h post-exercise: ↑EPCs; 48 h post-exercise: ↑EPCs
Ribeiro et al. (2017)	EG 1: 13 healthy women (20.7 ± 1.7 yrs)	Modality: Resistance exercise (3 x 12 reps including barbell bench press standing barbell curl, dumbbell squat and standing dumbbell upright row); Intensity: EG 1: 60% 1RM; EG 2: 70% 1RM; EG 3: 80% 1RM; Duration: ~30 min	CD34 ⁺ /KDR ⁺ /CD45 ^{dim} (% Leucocytes)	EG 1: Post-exercise: ↑EPCs
	EG 2: 12 healthy women (21.0 ± 0.9 yrs)			EG 2: Post-exercise: ↑EPCs; 6 h post-exercise: ↑EPCs; 24 h post-exercise: ↑EPCs
	EG 3: 13 healthy women (20.9 ± 1.4 yrs)			EG 3: ↑EPCs; 6h post-exercise: ↑EPCs
Ross et al. (2014)	EG: 13 healthy men (22.4 ± 0.5 yrs)	Modality: Resistance exercise (3 circuits x 15 reps, including leg press, seated chest press, leg curl, Lat pulldown, knee extension, triceps pushdown) with 1 min rest between each circuit; Intensity: 15RM; Duration: 12.1 ± 0.6 min	CD34 ⁺ /KDR ⁺ /CD45 ^{dim} (Cells/mL)	10 min post-exercise: No change 2 h post-exercise: ↑EPCs 24 h post-exercise: No change
Montgomery et al. (2019)	EG: 9 healthy men (21 ± 1 yrs)	Modality: Resistance (Unilateral knee extension on an isokinetic dynamometer; 4 sets in total including 1 x 30 reps and 3 x 15 reps with 30 s recovery); Intensity: Not reported; Duration: Not reported	CD34 ⁺ /KDR ⁺ ; CD34 ⁺ /CD45 ^{dim} /KDR ⁺ (Cells/mL)	Post-exercise: ↑CD34 ⁺ /KDR ⁺ ; CD34 ⁺ /CD45 ^{dim} /KDR ⁺ : No change 30 min post-exercise: ↑CD34 ⁺ /KDR ⁺ ; ↑CD34 ⁺ /CD45 ^{dim} /KDR ⁺
<i>Studies comparing HIIT to MICON</i>				
O'Carroll et al. (2019)	EG: 12 healthy active (29 ± 2 yrs; 67% men)	Modality: Aerobic (cycle ergometer); Intensity: 70% VO _{2 peak} ; Duration: 45 min Modality 2: Aerobic (Interval exercise; 6 x 20 s	CD34 ⁺ /CD45 ^{dim} /KDR ⁺ (Cells/mL)	Modality 1 and 2: No change at post-exercise, 2 h post-exercise or 24 h post-exercise

		sprints with 2 min low active or passive recovery; Intensity: Maximum; Duration: 12 min		
Harris et al. (2017)	EG: 15 healthy post-menopausal women (63 ± 4 yrs)	Modality 1: Aerobic (cycle ergometer); Intensity: 80% work rate at LT; Duration: 30 min Modality 2: Interval exercise (10:20 s, work: recovery); Intensity: 90% work rate at VO _{2 peak} ; Duration: 30 min Modality 3: Interval exercise (cycle ergometer) (30:60 s, work: recovery); Intensity: 90% work rate at LT; Duration: 30 min	CD34 ⁺ /KDR ⁺ ; CD34 ⁺ /KDR ⁺ /CD133 ⁺ (Cells/mL)	30 min post-exercise: no change on all the evaluated EPCs.
Sapp et al. (2019)	EG: 10 healthy moderately active men (22 ± 2 yrs)	Modality 1: Aerobic (cycle ergometer); Intensity: 60% PPO; Duration: 30 min Modality 2: Interval exercise (3:4 min, work: recovery); Intensity: 85% PPO; 40% PPO; Duration: 30 min	CD34 ⁺ /CD31 ⁺ /CD45 ^{dim/-} (Cells/500,000 events)	Modality 1: ↓ EPCs (by 34%) Modality 2: ↓ EPCs (by 21%)
Krüger et al. (2016)	EG: 23 healthy men (25.7 ± 3.2 yrs)	Modality 1: Aerobic (cycle ergometer); Intensity: 70% VO _{2 max} ; Duration: 30 min Modality 2: HIIT (cycle ergometer) (5 sets x 3 min at 90% PPO with 3 min unloaded active recovery); Duration: 27 min	CD34 ⁺ /KDR ⁺ /CD45 ⁻ (Cells/μl)	Modality 1: Post-exercise: ↑ EPCs; 3h and 24 h post-exercise: No change Modality 2: Post-exercise: ↑ EPCs; 3h post-exercise: No change; 24 post-exercise: No change

CPET, cardiopulmonary exercise test; EG, exercise group; EPCs, endothelial progenitor cells; HIIT, high intensity interval training; HR, heart rate; HR_{max}, maximum heart rate; IAT, individual anaerobic threshold; LT, lactate threshold; *MetS*, metabolic syndrome; MICON, moderate intensity continuous; mL, millilitres; PBMNCs, peripheral blood mononuclear cells; PPO, peak power output; Reps, repetitions; VO_{2 peak/max}, peak/maximal oxygen uptake; Yrs, years, μL, microliter; ↑, significant increase; ↓, significant decrease; %, percentage; ~, approximately; 1RM, one repetition maximum strength. Table 1 was adapted from Ferentinos et al. (2022a) and Ferentinos et al. (2022b).

Table 2 displays the outcomes of previous studies that investigated how single exercise sessions affect circulating levels of EPCs in patients with CVDs.

Table 2. Studies evaluating the effects of single exercise sessions in patients with cardiovascular diseases

Study	Participants	Exercise session characteristics	EPCs phenotype	Levels of EPCs
<i>Studies assessing maximal exercise testing (on a cycle ergometer)</i>				
Adams et al. (2004)	EG 1: 16 ischaemic CAD patients (65 ± 1.4 yrs; gender not reported) EG 2: 12 non-ischaemic CAD patients (60 ± 2.1 yrs; gender not reported) EG 3: 11 healthy participants (59 ± 4.4 yrs; gender not reported)	Modality: Aerobic (Symptom limited exercise test on cycle ergometer); Intensity: Maximal; Duration: EG 1: 8.18 ± 6.2 min; EG 2: 9.43 ± 8.4 min; EG 3: 12.40 ± 1.15 min	CD34 ⁺ /KDR ⁺ (Cells/mL)	24 h post-exercise: EG 1: ↑ EPCs; EG 2 and EG 3: No change 48 h post-exercise: EG 1: ↑ EPCs; EG 2 and EG 3: No change
Gevaert et al. (2019)	EG 1: 26 CHF patients with preserved ejection fraction ≥ 50% (74 ± 7 yrs; 38% men) EG 2: 26 sedentary healthy adults (73 ± 6 yrs; 38% men)	Modality: Aerobic (CPET on cycle ergometer); Intensity: Maximal; Duration: Not reported	CD34 ⁺ /KDR ⁺ /CD45 ^{dim} (Cells/10 ⁶ MNCs)	EG 1 and EG 2: No change
Kourek et al. (2020)	EG 1: 25 patients with CHF, NYHA II-III, and LVEF of 32 ± 9% (57 ± 10 yrs; 84% men) EG 2: 24 patients with CHF, NYHA II-III, and LVEF of 32 ± 8% (55 ± 9 yrs; 83% men)	Modality: Aerobic (CPET on cycle ergometer); Intensity: Maximal; Duration: not reported	CD34 ⁺ /CD45 ⁻ /CD133 ⁺ /KDR ⁺ ; CD34 ⁺ /CD133 ⁺ /KDR ⁺ ; CD33 ⁺ /CD45 ⁻ /CD133 ⁻ KDR ⁺ (Cells/10 ⁶ enucleated cells)	EG 1: ↑ CD34 ⁺ /CD45 ⁻ /CD133 ⁺ /KDR ⁺ ; No change on the levels of CD34 ⁺ /CD133 ⁺ /KDR ⁺ ; ↑ CD33 ⁺ /CD45 ⁻ /CD133 ⁻ KDR ⁺ EG 2: ↑ All the evaluated EPCs
Rummens et al. (2012)	EG 1: 60 CAD patients after revascularisation (61 ± 1 yrs; 100% men) EG 2: 25 healthy men (55 ± 1 yrs)	Modality: Aerobic (CPET on cycle ergometer); Intensity: Maximal; Duration: Not reported	CD34 ⁺ /KDR ⁺ ; CD34 ⁺ /CD133 ⁺ /KDR ⁺ ; CD34 ⁺ /CD133 ⁻ /KDR ⁺ (Cells/mL)	EG 1 and EG 2: ↑ CD34 ⁺ /KDR ⁺ and ↑ CD34 ⁺ /CD133 ⁻ /KDR ⁺ ; No change on the levels of CD34 ⁺ /CD133 ⁺ /KDR ⁺ The increase in CD34 ⁺ /CD133 ⁻ /KDR ⁺ EPCs was significantly smaller in patients with CAD compared to healthy adults
Van Craenenbroeck et al. (2009)	EG 1: 10 patients with CHF type D personality, NYHA II-III, LVEF of 26.5 ± 2.1%; (56.3 ± 3.0 yrs; 100% men) EG 2: 25 patients with CHF, NYHA I-III, LVEF	Modality: Aerobic (CPET on cycle ergometer); Intensity: Maximal; Duration: 8-10 min	CD34 ⁺ /KDR ⁺ (% Lymphocytes)	EG 1: No change EG 2: No change

Van Craenenbroeck et al. (2010)	of $27.6 \pm 1.9\%$ (63.4 ± 2.6 yrs; 100% men) EG 1: 19 patients with severe CHF, NYHA II-III, LVEF of $22.8 \pm 1.8\%$ (63.0 ± 2.6 yrs; 79% men) EG 2: 22 patients with mild CHF, NYHA I-III, LVEF of $31.7 \pm 0.9\%$ (61.9 ± 2.5 yrs; 83% men) EG 3: 13 healthy participants (55.7 ± 1.6 yrs; 69% men)	Modality: Aerobic (CPET on cycle ergometer); Intensity: Maximal; Duration: 8-10 min	CD34 ⁺ /KDR ⁺ /CD3 ⁻ (Cells/ 10^6 events)	EG 1: No change EG 2: No change EG 3: No change
Van Craenenbroeck et al. (2011)	EG 1: 7 patients with CHF, NYHA II-III, LVEF of $28.3 \pm 2.9\%$ (65.1 ± 3.5 yrs; 71% men) EG 2: 4 healthy men (71.5 ± 3.0 yrs) EG 3: 4 healthy men (20.3 ± 0.9 yrs)	Modality: Aerobic (CPET on cycle ergometer); Intensity: Maximal; Duration: not reported	CD34 ⁺ /KDR ⁺ /CD3 ⁻ (Cells/ 10^6 events)	EG 1: No change; EG 2 and EG 3: $\uparrow$EPCs
<u>Studies assessing maximal exercise testing (on a treadmill)</u>				
Kaźmierski et al. (2015)	EG 1: 60 patients with premature CAD with NYHA I [43 (35–49) yrs; 72% men] EG 2: 33 healthy participants [42 (34–49) yrs; 82% men]	Modality: Aerobic (Standard Bruce test on treadmill); Intensity: Maximal; Duration: not reported	CD34 ⁺ /CD133 ⁺ /KDR ⁺ (Cells/ μ L)	EG 1: 15 min post-exercise: No change; 60 min post-exercise: $\uparrow$EPCs EG 2: 15 min post-exercise: $\uparrow$EPCs; 60 min post-exercise: $\uparrow$EPCs
Sandri et al. (2011)	EG: 23 patients with stable PAD, Fontaine IIb (61.4 ± 22.1 yrs; 96% men)	Modality: Aerobic (Symptom limited treadmill exercise test); Intensity: Maximal; Duration: not reported	CD34 ⁺ /KDR ⁺ ; CD133 ⁺ /KDR ⁺ (Cells/mL)	24 h post-exercise: $\uparrow$ CD34 ⁺ /KDR ⁺ (by 212%); $\uparrow$ CD133 ⁺ /KDR ⁺ (by 278%)
Scalone et al. (2013)	EG 1: 20 patients with microvascular angina (61 ± 10 yrs; 50% men) EG 2: 20 patients with stable angina CAD (63 ± 8 yrs; 50% men) EG 3: 20 healthy participants with ≥ 1 CVRFs (59 ± 8 yrs; 50% men)	Modality: Aerobic (Standard treadmill Bruce test); Intensity: Maximal; Duration: EG 1: 433 $\pm$ 121 s; EG 2: 380 $\pm$ 104 s; EG 3: 541 $\pm$ 135 s	CD34 ⁺ /KDR ⁺ /CD45 ⁻ (Cells/ 10^5 mononuclear cells)	EG 1, EG 2 and EG 3: No change Whole group: $\uparrow$ EPCs
Shaffer et al. (2006)	EG 1: 15 PAD patients (69 yrs; gender not reported) EG 2: 13 healthy participants (63 yrs; gender not reported)	Modality: Aerobic [Gardner (EG 1 and 2) and Bruce treadmill protocols (EG 3)]; Intensity: Maximal; Duration: EG 1 and 2: 10 min	CD133 ⁺ /KDR ⁺ ; CD34 ⁺ /KDR ⁺ ; CD133 ⁺ /CD34 ⁺ /KDR ⁺ ; CD133 ⁺ /CD34 ⁺ /KDR ⁺ /CD31 ⁻ ; CD34 ⁺	EG 2: $\downarrow$CD133⁺/CD34⁺/KDR⁺/CD31⁻ (by ~63%) No other significant

EG 3: 9 healthy participants (33 yrs; gender not reported)	max unless stopped due to symptoms and EG 3: 15 min max	KDR ⁺ /CD146 ⁺ /CD31 ⁻ (% Live events)	change observed
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CAD, coronary artery disease; CHF, chronic heart failure; CPET, cardiopulmonary exercise test; CVRFs, cardiovascular risk factors; EG, exercise group; EPCs, endothelial progenitor cells; LVEF, left ventricular ejection fraction; mL, millilitres; MNCs, mononuclear cells; NYHA, New York Heart Association classification; PAD, peripheral arterial disease; yrs, years; μ L, microliter; $\uparrow$, significant increase; $\downarrow$, significant decrease; %, percentage. Table 2 was adapted from Ferentinos et al. (2022a).

Given the abovementioned, further research is warranted concerning the acute and chronic effects of exercise, especially focusing on multicomponent exercise, on the levels of EPCs and CECs in patients with CVRFs and patients with CVD. To provide further clarification regarding the acute effect of exercise on the potential for vascular repair, Original Study 1 investigated the impact of two single exercise sessions with different durations on the levels of EPCs and CECs in adults with CVRFs free from cardiac disease, while Original Study 2 assessed the effects of a CPET on the levels of EPCs and CECs in patients with HF.

Chronic Effects Of Exercise

In general, exercise training may augment the levels of EPCs in patients with CVDs (Ferentinos et al., 2022a; Ribeiro et al., 2013). For instance, 8 weeks of aerobic exercise performed three times per week at 60% of heart rate reserve (HRR) increased the levels of EPCs in patients with HF (Sarto et al., 2007). In line with this, a significant increase in EPCs was also observed in patients with peripheral arterial disease (PAD) after 3 and 6 weeks of aerobic exercise (i.e., intermittent walking at a speed that elicited claudication symptoms within 3–5 minutes, until achieving moderate claudication, followed by a brief rest to alleviate the symptoms) performed twice a week (Schlager et al., 2011b). However, controversial results were also found on this topic. A previous study (Sandri et al., 2005) found no changes in the levels of EPCs after 4 weeks of aerobic exercise at 70% of peak heart rate performed 5 times per week for 6 days a week for 10-minute durations in patients with CAD. Additionally, no changes were found after 4 weeks of aerobic exercise (walking at a 12% incline at 3.5 km/h) in patients with PAD (Sandri et al., 2005). Indeed, the differences in results can be attributed

to a possible influence of the clinical condition of the patients tested, as well as the type of exercise protocol applied (Ferentinos et al., 2022a).

Several literature reviews have been published aiming to elucidate the effects of exercise training in the levels of EPCs in patients with CVDs (Guo et al., 2017; Palmefors et al., 2014a; Recchioni et al., 2016; Ribeiro et al., 2013; Van Craenenbroeck & Conraads, 2013). Nevertheless, only one review conducted a meta-analysis (Pearson & Smart, 2017) that summarized the effects of exercise training on the levels of EPCs in patients with HF. However, it was a secondary analysis of the study, which may have limited the inclusion of relevant papers and further interpretations on this issue. Additionally, other types of CVDs with high prevalence worldwide (e.g., CAD) were not included in the analysis. To contribute further evidence in this field, and as part of the present thesis presents, a systematic review and meta-analysis of randomized controlled trials investigating the effects of physical exercise on the circulating number and function of EPCs in patients with CVDs was conducted and is presented below (Cavalcante et al., 2019).

Additionally, recent reviews added some information on this topic by reinforcing that exercise training is able to improve the levels of EPCs in both healthy adults and those with CVRFs (Ferentinos et al., 2022a; Jo et al., 2020a; Mitsiou et al., 2022).

Table 3 presents the current data regarding the effects of exercise training on the levels of EPCs in adults free from cardiac disease (age range: 22 to 76 years old).

Table 3. Studies that evaluated the effects of exercise training (chronic effects) in adults free from cardiac disease

Study	Participants	Exercise training characteristics	EPCs phenotype	Levels of EPCs
<i>Studies assessing MICON exercise</i>				
Cesari et al. (2012)	Compliant group: 21 overweight/obese Non-compliant group: 19 overweight/obese Whole group: 40 (Median age: 48 yrs; 55% men)	Modality: Aerobic exercise (walking/running); Length: 3 months; Frequency: 3x/week; Intensity: ≤ IAT; Duration: 45 min	CD34 ⁺ /KDR ⁺ ; CD133 ⁺ /KDR ⁺ ; CD34 ⁺ /CD133 ⁺ /KDR ⁺ (Cells/10 ⁶ events)	Compliant group: ↑ All the evaluated EPCs Non-compliant group: No change on all the evaluated EPCs

Landers-Ramos et al. (2016)	11 healthy (61.0 ± 2.1 yrs; 36% men)	Modality: Aerobic exercise (walking/running); Length: 10 days; Frequency: Daily; Intensity: ~70% VO _{2 max} ; Duration: 60 min/day	CD34 ⁺ /KDR ⁺ (Cells/10 ⁵ events)	↑ EPCs (by 104%)
Niemi et al. (2018)	EG 1: 17 healthy lean sedentary (23.9 ± 5.4 yrs; 53% men)	Modality: Aerobic (treadmill or bike); Length: 6 weeks; Frequency: 3x/week; Intensity: 60–75% HRR; Duration: 30–60 min	CD45 ⁺ /CD34 ⁺ /CD31 ⁺ (Cells/μl)	EG 1: No change
	EG 2: 10 healthy obese sedentary (29.0 ± 8.0 yrs; 30% men)			EG 2: No change
Thijssen et al. (2006)	EG: 8 healthy sedentary men (67–76 yrs)	Modality: Aerobic (cycle ergometer); Length: 8 weeks; Frequency: 3x/week; Intensity: 65% HRR up to 85% HRR; Duration: 20 min	CD34 ⁺ /KDR ⁺ (Cells/μl)	↓ EPCs (by 45.7%)
Xia et al. (2012)	EG: 25 healthy elderly men (67.8 ± 3.4 yrs)	Modality: Aerobic exercise (treadmill); Length: 12 weeks; Frequency: 3x/week; Intensity: Not reported; Duration: 30 min	CD34 ⁺ /KDR ⁺ ; CD133 ⁺ /KDR ⁺ (% PBMNCs)	↑ All the evaluated EPCs
Yang et al. (2013)	EG 1: 10 healthy elderly sedentary men (61 ± 3 yrs)	Modality: Aerobic (treadmill); Length: 12 weeks; Frequency: 3x/week; Intensity: 4.0 METS for older sedentary and 5.5 METS for young sedentary; Duration: 30 min	CD34 ⁺ /KDR ⁺ (% PBMNCs)	EG 1: ↑ EPCs (by ~83%)
	EG 2: 10 healthy young sedentary men (27 ± 3 yrs)			EG 2: ↑ EPCs (by ~22%)

Studies assessing HIIT

Rakobowcuk et al. (2012)	EG 1: 9 healthy (23.7 ± 3.4 yrs; 33% men) EG 2: 11 healthy (23.1 ± 2.5 yrs; 36% men)	Modality: Aerobic; Length: 6 weeks; Frequency: 3x/week; Intensity: EG 1: Repeated 10 s interval at 120% PWR with 20 s recovery at 20 W; EG 2: Repeated 30 s intervals at 120% PWR with 60 s recovery at 20 W; Duration: Progressed from 30 to 40 min/session	CD34 ⁺ /CD133 ⁺ /KDR ⁺ ; CD34 ⁺ /KDR ⁺ (Cells/mL)	EG 1 and EG 2: No change on all the evaluated EPCs
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Studies comparing HIIT vs. MICON

Tsai et al. (2016)	EG 1: 20 healthy sedentary men (22.2 ± 1.6 yrs) EG 2: 20 healthy sedentary men (22.3 ± 1.0 yrs) CG: 20 healthy sedentary men (22.4 ± 2.1 yrs)	Modality: Aerobic; Length: 6 weeks; Frequency: 5x/Week; Intensity: EG 1: HIIT cycle ergometer 5 sets of 3 min at 80% VO ₂ peak with 3 min recovery at 40% VO ₂ peak; EG 2: Aerobic exercise on a cycle ergometer at 60% VO ₂ peak; Duration: 30 min	CD34 ⁺ /KDR ⁺ ; CD34 ⁺ /KDR ⁺ /CD133 ⁺ ; CD34 ⁺ /KDR ⁺ /CD31 ⁺ (Cells/mL)	EG 1: ↑ All the evaluated EPCs EG 2: ↑ CD34 ⁺ /KDR ⁺ and CD34 ⁺ /KDR ⁺ /CD133 ⁺ ; No change on CD34 ⁺ /KDR ⁺ /CD31 ⁺ CG: No change on all the evaluated EPCs
<i>Studies comparing MICON exercise to other aerobic exercise protocols</i>				
Jo et al. (2020b)	EG 1: 21 post-menopausal women (> 50 yrs) EG 2: 13 post-menopausal women (> 50 yrs) CG: 13 post-menopausal women (> 50 yrs)	EG 1: Modality: Aerobic (Running based exergame); Length: 12 weeks; Frequency: Daily; Intensity: 120 ± 19 bpm; Duration: 40 min EG 2: Modality: Aerobic exercise (Running/ jogging); Length: 12 weeks; Frequency: Daily; Intensity: 60–80% HRR; Duration: 40 min	CD34 ⁺ /KDR ⁺ (Cells/μl)	EG 1: No change EG 2: ↑ EPCs CG: No change

CG, control group; EG, exercise group; EPCs, endothelial progenitor cells; HIIT, high intensity interval training; HRR, heart rate reserve; IAT, individual anaerobic threshold; METS, metabolic equivalents; MICON, moderate intensity continuous training; mL, millilitre; PBMNCs, peripheral blood mononuclear cells; PWR, peak work rate; VO₂ peak/max, peak/maximal oxygen uptake; W, watts; yrs, years; μL, microliter; %, percentage; ↑, significant increase; ↓, significant decrease. Table 3 was adapted from Ferentinos et al. (2022b).

The total of nine studies (Table 3) that evaluated the effects of exercise on the levels of EPCs in adults free from cardiac disease included samples ranging from 22 to 76 years old, with a higher prevalence of men (62.4%) (Ferentinos et al., 2022b). In brief, the exercise training protocols tested in adults free from cardiac disease included moderate intensity continuous training (MICON) training, high-intensity interval training (HIIT), HIIT versus MICON, and MICON versus exergame (an exercise modality based on an interactive video game). The most tested modality was MICON, over periods ranging from 10 days to 12 weeks (Cesari et al., 2012; Landers-Ramos et al., 2016; Niemiro et al., 2018; Thijssen et al., 2006; Xia et al., 2012; Yang et al., 2013). The most common frequency

observed was 3 sessions per week, with sessions lasting 20 to 60 min/session. In general, the MICON protocol has shown to increase the levels of EPCs in older adults, in addition to improving endothelial function and arterial stiffness (Ferentinos et al., 2022b). The few protocols that tested HIIT, in general, demonstrated that short-term HIIT is not capable of increasing the levels of circulating EPCs, while long-term HIIT (with 4-minute bouts) seems to provide positive and superior results compared to the MICON (Table 3). The conflicting results among the studies can be attributed to the varying age ranges of the participants, the differences in prevalence of CVRFs, and variations in the characteristics of the training protocols (Ferentinos et al., 2022b). Additionally, no studies have investigated the effects of multicomponent exercise programs on the levels of EPCs. The original study 3 of this thesis aims to provide further evidence regarding the impact of an 18-week multicomponent exercise protocol with light to moderate intensity on the capacity to mobilize EPCs and release CECs in older adults with CVRFs free from cardiac disease.

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Effects of exercise on endothelial progenitor cells in patients with cardiovascular disease: A systematic review and meta-analysis of randomized controlled trials

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Abstract

The therapeutic potential of exercise training in the mobilization of endothelial progenitor cells (EPCs) into the peripheral blood in patients with cardiovascular disease is not yet clear. A systematic review and meta-analysis was performed in order to assess the effectiveness of exercise training in increasing the number of circulating EPCs in patients with cardiovascular disease. A literature search was conducted across the PubMed, Scopus, Web of Science and EBSCO databases, including the reference lists of relevant papers. The quality of randomized clinical trials was evaluated using the PEDro scale. The primary outcome data were circulating EPC levels. Six studies (236 participants) - three on heart failure (n = 111), one on peripheral arterial disease (n = 40) and two on coronary artery disease (n = 85) - were included. There was an increase in EPC levels in the exercise training groups (effect size [ES] = 0.57; 95% CI: 0.01 - 1.12), with considerable heterogeneity ($I^2 = 75.6\%$; $p < 0.001$). In subgroup analyses,

patients with heart failure showed a significant increase in EPCs in the exercise training groups (ES = 0.52; 95% CI: 0.15 - 0.90), with low heterogeneity ($I^2 = 0.0\%$; $p=0.648$), while no significant increase (ES = 0.67; 95% CI: -0.70 - 2.04; $I^2 = 91.2\%$; $p < 0.001$) was observed in patients with arterial disease. The only study in patients with peripheral arterial disease showed a significant increase in EPC levels.

Keywords: endothelial progenitor cells; flow cytometry; cardiovascular disease; physical exercise; cardiac rehabilitation

Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide (Piepoli et al., 2016). In order to decrease the high mortality burden attributable to CVD, many clinical approaches have focused on endothelial health, as a therapeutic target and an indicator of prognosis (Brainin et al., 2018; Brevetti et al., 2003; Shechter et al., 2009). The endothelium has essential functions in cardiovascular health, including regulating the balance of thrombotic and anticoagulant factors, regulating vascular tone and blood flow, self-repair, and promotion of angiogenesis and blood perfusion (Ribeiro et al., 2009; Versari et al., 2009; Widmer & Lerman, 2014). Conversely, dysfunctional and/or damaged endothelium increases the risk for progression of atherosclerosis (Lusis, 2000), and is considered an underlying cause in the development of CVD and an independent predictor of cardiovascular events (Mendis et al., 2011; Versari et al., 2009). Endothelial health is modulated by various behavioral factors such as smoking (Messner & Bernhard, 2014), diet (Schwingshackl & Hoffmann, 2014) and physical exercise (Pearson & Smart, 2017). Exercise training is a class IA intervention in patients with stable angina, previous myocardial infarction, and coronary artery bypass graft surgery or percutaneous coronary intervention (Perk et al., 2012), and in patients with heart failure (HF) (Ponikowski et al., 2016). Among other benefits, exercise training improves cardiorespiratory fitness and quality of life (Kato et al., 2017; Lin et al., 2015), and mitigates progression of atherosclerosis by enhancing vascular homeostasis (Padilla et al., 2011) and endothelial function (Ashor et al., 2015; Pearson & Smart, 2017).

The number of endothelial progenitor cells (EPCs) is positively correlated with vascular function and inversely correlated with the Framingham risk score (Hill et al., 2003), and predicts morbidity and mortality in patients with CVD (Van Craenenbroeck & Van Craenenbroeck, 2016; Werner et al., 2005). EPCs are mobilized to sites of endothelial injury and differentiate into mature endothelial cells, repairing and maintaining vascular structure, in addition to mediating paracrine mechanisms and promoting angiogenesis. The role of EPCs in vascular health is thus crucial to the treatment of CVD (Simard et al., 2017).

Although some previous studies have shown that exercise training induces the mobilization of EPCs from the bone marrow to the peripheral blood (Ribeiro et al., 2013; Ribeiro et al., 2017; Schlager et al., 2011a; Van Craenenbroeck et al., 2008), others have not demonstrated this effect (Hansen et al., 2011; Van Craenenbroeck et al., 2015). This topic has been extensively reviewed; several narrative reviews (Aicher et al., 2005; Brixius et al., 2006; De Biase et al., 2013; Guo et al., 2017; Lenk et al., 2011; Linke et al., 2008; Moebius-Winkler et al., 2011; Recchioni et al., 2016; Ross et al., 2016; Thijssen et al., 2009c; Van Craenenbroeck & Conraads, 2010; Van Craenenbroeck & Conraads, 2013; Volaklis et al., 2013; Wahl et al., 2007; Wahl et al., 2008; Wang et al., 2015; Witkowski et al., 2011) and systematic reviews (Palmefors et al., 2014b; Pearson & Smart, 2017; Ribeiro et al., 2013) can be found in the literature summarizing the effects and potential mechanisms by which exercise mobilizes EPCs. Nevertheless, only Pearson & Smart (2017) have performed a meta-analysis, which also included non-randomized controlled trials (RCTs). Additionally, analysis of the impact of exercise on EPC numbers was a secondary aim of their analysis (Pearson & Smart, 2017), which may have limited further interpretations on this issue, and was restricted to studies enrolling patients with HF. In view of the above, the aim of this study is to conduct a systematic review and meta-analysis of RCTs investigating the effects of physical exercise on the circulating number and function of EPCs in patients with CVD.

Databases and search strategy

This systematic review and meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Ottawa Hospital Research Institute & Oxford, 2015). Relevant studies

were identified by searching the PubMed, Scopus, Web of Science and EBSCO databases. The search terms were the following: “cardiovascular disease*” OR “chronic disease*” OR “heart disease*” OR hypertension OR “peripheral arterial disease” OR diabetes mellitus OR “coronary artery disease” OR “heart failure” OR myocardial infarction OR “vascular disease*” in combination with “endothelial progenitor cells” OR “progenitor cells” OR “colony-forming units” OR “stem cells” OR “circulating angiogenic cells” in combination with “exercise training” OR exercise OR “cardiac rehabilitation” OR “physical exercise” OR “physical activity” OR “exercise therapy” OR “rehabilitation exercise” and avoiding the terms infant* OR child* OR rat* OR mice OR mouse OR animal* OR cancer OR neoplasm* OR neoplasia. All the keywords were MeSH terms, with the exception of “acute myocardial infarction”, “progenitor cells” and “circulating angiogenic cells”. Considering that EPCs were first isolated by Asahara et al. (1997) we limited the search to studies published between January 1997 and January 31, 2018. In addition, we manually searched the reference lists of landmark studies and review articles on this topic to identify any relevant studies that electronic searches might have missed.

Study selection

Studies were eligible if they (i) were RCTs; (ii) included adults (≥ 18 years old) with established CVD; (iii) compared an experimental group receiving a structured program of exercise training, either exclusively or as a component of a cardiac rehabilitation program; (iv) included a control group receiving only standard medical care (that did not perform exercise training); (v) included an exercise training program lasting at least one week; (vi) assessed circulating EPC levels before and after the intervention; and (vii) were written in English. We excluded literature review papers, letters to the editor, abstracts published in conference proceedings, studies that assessed the acute effects of a single exercise session, studies involving healthy subjects or patients with other clinical conditions (e.g. kidney disease), and animal model studies.

Initially a single author screened the titles and discarded duplicates. Subsequently, two authors independently determined whether studies fulfilled the criteria for inclusion in this review through examination of screened titles and abstracts. The same two authors then analyzed the full articles and used a

standardized form to determine eligibility for inclusion in the review. Any disagreements regarding the inclusion of studies were resolved by discussion with a third author. The process of identification and selection of the studies is displayed in Figure 1.

Data extraction and assessment of methodological quality

Data were extracted for the main outcome (circulating EPC levels) as well as for the study design, population, details of the intervention (frequency, mode, duration, intensity and characteristics of the exercise) and control groups, antibodies used to identify EPCs, units of EPC measurement, functionality of EPCs in vitro (proliferative capacity, migratory capacity, number of colony forming unit endothelial cells), time of blood sample collection for EPC analysis, and exercise capacity. The methodological quality of the studies was determined using the Physiotherapy Evidence Database (PEDro) scale. This scale comprises 10 items related to internal validity and interpretability; a higher score indicates better methodological quality (Moseley et al., 2011). Based on the PEDro score, the quality of the included RCTs were described as following: 6-10 for high-quality studies; 4-5 for fair-quality studies; and ≤ 3 for poor-quality studies (Moseley et al., 2011). Two authors independently extracted the above-mentioned data using a customized form and evaluated methodological quality, while a third author resolved disagreements.

Statistical analysis

The data were analyzed using STATA/SE software, version 14 (StataCorp, College Station, TX, USA). The standardized mean difference between the pre-post of the intervention group versus the control group was calculated for EPC levels using Cohen's d as the effect size statistic. The significance of the pooled effect size was estimated based on 95% confidence intervals (CI). Data not provided in the main text or tables were obtained by contacting the authors or (when there was no response) extracted from figures. One study (Sandri et al., 2016) included two intervention groups (a younger group ≤ 55 years old and an older group ≥ 65 years old); the data from these groups were analyzed as independent samples.

The heterogeneity of results across studies was quantified by I^2 statistics as follows: low risk of heterogeneity (0-40%); moderate risk of heterogeneity (30-

60%); substantial heterogeneity (50-90%); or considerable heterogeneity (75-100%). Significance levels were expressed as p-values (Moher et al., 2009).

Subgroup analyses were performed based on the clinical condition: HF or arterial disease (peripheral and coronary).

Random-effects meta-regression was used to assess whether results differed according to the mean age of participants and the length of the intervention, since these could be considered sources of heterogeneity.

Publication bias was identified by visual inspection of funnel plots and the method proposed by Egger (Sterne et al., 2001).

Systematic review

The initial search identified 651 titles. After exclusion of 227 duplicates, the titles, and abstracts of the remaining 424 articles were analyzed, following which 332 articles were excluded, resulting in 92 studies qualifying for full-paper assessment. Of the 92 potentially eligible articles, only four (Eleuteri et al., 2013; Gagliardi et al., 2016; Sandri et al., 2016; Schlager et al., 2011a) met the inclusion criteria (Figure 1). After screening the reference lists of relevant articles on this topic, two additional papers, not retrieved from the database search, were found (Luk et al., 2012; Mezzani et al., 2013). A total of six papers were therefore included in the systematic review and meta-analysis.

These six studies included 236 participants, 119 in exercise training and 117 in control groups (Table 1). The sample size ranged from 21 to 64 participants and over all the studies involved more men ($n = 189$, almost 80%) than women ($n = 47$). Two studies (Gagliardi et al., 2016; Luk et al., 2012) included coronary artery disease (CAD) patients ($n = 85$), three studies (Eleuteri et al., 2013; Mezzani et al., 2013; Sandri et al., 2016) involved patients with HF with reduced ejection fraction ($n = 111$), and one study included patients with peripheral arterial disease (PAD) ($n = 40$) with mild to severe claudication (Schlager et al., 2011a). All studies identified and quantified circulating EPCs by flow cytometry using a combination of specific antibodies: three studies used the specific antibodies CD34⁺/KDR⁺ (Gagliardi et al., 2016; Luk et al., 2012; Sandri et al., 2016), two CD45^{dim}/CD34⁺/KDR⁺ (Eleuteri et al., 2013; Mezzani et al., 2013) one CD34⁺/CD133⁺/KDR⁺ (Schlager et al., 2011a) and one CD133⁺/KDR⁺ (Sandri et al., 2016). Three studies quantified EPCs as the percentage of mononuclear cells

(Eleuteri et al., 2013; Mezzani et al., 2013; Schlager et al., 2011a), two as the absolute number per ml of peripheral blood (Sandri et al., 2016; Schlager et al., 2011a), one as the percentage of absolute cell counts divided by lymphocyte counts (Luk et al., 2012), and one as cells per 100 000 laser detection events (Gagliardi et al., 2016). Blood collection for EPC determination was performed 24-48 h (Gagliardi et al., 2016), 72 h (Schlager et al., 2011a), and up to a week (Eleuteri et al., 2013) after the last exercise session. Some studies did not provide the time of blood collection (Luk et al., 2012; Mezzani et al., 2013; Sandri et al., 2016). Only one study reported the time of day of blood collection, between 8:00 and 9:00 am (Eleuteri et al., 2013).

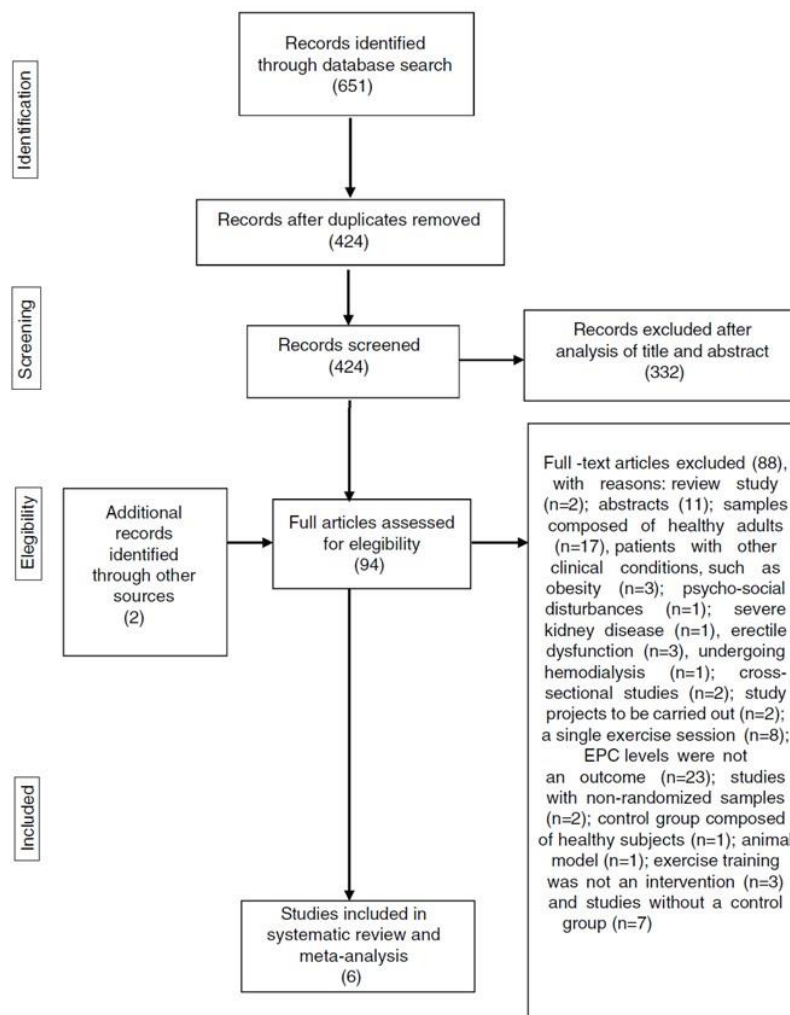


Figure 1 Flow diagram of the literature search.

Table 1 Main features of the included studies.

Study	Sample	Exercise training characteristics	EPC determination	Main results (number and functionality of EPCs)	PEDro score
Schlager et al. ²⁵	40 PAD patients with intermittent claudication EG: 20 (69±8 years; 13 men/7 women) CG: 20 (70±1 years; 11 men/9 women)	Mode: aerobic Length: 24 wks Frequency: 2 sessions/wk Intensity: walking speed that elicited claudication symptoms within 3-5 min Session: 50 min	CD34+/CD133+/KDR+ (% MNCs and absolute number/ml peripheral blood)	Number: ↑ percentage of EPCs Functionality: ↑ migratory capacity; ↑ CFU-EC (well)	7/10
Luk et al. ⁵⁶	64 stable CAD patients EG: 32 (67.7±9 years; 24 men/8 women) CG: 32 (66.6±7 years; 24 men/8 women)	Mode: aerobic and resistance; Length: 8 wks Frequency: 3 sessions/wk Intensity: 80% HRmax (aerobic exercise) and perceived exertion and % HRmax (resistance exercise) Session: 60 min	CD34+/KDR+ (% absolute cell counts divided by lymphocyte counts)	Number: no significant change Functionality: not assessed	6/10
Eleuteri et al. ⁵³	21 male patients with HF EG: 11 (66±2 years) CG: 10 (63±2 years)	Mode: aerobic; Length: 12 wks Frequency: 5 sessions/wk Intensity: HR at ventilatory threshold Session: 40 min	CD45dim/CD34+/KDR+ (% MNCs)	Number: ↑ percentage of EPCs Functionality: not assessed	5/10
Mezzani et al. ⁵⁵	30 male patients with HF EG: 15 (65±7 years) CG: 15 (63±7 years)	Mode: aerobic Length: 12 wks Frequency: 5 sessions/wk Intensity: HR at ventilatory threshold Session: 40 min	CD45dim/CD34+/KDR+ (% MNCs)	Number: ↑ percentage of EPCs Functionality: not assessed	6/10
Gagliardi et al. ⁵⁴	21 stable CAD patients EG: 11 (59.5±2 years; 7 men/4 women) CG: 10 (65.4±1.6 years; all men)	Mode: aerobic Length: 12 wks Frequency: 3 sessions/wk Intensity: not available Session: not available	CD34+/KDR+ (cells/100 000 laser detection events)	Number: no significant change Functionality: not assessed	6/10
Sandri et al. ⁵⁰	69 HF patients Group 1 (aged ≤55): EG: 15 (50±5 years; 12 men/3 women), CG: 30 (49±5 years; 13 men/2 women) Group 2 (aged ≥65): EG: 15 (72±4 years; 12 men/3 women), CG: 15 (72±3 years; 12 men/3 women)	Mode: aerobic Length: 4 wks Frequency: 5 sessions/wk Intensity: 70% VO _{2max} Session: 30 min (4 sessions) plus 60 min (1 session)	CD34+/KDR+ and CD133+/KDR+ (cells/ml)	Number: ↑ EPCs in both EGs Functionality: ↑ migratory capacity in both EGs	5/10

↑: increased; CAD: coronary artery disease; CFU-EC: colony forming unit endothelial cells; CG: control group; EG: experimental group; HF: heart failure; HR: heart rate; HR_{max}: maximum heart rate; MNCs: mononuclear cells; PAD: peripheral arterial disease; VO_{2max}: maximum oxygen uptake; wk: week.

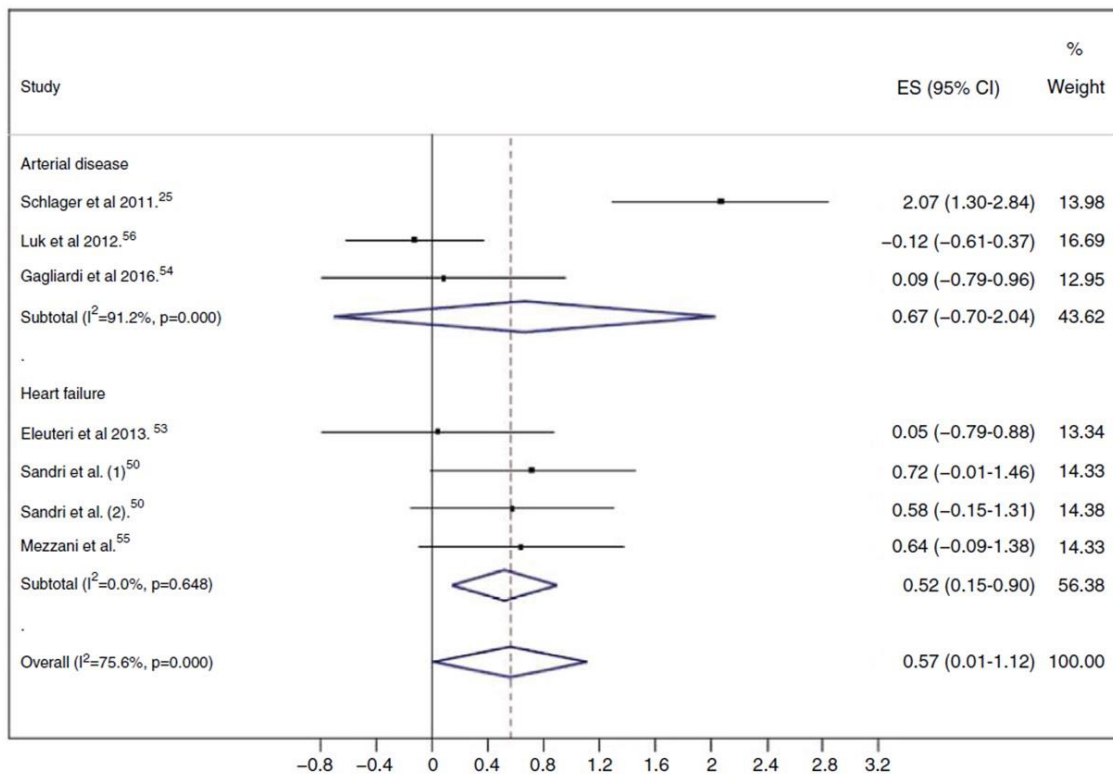


Figure 2 Forest plots of effect size for exercise training interventions in patients with cardiovascular disease according to clinical condition. CI: confidence interval; EPCs: endothelial progenitor cells; ES: effect size.

Regarding the characteristics of exercise training, all studies used aerobic exercise, either exclusively (Eleuteri et al., 2013; Gagliardi et al., 2016; Mezzani et al., 2013; Sandri et al., 2016; Schlager et al., 2011a) or combined with resistance exercise (Luk et al., 2012). The length of exercise interventions ranged from four (Sandri et al., 2016) to 24 weeks (Schlager et al., 2011a), with a frequency of two (Schlager et al., 2011a), three (Gagliardi et al., 2016; Luk et al., 2012), or five (Eleuteri et al., 2013; Mezzani et al., 2013; Sandri et al., 2016) sessions per week. The duration of the exercise sessions ranged from 30 to 60 min. Exercise intensity was determined using claudication symptoms (Schlager et al., 2011a), or heart rate at the ventilatory anaerobic threshold (Eleuteri et al., 2013; Mezzani et al., 2013) at 80% of maximum heart rate (HR_{max}) (Luk et al., 2012) or at 70% of maximum oxygen uptake (Sandri et al., 2016). Only one study involved resistance exercise determined by intensity assessed through perceived exertion and HR_{max} , at a similar intensity to aerobic exercise (80% of HR_{max}) (Luk et al., 2012). One study did not report either exercise session duration or exercise intensity (Gagliardi et al., 2016).

Meta-analysis

When exercise training was compared to control, EPC levels were higher in the exercise training groups (ES = 0.57; 95% CI: 0.01 - 1.12), with considerable heterogeneity ($I^2 = 75.6\%$; $p < 0.001$) (Figure 2).

Subgroup analyses and meta-regression

Subgroup analyses were performed based on the clinical condition, i.e. patients with HF and patients with arterial disease (CAD or PAD). Studies enrolling patients with CAD and PAD were grouped together, because only one study in patients with PAD was included in this review. In patients with HF, a significant increase in circulating EPC levels was seen in the exercise training group (ES = 0.52; 95% CI: 0.15 - 0.90), with low heterogeneity ($I^2 = 0.0\%$; $p = 0.648$) (Figure 2).

In patients with arterial disease, there was no significant increase in EPC levels in the exercise training groups (ES = 0.67; 95% CI: -0.70 - 2.04), with considerable heterogeneity ($I^2 = 91.2\%$; $p < 0.001$) (Figure 2). The two studies (Gagliardi et al., 2016; Luk et al., 2012) that enrolled patients with CAD showed no changes in circulating EPC numbers after the exercise training program, while the study (Schlager et al., 2011a) that assessed the effects of aerobic exercise in the mobilization of EPCs in patients with PAD and intermittent claudication showed a significant increase in circulating EPC levels.

The random-effects meta-regression model showed that age (Figure 3) and length of the intervention (Figure 4) are not related to heterogeneity across studies ($p = 0.821$ for age, $p = 0.113$ for length of the intervention).

Sensitivity analysis

The pooled effect size estimate was not significantly altered in magnitude or direction when individual study data were removed from the analysis one at a time.

Study quality and publication bias

The studies had a mean score of 6 on the PEDro scale, ranging from 5 to 7. Two studies scored 5, which indicates fair methodological quality; the remaining studies were classified as high methodological quality RCTs (Table 1). There was no significant publication bias for exercise training intervention versus

control, as demonstrated by the asymmetry of the funnel plot (Figure 5) and Egger's test ($p = 0.324$).

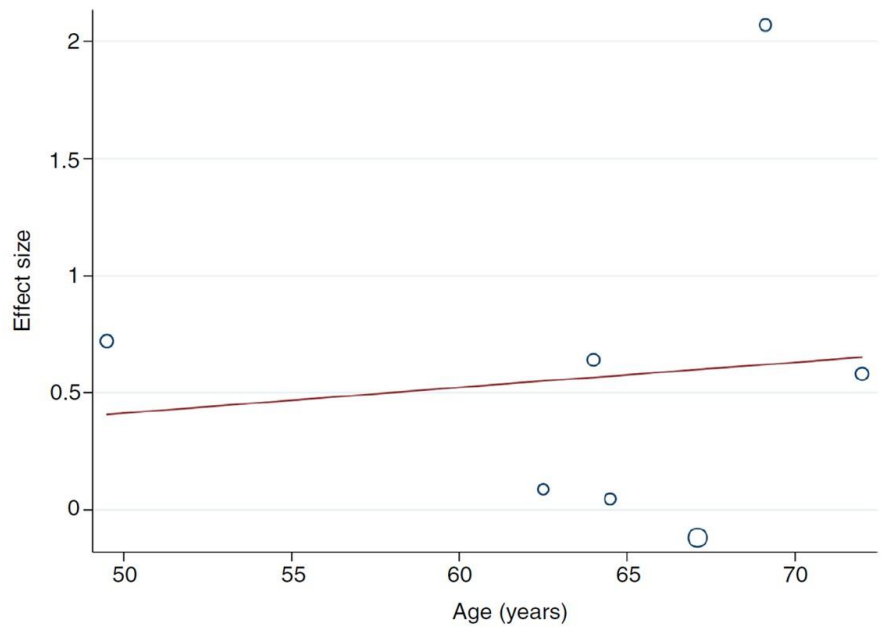


Figure 3 Random effects meta-regression for age.

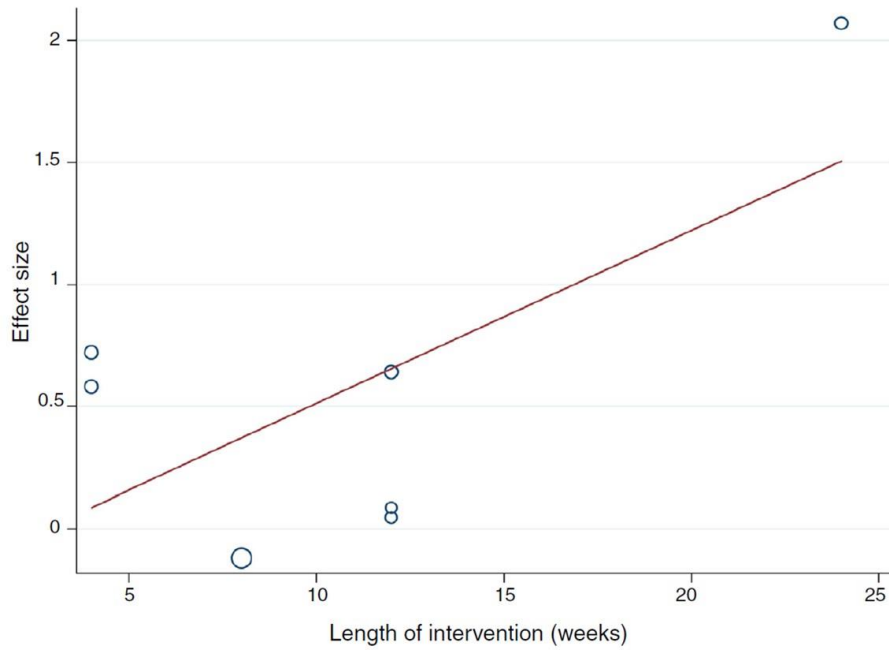


Figure 4 Random effects meta-regression for length of intervention.

Discussion

The present systematic review and meta-analysis provides a synthesis of the evidence, which suggests that exercise training interventions are successful

in the mobilization of EPCs from bone marrow to circulation in patients with HF. Furthermore, the only study in patients with PAD also showed a significant increase in circulating EPC levels.

Our pooled data demonstrated that exercise training had a significant effect on EPCs in patients with HF. This finding is consistent with previous studies in patients with HF, which show an increase in the circulating levels and in-vitro functionality of EPCs after an aerobic exercise program (Gatta et al., 2012; Sarto et al., 2007; Van Craenenbroeck et al., 2010). A previous systematic review and meta-analysis quantifying the effect of exercise training on endothelial function in heart failure patients, assessed by flow-mediated dilation, but examining as a secondary aim the effects of exercise training on EPCs, also showed a significant improvement in EPCs as a result of exercise training (Pearson & Smart, 2017).

Various mechanisms may explain the beneficial effects of exercise training on EPC levels. In brief, exercise training influences the mobilization of EPCs by increasing shear stress and levels of nitric oxide and angiogenic factors, particularly VEGF, stromal cell-derived growth factor-1, hypoxia-inducible factor1 (HIF-1) and matrix metalloproteinase-9 (De Biase et al., 2013; Ribeiro et al., 2013; Ribeiro et al., 2017). Ischemia is also a powerful stimulus for EPC mobilization, mainly due to the release of angiogenic factors such as VEGF and HIF-1 (De Biase et al., 2013; Forsythe et al., 1996; Takahashi et al., 1999). In this regard, the increase in EPCs observed in the study enrolling patients with PAD (Schlager et al., 2011a) could be explained by exercise-induced transitory ischemia, since patients were trained until moderate claudication was elicited. Interestingly, a previous study showed that four weeks of aerobic exercise-induced transitory ischemia was associated with a 5.2-fold increase in CD34⁺/KDR⁺ cells in patients with PAD, whereas no changes were observed in the PAD and CAD groups who underwent non- exercise-induced ischemia (Sandri et al., 2005).

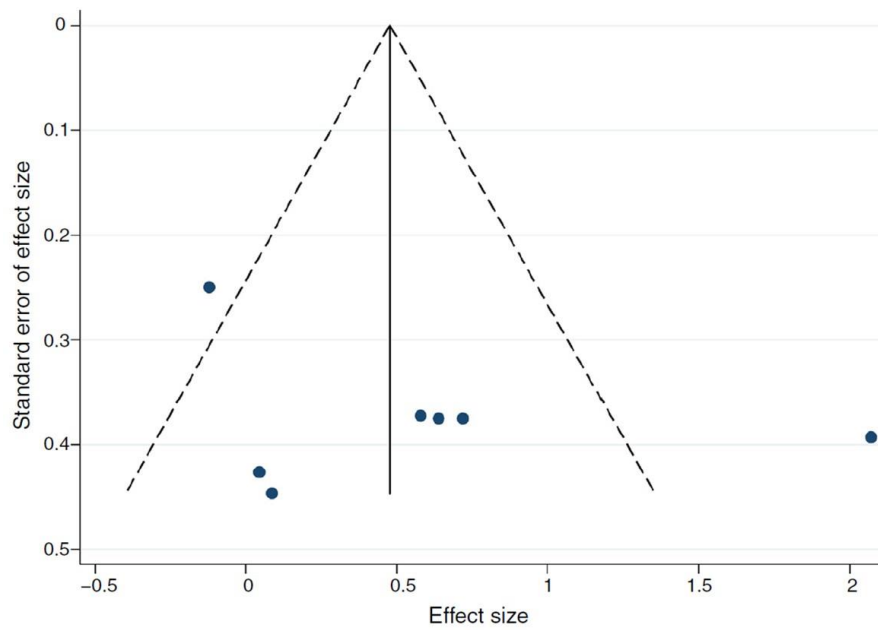


Figure 5 Assessment of potential publication bias by Egger's test.

The absence of changes in EPC levels in patients with CAD in the RCTs included in this systematic review and meta-analysis has also been observed in other non-randomized trials (Hansen et al., 2011; Van Craenenbroeck et al., 2015). Nonetheless, conflicting responses to exercise training have been found in patients with CAD; some studies failed to show an increase in circulating EPC levels after continuous aerobic exercise at moderate intensity (Hansen et al., 2011; Van Craenenbroeck et al., 2015) or interval training (Van Craenenbroeck et al., 2015), while others showed an increase in EPC levels in response to an aerobic exercise program (Paul et al., 2007; Steiner et al., 2005) or combined exercise training (Laufs et al., 2004). The lack of detailed descriptions regarding the dose of resistance training (number of series of exercise, number of repetitions, and resting time between series) (Luk et al., 2012) and aerobic training (session duration and exercise intensity) (Gagliardi et al., 2016) in the included studies hinders comparisons between results and replication of their methodologies. The possibility that the exercise training protocol delivered a suboptimal shear stress stimulus that was insufficient to increase the mobilization of EPCs is noted as a possible explanation for the results observed in patients with CAD. Due to these limitations, these results should be interpreted with caution and future randomized trials are clearly needed in this population to

enhance understanding of the effects of exercise training on EPCs in patients with CAD.

Study limitations

Some limitations of this review should be acknowledged. A major limitation is the considerable heterogeneity among studies. Differences in the methodological assessment of EPCs may have contributed to this level of heterogeneity. EPCs are a relatively scarce cell population, which makes their quantification by flow cytometry a challenging task (Van Craenenbroeck et al., 2013). The best EPC phenotype to determine EPCs in a clinical setting appears to be CD34⁺/KDR⁺/CD45^{dim} (Fadini et al., 2012; Schmidt-Lucke et al., 2010; Van Craenenbroeck et al., 2013). The results of different studies may also to some extent be influenced by the lack of consistency in isolation strategies, use of lineage markers to determine EPCs, and units used to report EPCs (Simard et al., 2017). In this regard, the use of cells per ml as units to quantify EPCs (Sandri et al., 2016) is a major limitation, due to the hemodilution that can be seen in patients with HF. Also, several studies did not clearly state the time of day of blood collection and the time from the last exercise session, as EPCs appear to display diurnal variation (Thomas et al., 2008) and the timing of blood collection post-exercise could reflect the effect of the last exercise session (Adams et al., 2004; Ribeiro et al., 2017). The differences between studies in disease severity, medication use and cardiovascular risk factors may also have contributed to the high heterogeneity. Data from some studies were extracted from figures, which is another potential source of error. The lack of a clear description of the characteristics (e.g., intensity, mode, duration, frequency) of the exercise training programs was also a limitation.

Future prospects

This analysis has several implications for further research. In most studies, the sample was composed mainly (or exclusively) of male patients; therefore, future studies should include mixed-gender samples and sub-analysis by gender, when possible, to ascertain the possible existence of between-gender differences, since hormones could influence EPC levels (Strehlow et al., 2003). It would be useful to assess endothelial function and correlate any changes with changes in EPC levels; the only study included in this review that assessed the

relation between endothelial function and EPCs found no correlation. Although the effect of exercise on both endothelial function and EPC levels shares common mechanisms (decreasing inflammatory cytokine concentrations and increasing endothelial nitric oxide synthase activity and endothelium-dependent nitric oxide production) (De Biase et al., 2013; Landmesser & Drexler, 2005; Pearson & Smart, 2017), the relation between the two variables still lacks clarification. Future studies should use standard gating strategies and assessment protocols for the quantification of EPCs.

Conclusions

This meta-analysis found that exercise training improves the number of circulating EPCs in patients with HF with reduced ejection fraction. This increase in circulating EPC levels may enhance endothelial function and repair. However, whether the same effects occur in patients with preserved ejection fraction, or whether the positive results observed in the only study in patients with PAD are replicated in further studies, is still unclear.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Chapter III

Original Studies

This study was published in Cavalcante, S., Teixeira, M., Duarte, A., Ferreira, M., Simões, M. I., Conceição, M., Costa, M., Ribeiro, I. P., Gonçalves, A. C., Oliveira, J., & Ribeiro, F. (2022). Endothelial progenitor cell response to acute multicomponent exercise sessions with different durations. *Biology (Basel)*, 11(4). doi:10.3390/biology11040572

Endothelial Progenitor Cell Response to Acute Multicomponent Exercise Sessions with Different Durations

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Simple Summary: Reports suggest that an acute bout of exercise promotes the mobilization of endothelial progenitor cells from bone marrow, increasing the circulating levels of endothelial progenitor cells. The impact of the intensity of acute exercise on the circulating levels of endothelial progenitor cells was previously described. Yet, the question of whether the duration of the exercise session impacts the mobilization of endothelial progenitor cells has not been tested among adults with cardiovascular risk factors. In this study, a 30 min session vs. a 45 min session promoted a significant increase in the circulating

number of endothelial progenitor cells. The findings show a multicomponent exercise session of only 30 min has the potential to induce beneficial effects for vascular health.

Abstract

It is widely accepted that exercise training has beneficial effects on vascular health. Although a dose-dependent relation has been suggested, little is known about the effects of different exercise durations on endothelial markers. This study aimed to assess the effect of single exercise sessions with different durations in the circulating levels of endothelial progenitor cells (EPCs) and endothelial cells (CECs) among adults with cardiovascular risk factors. Ten participants performed two multicomponent exercise sessions, one week apart, lasting 30 and 45 min (main exercise phase). Before and after each exercise session, blood samples were collected to quantify EPCs and CECs by flow cytometry. The change in EPCs was significantly different between sessions by 3.0% (95% CI: 1.3 to 4.7), being increased by $1.8 \pm 1.7\%$ ($p = 0.009$) in the 30 min session vs. $-1.2 \pm 2.0\%$ ($p > 0.05$) in the 45 min session. No significant change was observed in CECs [-2.0% , 95% CI: (-4.1 to 0.2)] between the sessions. In conclusion, a multicomponent exercise session of 30 min promotes an acute increase in the circulating levels of EPCs without increasing endothelial damage (measured by the levels of CECs) among adults with cardiovascular risk factors.

Keywords: exercise; vascular health; aging; endothelial progenitor cell; flow cytometry

1. Introduction

Aging is associated with vascular endothelial cell senescence and endothelial dysfunction [1], which, in turn, are related to cardiovascular disease development and progression [2,3]. Endothelial cells are considered potential targets for the prevention and treatment of atherosclerotic vascular diseases [4]. Endothelial progenitor cells (EPCs) are bone-marrow-derived cells that play important roles in endothelial cell repair, vascular integrity, and neovascularization [5,6]. EPCs are circulating precursors of endothelial cells that,

when attracted to sites of endothelium damage and ischemia tissues [7,8], could promote endothelial repair/regeneration by stimulating the proliferation of mature endothelial cells via a paracrine mechanism, or by incorporating the vessels and differentiating into mature endothelial cells [9–11]. Aging [12] and other cardiovascular risk factors [13] are linked to a reduced circulating number and functionality of EPCs; moreover, the circulating number of EPCs seems to predict clinical events in cardiovascular disease [14]. Thus, finding effective strategies of increasing the mobilization of EPC from the bone marrow to the circulation is a promising area of investigation in both primary and secondary prevention of cardiovascular disease. Circulating endothelial cells (CECs) are considered to be an indicator of endothelial injury, because their levels are correlated with endothelial function [15]. The levels of CECs, measured 48 h after acute coronary syndrome, were shown to be an independent predictor of mortality and 1-year major cardiovascular end points at 30 days and 1 year after the acute syndrome [16]. The imbalance between the levels of EPCs and CECs may indicate a lower vascular repair capacity, which makes them potential biomarkers for vascular health [17].

Physical exercise is widely recommended to prevent cardiovascular diseases in adults [18,19] and may counteract the effects of aging on endothelial function [20–22]. Exercise seems to maintain endothelial structure/functionality and vascular health, yet the precise mechanisms mediating these effects are yet to be elucidated. Even in patients with heart failure, a disease in which the circulating levels of EPCs are diminished in comparison to an age matched group [23–25], an exercise training intervention lasting from four weeks to six months [26–28] is effective in improving EPCs number. Proposed mechanisms include the upregulation of nitric oxide and antioxidant factors, the reduced vascular wall low-grade inflammation, and increased circulating levels of EPCs [8,21]. The long-term beneficial effects of physical exercise occur under cumulative molecular responses to repetitive acute exercise bouts [29]. In this regard, a single exercise session/bout can acutely increase the levels of EPCs [30]; however, few studies have tested whether the mobilization of EPCs from the bone marrow into circulation, and the desquamation of mature endothelial cells, depend on the characteristics (e.g., duration, intensity) of the exercise session

[30–35]. Among these, only one study tested the acute effects of different durations of exercise in the circulating levels of EPCs among young adults [33].

The duration of the exercise sessions of physical exercise programs among late middle-aged and older adults varies between approximately 9 and 90 min per session [36,37], with 45 min being the most common [36]. There is scarce information on the optimal exercise session duration to optimize the mobilization of EPCs from bone marrow to circulation. Additionally, the number of studies focusing on the response of EPCs and CECs to exercise in late middle-aged and older adults with cardiovascular risk factors is limited. Therefore, this study investigated the effect of single exercise sessions of different durations (30 min vs. 45 min) on the circulating levels of EPCs and CECs among adults with cardiovascular risk factors.

2. Materials and Methods

2.1. Study Design and Participants

Fifteen physically active adults participating in a primary health care center's physical activity program were invited to participate in this study. Inclusion criteria: (i) age > 50 years old; (ii) men and women with regular exercise (2 times per week) during the last 6 months; (iii) adults with established cardiovascular disease or at least one cardiovascular risk factor (e.g., diabetes, hypertension, dyslipidemia). Exclusion criteria: any limitation precluding physical efforts, medical history of cancer, or diseases of the hematopoietic system. Five participants did not comply with the eligibility criteria; hence, ten adults participated in this study. A within-subjects, repeated-measures design was used. All participants completed two multicomponent exercise sessions of different durations (30 or 45 min) on two separate days (one week apart). Both exercise sessions started at the same time of the day (6:00 p.m.). The participants were asked not to engage in any form of exercise on the day of each session. A one-week interval between sessions was used to minimize carry-over effects between exercise sessions. The local ethics committee approved the study (Ref. 174211). All participants provided written informed consent and all procedures were conducted according to the Declaration of Helsinki.

2.2. Demographics and Clinical Data

Body weight and height were obtained with a scale and a stationary stadiometer, respectively, and body mass index (kg/m^2) was calculated. Waist circumference (WC) was measured in the midpoint between the superior iliac crest and the lower margin of the last rib while the participants stood in an erect position with their arms at their sides and their feet close together. Clinical data, medication, and sociodemographic variables (e.g., age, smoking habits) were collected. Resting peripheral blood pressure and heart rate were assessed using an automatic sphygmomanometer (Omron M6, Omron Healthcare, Hoofddorp, The Netherlands) according to the recommendations of the European Society of Hypertension/European Society of Cardiology [38].

2.3. EPCs and CECs Quantification by Flow Cytometry

Before and immediately after each exercise session, 3 mL of venous blood was collected into EDTA tubes and treated, according to the manufacturer's instructions, with TransFix (Cytomark, Caltag Medsystems Ltd., Buckingham, UK) at a 1:5 ratio immediately after collection. Transfix is a cellular antigen stabilization reagent that stabilizes cell populations and permits blood analysis for up to seven days after blood collection [39]. Blood samples were stored in the dark box, at room temperature, until the flow cytometry quantitative assessment of circulating EPCs and CECs (two–three days after blood collection). All the staining and analysis procedures were conducted as previously described [40]. In short, whole blood samples were incubated for 10 min with FcR-blocking reagent to block unwanted binding of antibodies to human-Fc-receptor-expressing cells. All staining procedures were executed at room temperature. Samples were incubated with BV410 CD34 (BD Horizon), PE CD309 (VEGFR-2/KDR; BD Pharmingen), FITC CD144 (BD Pharmingen), BV510 CD45 (BD Horizon), and APC CD133/1 (Miltenyi Biotec), according to the instructions of the manufacturers. After the lysis of the erythrocytes, at least 500.000 CD45⁺ and a minimum of 100 CD34⁺ cells were acquired on a BD FACS Canto II™ system (BD Biosciences, Franklin Lakes, NJ, USA) using BD FACSDiva™ version 6.1.3 software. All samples were analyzed in duplicate. Data were analyzed using Infinicyt™ (Cytognos, Salamanca, Spain). The EPCs were defined as CD45^{low}/CD34⁺/CD309⁺/CD133⁺/CD144⁻ cells, and the CECs were defined as CD45^{low}/CD34⁺/CD309⁺/CD133⁻/CD144⁺. EPCs and CECs are expressed as a

percentage of leukocytes (CD45⁺ cells). The within-day coefficient of variation of EPCs and CECs quantification was < 5%.

2.4. Exercise Sessions

All participants were familiarized with the type and intensity of exercise. Both exercise sessions took place in the afternoon, between 6:00 and 7:00 pm. Each participant was asked to wear a heart rate monitor (Polar Electro Oy, Kempele, Finland) for monitoring of heart rate throughout the exercise session. The Borg scale was used to assess the rate of perceived exertion (RPE) during the exercise sessions. Each exercise session consisted of three phases: a warm-up phase (5 min of active stretching and mobility exercises of the upper and lower limbs), a main multicomponent exercise phase, and a cool-down phase (composed of 5 min of walking at a light intensity and static stretching). The main multicomponent exercise phase (30 or 45 min) of each session was composed of balance, strength, and aerobic exercise at moderate intensity (12–14 on RPE, 50–60% heart rate reserve). The multicomponent exercise was performed with an interval training design, including periods of walking at light intensity (1 min) with 1 min periods of brisk walking/running intersected with callisthenic exercises (e.g., 1 min light intensity walking–1 min brisk walking/running– 1 min light intensity walking–1 min strength exercise–1 min light intensity walking–1 min brisk walking/running, and so on). Each round of the 1 min callisthenic exercises comprised 1 set of 8–12 repetitions of each exercise (i.e., squat, shoulder abduction, front support, and plantar flexion, in sequence). The upper and lower limbs callisthenic exercises were performed with intensity assessed by the participant's rating of perceived exertion (12–14 on RPE).

2.5. Statistical Analysis

All statistical analyses were carried out using the IBM SPSS Statistics 25 software. Normality of the data was tested with the Shapiro–Wilk test. Data are presented as mean $\pm$ standard deviation (SD); mean differences are expressed with their two-sided 95% confidence interval (CI). Paired Student *t*-tests were performed for within-session comparisons from baseline to the end of the session. Between-session differences at baseline and in the change from baseline to the end of the session were tested with unpaired Student *t*-tests. The value of significance was set at $p < 0.05$.

3. Results

3.1. Participant Characteristics

From the 15 participants who were invited to participate in the study, 5 did not comply with the eligibility criteria. Thus, 10 participants agreed to participate and completed both exercise sessions. The participants were mostly women (70%), the mean (SD) age was 67.1 ± 8.6 years, and the most prevalent cardiovascular risk factor was overweight/obesity (60%) followed by dyslipidemia (50%). The heart rate was similar during the exercise sessions (45 min: 114.3 ± 13.4 vs. 30 min: 116.3 ± 18.0 bpm, $p = 0.64$). The characteristics of the participants are summarized in Table 1.

3.2. Effects on EPCs and CECs

The circulating levels of EPCs and CECs were similar before the two exercise sessions (at baseline, $p > 0.05$) (Figure 1). The change in EPCs was significantly different between sessions by 3.0% ($p = 0.002$), being increased by $1.8 \pm 1.7\%$ ($p = 0.009$) in the 30 min session vs. $-1.2 \pm 2.0\%$ ($p > 0.05$) in the 45 min session (Figure 1, Panel EPCs). After the 30 min exercise session, the levels of CECs decreased significantly ($p = 0.03$), while after the 45 min session, the levels of CECs remained unchanged (Figure 1, CECs panel); nevertheless, no significant change was observed between sessions in CECs (Figure 1, CECs panel).

Table 1. Characteristics of the Participants

Age (years)	67.1 ± 8.6
Sex (Male/Female)	3/7
Weight (kg)	65.9 ± 12.4
Height (cm)	158.8 ± 8.8
Systolic blood pressure (mmHg)	129.6 ± 19.4
Diastolic blood pressure (mmHg)	77.7 ± 11.4
Heart rate (bpm)	69.4 ± 7.6
Waist circumference (cm)	93.0 ± 13.2
Body mass index (kg/m ²)	26.0 ± 3.3
Cardiovascular risk factors (<i>n</i> , %)	
Obesity class I	2 (20)
Overweight	4 (40)
Hypertension	3 (30)
Type 2 Diabetes mellitus	2 (20)
Dyslipidemia	5 (50)
Current smoker	1 (10)
Medication (<i>n</i> , %)	
Statins	3 (30)
Diuretics	1 (10)
Angiotensin II receptor blockers	1 (10)
Anticoagulants	1 (10)
Calcium channel blockers	1 (10)

Data are presented as means ± SD or number (%).

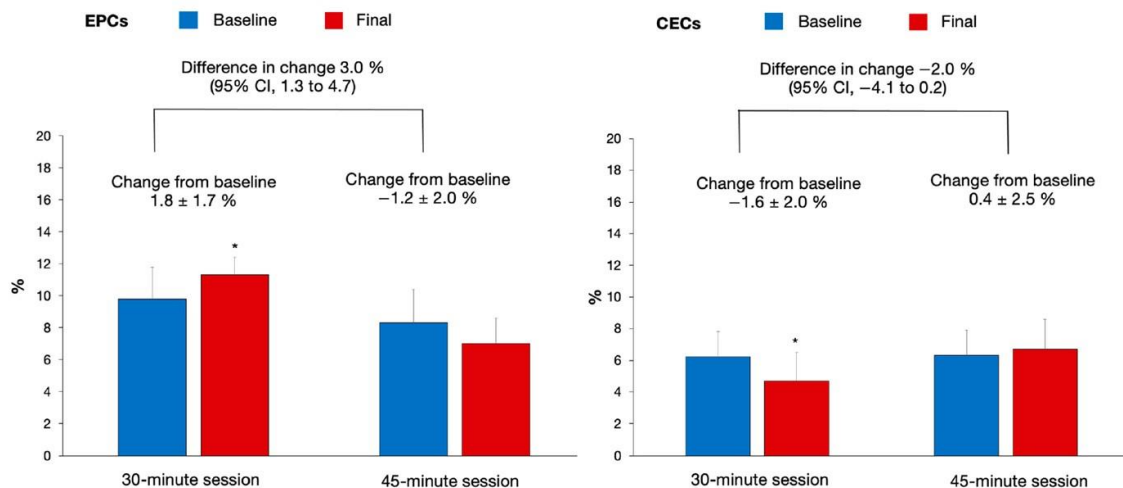


Figure 1. Effects of an acute session of exercise of different durations in the circulating levels of endothelial progenitor cells (EPCs) and endothelial cells (CECs). Data are presented as the number of circulating cells (%) × 10⁻³. * Significant difference from baseline, *p* < 0.05.

4. Discussion

The present study tested the acute effects of multicomponent exercise sessions with different durations (30 and 45 min) on the circulating levels of EPCs and CECs in adults with cardiovascular risk factors. Our results suggest that only the 30 min exercise session significantly enhanced the levels of EPCs and decreased CECs in the peripheral circulation. The mobilization of EPCs from bone marrow was significantly higher in the 30 min session in comparison to the 45 min exercise session.

To the best of our knowledge, the present study is the first to assess the acute effects of two multicomponent exercise sessions with different durations on levels of EPCs and CECs among adults with cardiovascular risk factors. Our findings are partly in agreement with the results of a previous study, assessing the acute effects of two continuous moderate intensity aerobic exercise sessions with different durations (i.e., 30 and 10 min) on the levels of EPCs among healthy young men [33]. The authors observed that only the 30 min session acutely enhanced the levels of EPCs [33]. Other studies have also showed that 30 min of continuous aerobic exercise in young athletes [32] and resistance exercise in young women [30] increased the circulating levels of EPCs immediately after an exercise session. Regarding the effects of a 45 min exercise session, previous studies showed that a 45 min session of aerobic exercise at 70% $\text{VO}_{2\text{ peak}}$ did not alter the levels of EPCs immediately after exercise in young adults [41] or 60 min after exercise in non-diabetic young men [42]. We anticipated observing an increase in the circulating levels of EPCs after the 45 min exercise session. One potential explanation of our results could be that continuous shear stress for a longer period (45 min vs. 30 min) might increase the activation of endothelial cells to a greater extent, which, in turn, would increase the attraction of EPCs to sites of endothelial activation to stimulate the proliferation of mature endothelial cells or to be incorporated into the vessels, differentiating into mature endothelial cells. This would explain the lack of significant changes in the circulating levels of EPCs immediately after the 45 min session.

Our results showed a decrease in the levels of CECs only after the 30 min session. A previous study [43] found no alterations in the levels of CECs immediately after 30 min of continuous or high-intensity interval exercise in young men. Another study [42] also found no alterations in CECs after 60 min or on the morning after a 45 min session of continuous aerobic exercise. On the other hand, a maximal exercise test increased the levels of CECs in cardiac patients [44,45] but decreased CECs levels at 30 min [45] and 4 h [44] after the exercise test. Taken together, these results suggest that a single 30 or 45 min exercise session does not promote substantial endothelial damage expressed by the CECs count. The possibility of improving vascular repair without increasing the levels of CECs in response to exercise is promising in the context of the prevention of cardiovascular disease.

The exercise characteristics (i.e., mode, intensity, and duration) may have an important impact on vascular response [46]. Indeed, previous studies showed that aerobic exercise protocols (~30 min exercise sessions) at moderate and vigorous intensities increased the number of EPCs [32,33]. Nonetheless, a 10 min session of aerobic exercise at moderate intensity did not change the levels of EPCs in young men [33]; additionally, no alterations were found after 30 min of moderate-intensity continuous aerobic exercise, moderate-intensity interval exercise, or heavy-intensity interval exercise in postmenopausal women [31]. In addition to the characteristics of the exercise session, the level of participants' physical activity/exercise capacity could explain the difference between our results and the results of previous studies [47]. Nontrained adults submitted to an unhabitual stimuli (i.e., exercise) may present different EPCs and CECs mobilization in comparison with trained subjects. One of the mechanisms that is related to the mobilization of EPCs is exercise-induced shear stress, which leads to the upregulation of nitric oxide [48]. Different modes of exercise can induce different patterns of shear stress [49], which can result in specific responses in vascular function [46,50]. Thus, the quest for the optimal set of exercise session characteristics (i.e., intensity, mode, and duration) to mobilize EPCs from bone marrow to circulation seems a relevant research topic. More studies are needed comparing similar durations of exercise sessions at different intensities and modes in the levels of EPCs and CECs. A strong point of our study is the study of a multicomponent exercise session, since multicomponent exercise is particularly important for late middle-age and elderly adults.

Some limitations should be acknowledged. First, the present study collected the blood samples at a single timepoint after each exercise session, which limited the assessment of the transitory effect over time after both exercise sessions. Second, despite being similar to previous studies in this field [31,41,43], the sample size is also a limitation. We recruited a small sample without performing a previous sample size calculation, which limits the generalizability of our results. This initial study generates data that can be used to determine the sample size in future studies aiming to confirm our findings. Third, the participants did not present a common risk factor, i.e., they presented different cardiovascular risk factors, which could affect the mobilization of EPCs in different degrees. Fourth, further analysis of endothelial damage markers (e.g., assessment of

endothelial-derived microparticles [51]), and inflammatory cytokines, such as tumor necrosis factor-alpha [42], could have provided additional information on the effect of exercise on endothelial damage. Finally, since all participants included in this study were trained subjects, it is not clear if the same (or even a more pronounced) effect would be observed in nontrained adults.

5. Conclusions

In conclusion, the main results of this study suggest that a shorter bout of multicomponent exercise (30 min) could promote an acute increase in the circulating levels of EPCs without increasing endothelial damage (measured by the levels of CECs) among adults with cardiovascular risk factors.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the local ethical committee (ARS Centro Ref. 174211).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study and additional information are available on request to the corresponding author.

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Maximal Exercise Improves the Levels of Endothelial Progenitor Cells in Heart Failure Patients

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Abstract

The impact of exercise on the levels of endothelial progenitor cells (EPCs), a marker of endothelial repair and angiogenesis, and circulating endothelial cells (CECs), an indicator of endothelial damage, in heart failure patients is largely unknown. This study aims to evaluate the effects of a single exercise bout on the circulating levels of EPCs and CECs in heart failure patients. Thirteen patients with heart failure underwent a symptom-limited maximal cardiopulmonary

exercise test to assess exercise capacity. Before and after exercise testing, blood samples were collected to quantify EPCs and CECs by flow cytometry. The circulating levels of both cells were also compared to the resting levels of 13 volunteers (age-matched group). The maximal exercise bout increased the levels of EPCs by 0.5% [95% Confidence Interval, 0.07 to 0.93%], from $4.2 \times 10^{-3} \pm 1.5 \times 10^{-3} \%$ to $4.7 \times 10^{-3} \pm 1.8 \times 10^{-3} \%$ ($p = 0.02$). No changes were observed in the levels of CECs. At baseline, HF patients presented reduced levels of EPCs compared to the age-matched group ($p = 0.03$), but the exercise bout enhanced circulating EPCs to a level comparable to the age-matched group ($4.7 \times 10^{-3} \pm 1.8 \times 10^{-3} \%$ vs. $5.4 \times 10^{-3} \pm 1.7 \times 10^{-3} \%$, respectively, $p = 0.14$). An acute bout of exercise improves the potential of endothelial repair and angiogenesis capacity by increasing the circulating levels of EPCs in patients with heart failure.

Keywords: acute exercise; cardiovascular disease; endothelium; flow cytometry

1. Introduction

Heart failure (HF) is a clinical syndrome characterized by structural and functional alterations in the cardiovascular system [1]. Patients with HF present abnormal hemodynamic alterations, such as increased intracardiac pressures and/or depressed cardiac output, which can be evidenced during physical efforts and/or at rest [1]. The endothelium plays a paramount role in hemodynamic control and vascular function [2,3]. Endothelial dysfunction is related to the progression of HF [4], being an independent predictor for HF [5] and a potential treatment target [6]. In a healthy endothelium, there is a delicate balance between endothelial injury (e.g., assessed by the levels of endothelial damage biomarkers, such as circulating endothelial cells (CECs)) and the endogen repair capacity (e.g., assessed by the circulating levels of pro-angiogenic and endothelial repair/maintenance factors, such as endothelial progenitor cells (EPCs)) [7].

The EPCs are bone-marrow-derived cells with the potential to migrate and differentiate in mature endothelial cells. EPCs are attracted to sites of endothelial damage, contributing to endothelial repair, maintenance, and angiogenesis [8]. Indeed, the EPCs can also be described through their biological properties and the time of growth in vivo culture, namely as early EPCs and late EPCs. Early EPCs are known to participate in the formation of vessels through paracrine

mechanisms, while late EPCs participate in endothelial tubulogenesis [9]. The number of EPCs is positively related to endothelial function [10] and has been used for the assessment of endothelial dysfunction [4]. Functionality and levels of EPCs are reduced in HF patients [11,12] and tend to decline with aging [13]. Indeed, reduced circulating levels of EPCs are related to increasing hazard ratios for all-cause mortality and cardiovascular death, independently of N-terminal pro B-type natriuretic peptide (NTproBNP) levels [14], and circulating levels of EPCs are considered a strong and independent predictor of mortality in HF [15,16].

The CECs are mature endothelial cells that have detached from the endothelial layer of the vessel after endothelial injury [17], being the most direct cellular marker of endothelial damage [18–20]. CECs are present at low levels in healthy subjects [21], whereas high levels are present in cardiovascular diseases [22], including HF [21,22]. Recently, CECs were proposed as a diagnostic biomarker for HF with preserved ejection fraction (HFpEF) [23], which highlights the potential of the CEC count for clinical settings.

Physical exercise can improve exercise capacity and decrease HF hospitalizations, being strongly recommended in the treatment of patients with HF [1]. One of the benefits of exercise training among patients with HF seems to be the increased mobilization of EPCs from bone marrow [24–26]. A growing number of studies have investigated the influence of isolated exercise sessions, namely maximal exercise, in the mobilization of EPCs in HF patients [27–32]. However, none considered the additional analysis of the effects of a maximal exercise bout on endothelial damage indicators. The optimization of strategies that could increase the endogenous mobilization of EPCs without inducing endothelial damage is of undeniable clinical importance. Thus, the present study aims to evaluate the impact of a maximal exercise bout on the levels of EPCs and CECs in HF patients. We hypothesized that a maximal exercise bout increases the levels of EPCs to those of age-matched individuals. Therefore, we assessed the circulating levels of EPCs and CECs in HF patients before and after a cardiopulmonary exercise test (CPET) and compared it with the resting circulating levels of age-matched adults free from cardiovascular disease.

2. Materials and Methods

2.1. Participants and Design of the Study

Thirteen patients with chronic HF were recruited from the cardiac rehabilitation program of the North Rehabilitation Center, Vila Nova de Gaia, Portugal. The eligibility was assessed by a clinician based on the following inclusion criteria: patients with HF according to the European Society of Cardiology (ESC) guidelines [1], age ≥ 45 years old; New York Heart Association (NYHA) class I–III; clinically stable. All patients were referred to the cardiac rehabilitation program after an HF diagnosis. In brief, the diagnosis was established after the assessment of clinical history, physical examination, brain natriuretic peptide evaluation, electrocardiogram, and echocardiography to confirm structural and/or functional alterations of the heart [1]. Participants with lung disease, peripheral artery disease, heart transplantation and/or exercise-limiting orthopedic disabilities were excluded. Additionally, a group of 13 age-matched adults was recruited to serve as a reference group for baseline EPC and CEC levels. The reference group was composed of adults with cardiovascular risk factors referred by their physicians for participation in a primary prevention program. The inclusion criteria were the presence of cardiovascular risk factors but free from cardiovascular disease; age ≥ 45 years old. The exclusion criteria were the same as for the HF group.

The clinical and sociodemographic data were retrieved from the clinical files and confirmed with the participants. Body weight, height, and the level of adherence to the Mediterranean Diet were assessed before the CPET. Adherence to the Mediterranean Diet was assessed with the 14-Item Mediterranean Diet Adherence Screener (MEDAS); the result of the MEDAS was obtained by summing the score (0 or 1) assigned to each question (0–14). A total score ≤ 5 indicates “weak adherence”, 6 to 9 “moderate to fair adherence,” and ≥ 10 “good or very good adherence” to the Mediterranean diet [33]. Blood collection was performed before and 30 min after the completion of the CPET to assess the number of EPCs and CECs.

All the participants provided written informed consent. The study procedures were in accordance with the Declaration of Helsinki and the study was approved by the Ethics Committee for Social and Health fields of the Santa Casa da Misericórdia do Porto (the entity that at that time was running the North Rehabilitation Center) (Ata No. 28, 28 March 2017).

2.2. Cardiopulmonary Exercise Test

Patients underwent a symptom-limited maximal CPET in the afternoon (between 2 and 4 pm), using a calibrated electronic treadmill (Bruce protocol). Patients were instructed to take all medications on the test day and were stimulated to exercise until exhaustion. Briefly, the cardiac rhythm was assessed by a 12-lead electrocardiogram throughout the CPET. Blood pressure was recorded at rest and during the CPET. Ventilation (VE), oxygen uptake (VO₂), and carbon dioxide output (VCO₂) were measured breath-by-breath using the CS- 200 gas analyzer Ergo Spiro (Schiller, Baar, Switzerland). The VO_{2 peak} (mL/kg/min) was considered the highest value reached at the end of the test, and the respiratory exchange ratio was registered for the evaluation of the level of effort. The VE/VCO₂ slope was calculated by automatic linear regression fitting the relationship between VE and VCO₂. The duration of the exercise test (min) was recorded. All the included HF patients completed the symptom-limited maximal CPET.

2.3. Quantification of Circulating Number of EPCs and CECs

Blood samples (3 mL) for cytometry analysis were collected into ethylenediaminetetraacetic acid (EDTA) tubes and treated, according to the manufacturer's instructions, with TransFix (Cytomark, Caltag Medsystems Ltd., Buckingham, UK) at a 1:5 ratio immediately after collection. Transfix can stabilize cell populations and permits blood analysis for up to seven days after blood collection [34]. Blood samples were collected in the afternoon between 2 and 4 pm (patients were not fasting), before and 30 min after the CPET, and stored in the dark at room temperature. The flow cytometry analysis (FACS-Calibur flow cytometer, Becton Dickinson, San Jose, CA, USA) was performed two to three days after the blood collection. Staining and analysis were performed using a protocol adapted from Ahmed, et al. [35], as previously reported [11]. For the quantitative assessment of circulating EPCs and CECs by flow cytometry, whole blood samples were incubated for 10 min with FcR-blocking reagent to block unwanted binding of antibodies to human Fc receptor-expressing cells. All staining procedures were executed at room temperature. Samples were incubated with BV410 CD34 (BD Horizon, BD, Franklin Lakes, NJ, USA), PE CD309 (VEGFR-2/KDR; BD Pharmingen, BD, Franklin Lakes, NJ, USA), FITC

CD144 (BD Pharmingen, BD, Franklin Lakes, NJ, USA), BV510 CD45 (BD Horizon, BD, Franklin Lakes, NJ, USA), and APC CD133/1 (Miltenyi Biotec, Cologne, Germany), according to manufacturer's instructions. After erythrocyte lysis, at least 500,000 CD45⁺ and a minimum of 100 CD34⁺ cells were acquired on a BD FACS Canto II™ system using BD FACSDiva™ version 6.1.3 software (BD Biosciences, Franklin Lakes, NJ, USA). All samples were analyzed in duplicate. Data were analyzed using Infinicyt™ (Cytognos, Salamanca, Spain). The EPCs were defined as CD45^{low}/CD34⁺/CD309⁺/CD133⁺/CD144⁻ cells [36] and the CECs as CD45^{low}/CD34⁺/CD309⁺/CD133⁻/CD144⁺ cells [7,23,37]. EPC and CEC counts were expressed as % leukocytes (CD45⁺ cells). The intra-assay variation was <5%.

2.4. Statistical Analysis

Exploratory analysis and Shapiro-Wilk tests were performed to determine the normality of the data distribution. Variables are expressed as mean $\pm$ standard deviation, mean differences with their 2-sided 95% CIs or absolute number and percentage. Paired sample T-tests were performed for the within-group comparisons (EPCs and CECs) from baseline to 30 min after the CPET. Between-group comparisons were performed using the Student's independent t-test (EPCs, CECs, and clinical characteristics), Chi-squared (χ^2) test, or Fisher's exact test (clinical characteristics). Also, the HF patients were divided into two groups according to the severity of exercise intolerance (based on the median $\text{VO}_{2\text{ peak}}$: 17.0 mL/kg/min); then, a student's independent t-test was performed to assess whether those with lower cardiorespiratory fitness show a similar EPC response to those with higher fitness. The value of significance was set at a 1-sided p value ≤ 0.05 . Data analyses were made using IBM SPSS Statistics 26 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Participants' Characteristics

HF patients and age-matched adults free from cardiovascular disease were well balanced for age (67.8 ± 9.7 vs. 65.7 ± 7.1 years old, $p = 0.52$) and body mass index (27.7 ± 3.6 vs. 29.6 ± 5.6 kg/m², $p = 0.30$) (Table 1). Some

cardiovascular risk factors were more prevalent in HF patients, including diabetes mellitus (53.8%) and hypertension (84.6%). The HF patients showed a reduced left ventricular ejection fraction (LVEF) ($37.2 \pm 12.0\%$) and $\text{VO}_{2 \text{ peak}}$ ($16.8 \pm 4.1 \text{ mL/kg/min}$; $76.2 \pm 22.9\%$ of predicted). The $\text{VE/VCO}_{2 \text{ slope}}$ was 34.6 ± 10.9 and the duration of the CPET was $9.0 \pm 1.9 \text{ min}$. All patients completed the maximal exercise bout without signs or symptoms of ischemia. At baseline, HF patients presented a significantly lower number of circulating EPCs ($p = 0.03$) but similar levels of CECs ($p = 0.08$) when compared to the age-matched group (Table 1).

3.2. Effects of Maximal Exercise in the Levels of EPCs and CECs

In HF patients, a single maximal exercise bout increased the levels of EPCs by 0.5 [95% Confidence Interval (CI), 0.07 to 0.93%], i.e., from $4.2 \times 10^{-3} \pm 1.5 \times 10^{-3} \%$ to $4.7 \times 10^{-3} \pm 1.8 \times 10^{-3} \%$ ($p = 0.02$) (Figure 1a). The circulating levels of CECs did not change in response to the exercise bout ($3.8 \times 10^{-3} \pm 1.6 \times 10^{-3} \%$ to $3.8 \times 10^{-3} \pm 1.3 \times 10^{-3} \%$, $p = 0.98$) (Figure 1b). The EPC response to the exercise bout was similar among HF patients with lower ($<17.0 \text{ mL/kg/min}$) versus higher $\text{VO}_{2 \text{ peak}}$ ($\geq 17.0 \text{ mL/kg/min}$) (0.41 ± 0.77 vs. $0.69 \pm 0.71\%$, $p = 0.53$).

When comparing the levels of EPCs of the HF patients after the maximal exercise bout with the resting value of the age-matched group, we observed that the circulating level of EPCs in HF patients was not significantly different from the level of the age-matched participants ($4.7 \times 10^{-3} \pm 1.8 \times 10^{-3} \%$ vs. $5.4 \times 10^{-3} \pm 1.7 \times 10^{-3} \%$, respectively, $p = 0.14$) (Figure 1a).

Table 1. Clinical characteristics of the participants.

	Heart Failure Group (n = 13)	Age-Matched Group (n = 13)	p
Sex (males/females)	13 (11/2)	13 (2/11)	≤0.001
Age (years)	67.8 ± 9.7	65.7 ± 7.1	0.52
Anthropometric data			
Weight (kg)	75.6 ± 11.9	73.2 ± 16.7	0.68
Height (cm)	165.1 ± 9.6	156.7 ± 10	0.03
Body mass index (kg/m ²)	27.7 ± 3.6	29.6 ± 5.6	0.30
Cardiovascular risk factors, n (%)			
Overweight	5 (38.5)	6 (46.2)	0.69
Obesity	4 (30.8)	5 (38.5)	1.00
Hypertension	11 (84.6)	7 (53.8)	0.20
Diabetes mellitus	7 (53.8)	4 (30.8)	0.23
Dyslipidaemia	8 (61.5)	8 (61.5)	1.00
Heart failure characteristics			
HFrEF, n (%)	8 (61.5)	-	-
HFmrEF, n (%)	4 (30.8)	-	-
HFpEF, n (%)	1 (7.7)	-	-
LVEF, %	37.2 ± 12.0	-	-
NYHA functional class, n (%)			
I	2 (15.4)	-	-
II	11 (84.6)	-	-
Aetiology			
Ischemic, n (%)	7 (53.8)	-	-
Dilated, n (%)	6 (46.2)	-	-
Current medication, n (%)			
Statins	10 (76.9)	8 (61.5)	0.67
Antidiabetic medication	6 (46.2)	3 (23.1)	0.41
Diuretics	9 (69.2)	5 (38.5)	0.11
Angiotensin II receptor blockers	1 (7.7)	2 (15.4)	1.00
Angiotensin converting enzyme inhibitors	10 (76.9)	3 (23.1)	0.006
Calcium channel blockers	2 (15.4)	1 (7.7)	1.00
Beta-blockers	8 (61.5)	0 (0.0)	0.002
Mediterranean Diet Adherence			
Total scoring	8 ± 2	8 ± 2	0.47
Weak adherence, n (%)	0 (0.0)	1 (7.7)	1.00
Moderate to fair adherence, n (%)	11 (84.6)	8 (61.5)	0.37
Good or very good adherence, n (%)	2 (15.4)	4 (30.8)	0.64
Circulating Cells Populations			
Endothelial progenitor cells (%)	$4.2 \times 10^{-3} \pm 1.5 \times 10^{-3}$	$5.4 \times 10^{-3} \pm 1.7 \times 10^{-3}$	0.03
Endothelial cells (%)	$3.8 \times 10^{-3} \pm 1.6 \times 10^{-3}$	$4.9 \times 10^{-3} \pm 1.9 \times 10^{-3}$	0.08

Legend: HFrEF, heart failure with reduced ejection fraction; HFmrEF, heart failure with mid-range ejection fraction; HFpEF, heart failure with preserved ejection fraction; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

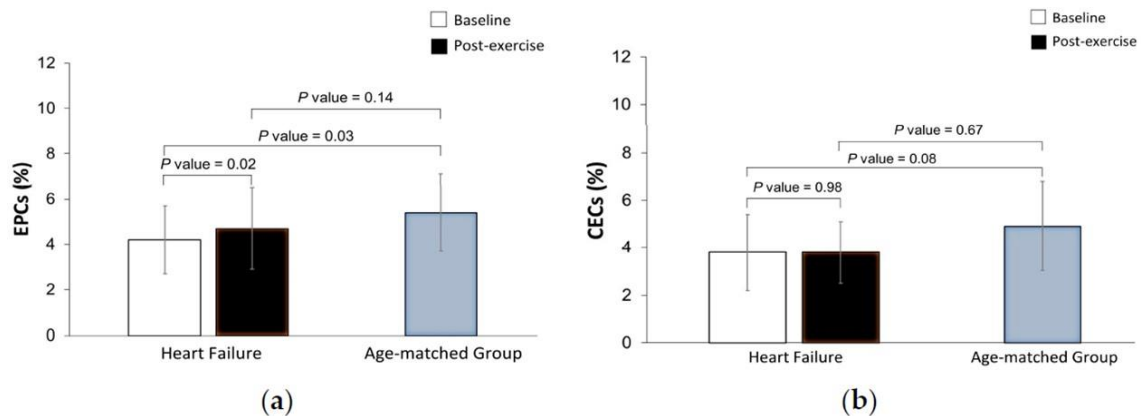


Figure 1. Effects of a maximal exercise bout on the circulating levels of endothelial progenitor cells (EPCs, Panel (a)) and circulating endothelial cells (CECs, Panel (b)) in heart failure patients, and comparison between baseline and post-exercise bout levels in the HF group with the resting circulating values of age-matched adults free from cardiovascular disease. EPCs and CECs count were expressed as % leukocytes (% of circulating cells $\times 10^{-3}$).

4. Discussion

The main findings of this study confirm our hypothesis that a maximal exercise bout increases the circulating levels of EPCs in HF patients, and that the number of EPCs in circulation after the exercise bout is similar to the baseline count of age-matched adults free from cardiovascular disease. Moreover, acute exercise improves EPC levels without increasing endothelial damage, assessed by measuring the levels of CECs.

The effects of acute exercise on EPCs are still uncertain, with previous studies reporting increased levels of EPCs after a maximal exercise test [27–29] while others describing no differences [30–32,38,39]. For instance, Kourek, et al. [27] observed an increase in the levels of EPCs after a maximal exercise bout performed on a cycle ergometer. The same authors tested the influence of HF severity on the ability to mobilize EPCs after a maximal exercise and concluded that the ability to mobilize EPCs after a maximal exercise was not affected by HF severity, regardless of the criteria ($VO_{2\text{ peak}}$, predicted $VO_{2\text{ peak}}$ or VE/VCO_2) used to determine HF severity [28]. Another study found a nearly four-fold increase in the number of EPCs [40]. Our preliminary results tend to indicate that the EPC response to a maximal exercise bout is similar irrespective of patients' $VO_{2\text{ peak}}$. On the other hand, Van Craenenbroeck, et al. [31] did not find significant changes in EPCs at several time points after a maximal exercise test (i.e., 10 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h, 48 h) in a group of patients with chronic HF. Some authors suggested that the blunted response of EPCs to exercise may be related to inflammatory factors and diminished availability of nitric oxide (NO) in response to exercise [41,42]. It must be highlighted that the literature is not consistent in the antibodies used to define or select cells that express properties attributed to EPCs, for instance, EPCs have been identified as $CD34^+/CD133^+/VEGFR_2$, $CD34^+/CD45^-/CD133^+/VEGFR_2$, and $CD34^+/CD45^-/CD133^+$ [27–29] or $CD34^+/KDR^+/CD3^-$ [30–32] or $CD34^+/KDR^+$ [38], $CD34^+/KDR^+/CD45^{\text{dim}}$ [39], and $CD133^+/CD144^+$ (or $AC133^+/VE\text{-}Cadherin^+$) cells [40]. Additionally, the different units used to count circulating EPCs (e.g., cells per event, percentage of cells within the lymphocyte or the total mononuclear cell population, or cells per volume) make comparisons between studies difficult. The exercise characteristics (e.g., intensity and duration) must be considered when evaluating

the physiological response of endothelial repair mechanisms to exercise. The intensity and type of exercise may have a bigger influence on EPC dynamics than the duration. Laufs and colleagues tested the acute effects of different durations of exercise and found that a 10-min aerobic exercise session at moderate intensity does not change the levels of EPCs, while a 30-min exercise session at moderate to vigorous intensity increases EPCs in healthy young men [43]. Moreover, a recent study tested chronic HF patients ($EF \leq 45\%$) and found that both 31 min of high-intensity interval exercise—4 min of aerobic exercise at 80% $VO_{2\text{ peak}}$ intermitted by 3 min of active recovery at moderate intensity (50% $VO_{2\text{ peak}}$)—and 53-min of continuous exercise at moderate intensity (50% $VO_{2\text{ peak}}$) (both exercise sessions were similar in total work) increased the levels of EPCs immediately and 40 min after the end of the exercise sessions [44]. In this sense, considering that in the present study, the exercise bout lasted an average of 9 min, and CPET is characterized by an effort of short duration and high intensity [45], we may assume that the intensity of exercise may be more important to the mobilization of EPCs than the duration of exercise.

Our results showed lower levels of EPCs in patients with HF at rest, which is in line with previous research [11,16,46]. Indeed, HF patients may present a down-regulation of endothelial nitric oxide synthase and increased oxidative stress that may affect the mobilization of EPCs [4,14]. These mechanisms participate in the progression of endothelial dysfunction and contribute to a lower capacity of endothelial repair and regeneration in HF [4,14].

The present study did not assess the mechanisms by which exercise would increase the number of EPCs in HF patients. However, a schematic diagram summarizing the potential pathways involved in EPCs dynamics is provided in Figure 2.

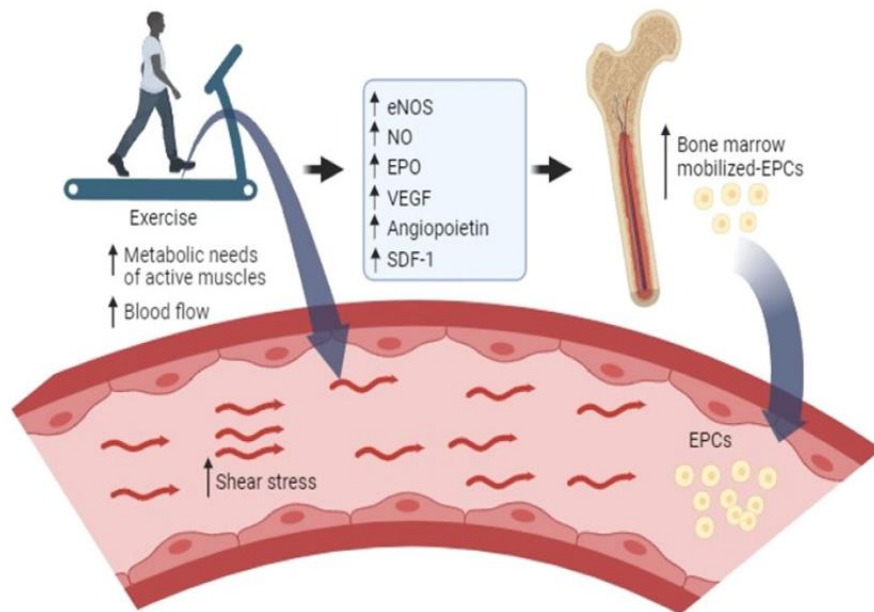


Figure 2. Summary of the potential factors involved in endothelial progenitor cell (EPC) mobilization during exercise. During exercise, the increase ($\uparrow$) in cardiac output and blood flow to active muscles increase the exercise-induced shear stress that stimulates the endothelial cells to increase the activity of endothelial nitric oxide synthase (eNOS) and produce higher amounts of nitric oxide (NO) [42]. The increase in NO [42,47,48], hypoxia-inducible factor (HIF)-1 and -2, erythropoietin (EPO) [25], and other angiogenic growth factors or chemokines, namely the vascular endothelial growth factor (VEGF), angiopoietin and stromal cell-derived factor (SDF)-1 bonding with the chemokine receptor (CXCR-4) [49,50] seem to mediate the mobilization of EPCs from bone marrow to peripheral blood [25]. The figure was designed by using the BioRender.com resource.

Regarding the levels of CECs, we did not find significant differences in response to maximal exercise in HF patients. Previous studies showed that a maximal exercise session [51] or 30 min of high-intensity interval exercise [52] did not change the CEC count in healthy young men. However, a previous study reported an increase in CEC levels immediately after a CPET, which was followed by a significant fall 30 min after exercise among coronary artery disease patients [19]. This biphasic pattern was also observed in 13 patients with effort angina (i.e., patients diagnosed during the exercise, which triggered angina pectoris or ST segment depression during the CPET) since the CEC count increased after exercise testing, followed by a decrease 4 h after the exercise test [53]. An acute exercise session [54] can lead to a biphasic acute response in endothelial function, i.e., a decrease in endothelial function followed by a significant improvement until reaching or overcoming the resting levels of endothelial function [54,55]. As recently discussed [54], acute exercise at high intensities can lead to endothelial damage, which would be reflected in decreased endothelial

function and increased circulating indicators of endothelial damage (e.g., CECs). Thus, the activation of vascular repair mechanisms (e.g., mobilization of EPCs) would increase the repair capacity of the endothelium. Therefore, the endothelial damage could be detectable only for a short period, i.e., until vascular repair mechanisms act to repair the endothelial damage [54]. Methodological differences, such as the lack of standardization of surface markers for the quantification of CECs, limit the comparisons between studies and may, at least partially, explain divergent results.

Our study presented some limitations. Firstly, we only collected blood at one time point after the CPET. Secondly, the present study lacks additional analysis of other endothelial damage markers (e.g., endothelial-derived microparticles [7] and inflammatory cytokines [41]), which could provide further information regarding the effects of maximal exercise on endothelial damage. Finally, age-matched subjects did not perform a CPET, which limits the comparison of the exercise-induced EPC response between them and HF patients. Despite these limitations, these preliminary results may provide new insights and perspectives into the effects of an acute exercise session on vascular health among HF patients. Future research with a larger sample and evaluating additional indicators of endothelial repair and vascular angiogenesis is necessary to confirm our findings.

5. Conclusions

A maximal exercise bout seems to increase the circulating levels of EPCs in HF patients without increasing endothelial damage (CEC count) in HF patients.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee for Social and Health fields of the Santa Casa da Misericórdia do Porto (the entity that at that time was in charge of the North Rehabilitation Centre) (Ata No. 28, 28 March 2017).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The identified data presented in this study will be made available for researchers who provide a methodologically sound proposal (e.g., individual participant meta-analysis).

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Endothelial progenitor cell response to a multicomponent exercise training program in adults with cardiovascular risk factors

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Abstract

Aim. To evaluate the effects of a multicomponent exercise program in the circulating levels of endothelial progenitor cells (EPCs) and endothelial damage among adults with cardiovascular risk factors. **Materials and methods.** Eleven adults with cardiovascular risk factors underwent an 18-week light-to-moderate intensity multicomponent exercise training program. Participants performed two 45 min supervised sessions per week. Blood samples were collected before and after the exercise program, and the levels of EPCs and circulating endothelial

cells (CECs) were quantified by flow cytometry. Results. The circulating levels of EPCs increased by $5.0 \pm 3.3\%$ ($5.0 \times 10^{-3} \pm 1.6 \times 10^{-3} \%$ to $10.0 \times 10^{-3} \pm 2.5 \times 10^{-3} \%$, $P < 0.001$) after the exercise training, whereas no change was observed in CEC count ($4.9 \times 10^{-3} \pm 2.6 \times 10^{-3} \%$ to $6.2 \times 10^{-3} \pm 1.5 \times 10^{-3} \%$, $P = 0.23$). Conclusions. A multicomponent exercise training program improves endothelial integrity, as reflected by the increased levels of EPCs without changes in endothelial damage. These findings provide evidence to support the use of multicomponent exercise to modulate vascular competence and improve endothelial health among adults with cardiovascular risk factors.

Keywords: preventive medicine; vascular health; aging; endothelial damage; flow cytometry

Introduction

The endothelium plays an essential role in vascular homeostasis. Mechanical damage and cardiovascular risk factors (Sabatier, Camoin-Jau, Anfosso, Sampol, & Dignat-George, 2009), including aging (Seals, Jablonski, & Donato, 2011), can affect regulatory functions of the endothelium and promote pro-inflammatory activation, senescence, and apoptosis of endothelial cells (Sabatier et al., 2009). Endothelial dysfunction is a hallmark of several cardiovascular diseases (CVD) and a predictor of cardiovascular events (Godo & Shimokawa, 2017).

The levels of endothelial progenitor cells (EPCs) and circulating endothelial cells (CECs) in peripheral blood are used to estimate the vascular competence, i.e., an endothelial phenotype characterized by homeostasis between endogenous repair capacity and endothelial damage (Sabatier et al., 2009). Reduced levels of EPCs and higher levels of CECs suggest a decreased endothelial integrity (Boos, Lip, & Blann, 2006; Farinacci et al., 2018; Sabatier et al., 2009). Traditionally, EPCs are thought to be bone marrow-resident cells that can be mobilized towards peripheral blood and attracted to sites of endothelium injury (Guo, Ledesma, Peng, Liu, & Xu, 2017; Saltzman et al., 2010; Wang, Lee, Lien, & Weng, 2014). Recent studies challenged this view (Medina et al., 2017; Salybekov, Kobayashi, & Asahara, 2022) as there is evidence showing that EPCs in circulation may not be originate from the bone marrow and that tissue-resident

EPCs promote neovascularization without a direct contribution from bone marrow cells (Fujisawa et al., 2019; Li, 2010; Li et al., 2019). Once there, EPCs play a paramount role in repairing/maintaining vascular integrity (Guo et al., 2017). The EPCs can migrate and differentiate into mature endothelial cells or by releasing paracrine factors for pro-angiogenic cytokines such as vascular endothelial growth factor (Guo et al., 2017; Yan, Liu, Ding, & Zhang, 2022), and participating in angiogenesis (Laurenzana et al., 2015). Low levels of EPCs have been observed in patients with CVD (Lopes et al., 2022; Vasa et al., 2001), relate to endothelial dysfunction (Werner et al., 2007) and the incidence of cardiovascular events (Schmidt-Lucke et al., 2005). Thus, the level of EPCs is considered a predictor of CVD and a potential target for preventive and personalized medicine (Kou et al., 2020; Ribeiro et al., 2013; Schmidt-Lucke et al., 2005). Since the circulating level of EPCs tends to decrease with aging (Ross, 2018), the development of preventive resources to increase these angiogenic cells are paramount to delay the deleterious effects of aging on vascular integrity.

The CECs are released to peripheral blood after pathological insult or endothelial injury (Boos et al., 2006), being considered an index of endothelial damage and an independent predictor of endothelial function (Chong et al., 2004). Contrary to EPCs, the levels of CECs are increased in patients with CVD (Chong et al., 2004; Farinacci et al., 2018). Clinically, CECs count is an independent predictor of death and a major cardiovascular endpoint at 30 days and 1 year in acute coronary syndrome (Lee, Lip, Tayebjee, Foster, & Blann, 2005), and a diagnostic biomarker for diabetic nephropathy and heart failure with preserved ejection fraction (Farinacci et al., 2018).

Exercise training is widely recommended to improve/maintain physical fitness and to prevent/treat CVD (Bull et al., 2020; Pelliccia et al., 2021), being multicomponent training programs (composed of strength, cardiorespiratory endurance, flexibility, and balance training) strongly indicated for older adults (Bull et al., 2020). Aerobic (Landers-Ramos et al., 2016; Van Craenenbroeck et al., 2010) and combined (i.e., aerobic combined with resistance exercise) (Choi et al., 2014; Van Craenenbroeck et al., 2010) exercise training can increase the circulating levels of EPCs in healthy (Choi et al., 2014; Landers-Ramos et al., 2016) and in patients with CVD (Ribeiro et al., 2013). There is mounting evidence showing multicomponent training programs improve functional capacity, health-

related quality of life, and balance, and reduce the falls rate (Li et al., 2022); however, scarce data exist regarding the effect of multicomponent exercise training delivered in a community setting on the estimation of vascular competence in adults with cardiovascular risk factors. Hence, this study aims to determine the effect of a multicomponent exercise training program on the circulating levels of EPCs and CECs in adults with cardiovascular risk factors.

Methods

Study design and participants

Eleven adults with cardiovascular risk factors registered in a primary health care center volunteered to participate in the exercise training program. Inclusion criteria: age ≥ 50 years old; men and women with at least one cardiovascular risk factor (e.g., diabetes mellitus, hypertension, dyslipidemia). The exclusion criteria were physical restrictions that limited regular physical efforts, medical history of cancer, or diseases of the hematopoietic system. Clinical data, medication, and blood collection were performed at baseline and at the end of the 18-week multicomponent exercise training program. The medication regimen, including the dosage, remained unchanged during the study. The present study was approved by the local ethics committee (ARS Centro Ref. 174211). All participants provided informed consent.

Procedures

Clinical data and medication were retrieved from clinical files and confirmed with the participant. Height and weight were measured with participants in light clothing and no shoes, using a standard stadiometer and a calibrated scale, respectively. Waist circumference was measured in the horizontal plane at the midpoint between the superior iliac crest and the lower margin of the last rib while the participants stand in an erect position with arms at the sides and feet close together. Cardiorespiratory fitness was assessed with the Chester Step Test, a submaximal test (to estimate maximum oxygen uptake, $\text{VO}_{2\text{ max}}$), validated for this population (Izquierdo et al., 2019), at the beginning of the study. The Test was performed according to test manual recommendations with a 15 cm step (Sykes, 2005); in brief, the test consists of five stages, each with a 2 min duration. The test stopped if the participant exceeded 80% of the

age-estimated maximal heart rate, reached a value above 14 on the Borg Perceived Exertion 6 to 20 scale or was unable to maintain the metronome-set pace.

Blood samples were collected at baseline and after the exercise program to determine the circulation levels of EPCs and CECs. The blood collections were performed at the same time of the day (at 6:00 pm) in a nonfasting state. After the exercise program, blood collection was performed 2 days after the last exercise session to avoid the acute effects of exercise. A total of 3 mL of blood was collected from the antecubital vein into ethylenediaminetetraacetic acid (EDTA) tubes and treated, according to the manufacturer's instructions, with TransFix (Cytomark, Caltag Medsystems Ltd, Buckingham, UK) at a 1:5 ratio immediately after collection for cytometry analysis. The TransFix promotes the stabilization of cell populations and allows the blood sample evaluation for up to 7 days after blood collection (Hoymans et al., 2012). Blood samples were stored in the dark at room temperature until flow cytometry analysis (2–4 days after collection). Staining and analysis were performed as previously described (Ahmed et al., 2016). For the flow cytometry quantitative assessment of circulating EPCs and CECs, whole blood samples were incubated for ten mins with FcR-blocking reagent to block unwanted binding of antibodies to human Fc receptor-expressing cells. All staining procedures were executed at room temperature. Samples were incubated with BV410 CD34 (BD Horizon), PE CD309 (VEGFR-2/KDR; BD Pharmingen), FITC CD144 (BD Pharmingen), BV510 CD45 (BD Horizon), and APC CD133/1 (Miltenyi Biotec), anti-CD3-APCCy7 (BD), according to manufacturer's instructions. After erythrocyte lysis, at least 500,000 CD45⁺ and a minimum of 100 CD34⁺ cells were acquired on a BD FACS Canto II™ system (BD Biosciences, Franklin Lakes, NJ, USA) using BD FACSDiva™ version 6.1.3 software. All samples were analyzed in duplicate. The average of the two readings was considered for analysis. Data were analyzed using Infinicyt™ (Cytognos, Salamanca, Spain). The EPCs were defined as CD45^{low}/CD34⁺/CD309⁺/CD133⁺/CD144⁻ cells (Schmidt-Lucke et al., 2010) and the CECs as CD45^{low}/CD34⁺/CD309⁺/CD133⁻/CD144⁺ (Farinacci et al., 2018; Kraan et al., 2012; Sabatier et al., 2009). EPCs and CECs count were expressed as a percentage of leukocytes (CD45⁺ cells). The within-day

coefficient of variation of EPCs quantification was < 5%. Blind analyses were used for all the quantification procedures.

Multicomponent exercise training program

The supervised exercise training program lasted 18 weeks (36 exercise sessions), with a weekly frequency of two sessions (60 min each session), and consisted of performing multicomponent exercise (i.e., the combination of aerobic, muscle strengthening, flexibility, and balance exercise) (Bull et al., 2020). The exercise sessions were preceded with approximately 5 min warm-up (i.e., active stretching and walking at a light intensity) and ended with approximately 5 min cool down (i.e., walking at a light intensity and static stretching). The main phase of each exercise session (45 min) comprised aerobic exercises (brisk walking, aerobic playful at a light-moderate intensity [11 to 14 on the Borg Scale], lasting 20 min), and resistance exercises (2–3 sets of 5–7 exercises for upper and lower body, e.g., bodyweight squat, hip flexion, plantar flexion, push-ups against wall, shoulders flexion/ abduction, triceps extension, and bicep curls, lasting 15–20 min). Body weight and external loads, such as dumbbells and elastics bands, were used to adjust the exercise intensity. Each resistance exercise set comprised 10–12 repetitions interspersed with approximately 30 s to 1 min active rest. In addition, the main phase comprised alternating flexibility exercises (mobility exercises and active stretching) and balance exercises (playful activities based on the development of the intrinsic and deep musculature to restore/develop the corporal balance, as well as reaction speed exercises, composed by alternated fast-changing in walking direction, lasting 5–10 min).

Statistical analysis

The SPSS Statistics 25 software (IBM, Armonk, NY, USA) was used for the statistical analysis. Data are presented as mean $\pm$ standard deviation (SD) and descriptive statistics are presented. Based on the distribution of variance and homogeneity, the paired sample t-test was used to compare the circulating levels of EPCs and CECs. The *p*-value was set at < 0.05.

Results

Participants

The characteristics of the participants are summarized in Table 1. The participants were 65.8 ± 8.6 years old and almost half of them presented arterial hypertension (45.5%) and dyslipidemia (45.5%). All the participants attended more than 80% of the exercise sessions.

Table 1 Participant characteristics at baseline	
	Mean $\pm$ SD or (n, %)
Age (years)	65.8 ± 8.6
Sex (male/female)	3/8
Weight (kg)	65.8 ± 12.6
Height (cm)	158.0 ± 8.6
Waist circumference (cm)	93.6 ± 13.1
Body mass index (kg/m^2)	26.2 ± 3.9
Systolic blood pressure (mm Hg)	129.0 ± 18.5
Diastolic blood pressure (mm Hg)	77.3 ± 10.9
Heart rate (bpm)	68.5 ± 8.4
Maximal oxygen uptake ($\text{ml O}_2/\text{kg}/\text{min}$)	30.0 ± 6.3
<i>Cardiovascular risk factors</i>	
Obesity class I	3 (27.3)
Preobesity/overweight	3 (27.3)
Hypertension	5 (45.5)
Diabetes mellitus	2 (18.2)
Dyslipidemia	5 (45.5)
Smoking	1 (9.1)
<i>Medication</i>	
Statins	3 (27.3)
Diuretics	2 (18.2)
Angiotensin II receptor blockers	3 (27.3)
Acetylsalicylic acid	1 (9.1)
Calcium channel blockers	2 (18.2)
<i>SD standard deviation, bpm beats per minute</i>	

Number of circulating endothelial markers after the exercise training program

After the exercise training program, the levels of EPCs increased by $5.0 \pm 3.3\%$ ($P < 0.001$; Fig. 1a). No alterations were observed in the levels of CECs ($P = 0.23$; Fig. 1b).

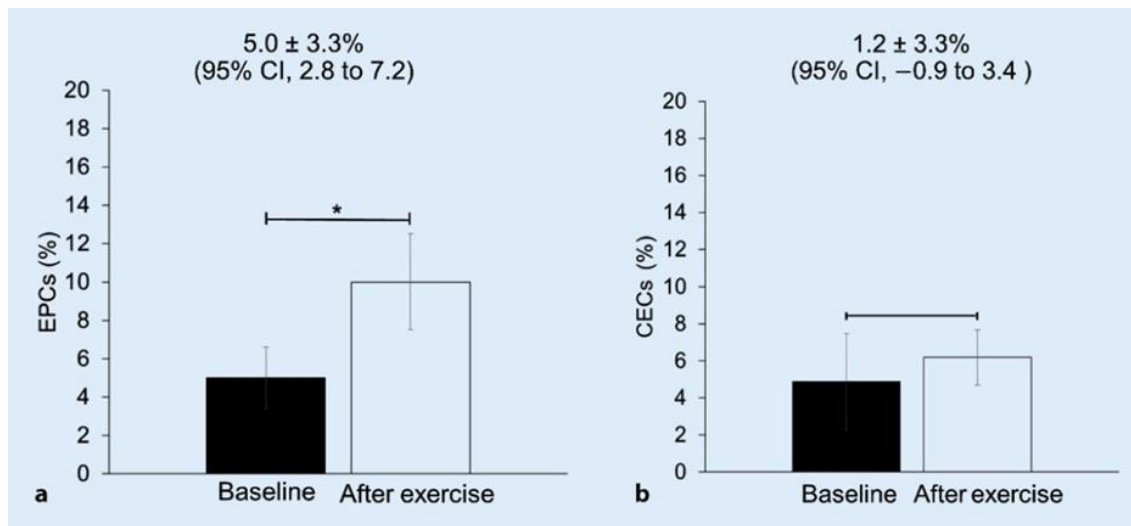


Fig. 1 Levels of circulating endothelial progenitor cells (EPCs; a) and endothelial cells (CECs; b). The evaluations before and after multicomponent exercise training are shown (x-axis) and the means of EPCs and CECs are expressed in the number of cells (%) $\times 10^{-3}$ (y-axis)

Discussion

The present study examined the effects of 18 weeks of multicomponent exercise in the circulating levels of EPCs and CECs. Exercise training increased the circulating levels of EPCs, whereas no change was observed in the levels of CECs. This study provides preliminary, new evidence regarding the role of multicomponent exercise training in vascular health in a primary care setting among adults with cardiovascular risk factors. The beneficial effects of higher levels of EPCs and lower levels of CECs on vascular health were previously described (Boos et al., 2006; Kou et al., 2020). Our findings may represent a new clue by which multicomponent exercise improves vascular competence and prevent CVDs. In this line, multicomponent exercise may represent an additional tool to be used in preventive medicine in this specific population.

The increase in the levels of EPCs observed in our study is in agreement with previous studies. In overweight and obese adults, 12 weeks of aerobic exercise (walking briskly/moderate running) at an intensity below or equal to the individual anaerobic threshold increased the levels of EPCs, but only in those subjects compliant with the exercise program (Cesari et al., 2012). Similarly, the levels of EPCs increased after 12 weeks of exercise training in older men (Yang et al., 2013). Herein, the authors suggested that the increased levels of EPCs following regular exercise training can attenuate the age-related decline in arterial elasticity (Yang et al., 2013). Even a shorter period of 10 days of aerobic exercise

on a treadmill daily performed at 65–75% heart rate reserve increased 104% the levels of CD34⁺/KDR⁺ circulating angiogenic cells (also identified as EPCs) (Cesari et al., 2012) in adults (Landers-Ramos et al., 2016).

Although the mechanisms of mobilization of EPCs towards peripheral blood by induced exercise are not yet clear in the literature, it has been suggested that exercise training may increase the number of EPCs by inducing shear stress, which leads to enhance in the bioavailability of nitric oxide, and hypoxia stimulus, which upregulates the hypoxia-inducible factor 1 and growth factors (Ribeiro et al., 2013). Indeed, a previous study (Ribeiro et al., 2017) found that the levels of hypoxia-inducible factor 1 α and EPCs increased in healthy young women after a single bout of resistance exercise. Exercise training may also decrease the concentration of inflammatory mediators, namely C-reactive protein (Fernandez-Rodriguez et al., 2022), regardless of age and sex (Fedewa, Hathaway, & Ward-Ritacco, 2017) and by this mean improve the circulating levels of EPCs. Adults with cardiovascular risk factors show an increased low-grade vascular wall inflammation (Libby, Ridker, & Maseri, 2002; Poredos, Poredos, & Gregoric, 2021). The increase of inflammatory mediators, namely C-reactive protein, can affect the bioavailability of nitric oxide (Singh, Devaraj, Vasquez-Vivar, & Jialal, 2007) and may decrease the circulating levels of EPCs (Aicher et al., 2003; Heinisch et al., 2022). Therefore, the improvement of the inflammatory profile in response to an exercise training intervention may also be a potential mechanism by which exercise improves endothelial integrity.

Different modes of exercise can induce distinct blood flow and shear rate (Thijssen et al., 2009a), and lead to specific vascular responses (Thijssen, Dawson, Tinken, Cable, & Green, 2009b). Although the present study showed an increase in the circulating level of EPCs after exercise training as in previous studies, scarce data exist on the effects of multicomponent exercise among adults with cardiovascular risk factors. More data are needed regarding the effects of different exercise modes, namely multicomponent exercise, on the pattern of shear stress and its influence on vascular/endothelial responses in releasing proangiogenic cells, namely EPCs in adults with cardiovascular risk factors.

In this study, exercise training did not change the levels of CECs. In accordance, 13 sessions of high-intensity intervals followed by 15 min of passive

recovery in a sitting position, did not change the levels of CECs in young men (Magalhaes et al., 2020). The shear stress stimulus elicited by exercise training seems to be enough to increase the levels of EPCs, without promoting endothelial injury, as evidenced by no change in the levels of CECs. The development of optimal personalized exercise training considering the specific needs of each age population in the prevention of CVD is widely indicated (Bull et al., 2020). Thus, an exercise training program capable of increasing the number of reparative/pro-angiogenic cells with no increase in endothelial damage is paramount for optimizing the beneficial effects of exercise training in adults with cardiovascular risk factors.

The present study presents some limitations. The absence of a control group and the small sample size limits the generalizability of the results. Also, the assessment of other pro-angiogenic factors (e.g., angiogenic T cells [Hur et al., 2007], VEGF [Mallick, Gurzeler, Toivanen, Nieminen, & Yla-Herttuala, 2022; Volaklis, Tokmakidis, & Halle, 2013] and SDF-1 α [Cun et al., 2021; Volaklis et al., 2013]) and indicators of vascular dysfunction (e.g., endothelial-derived microparticles [Sabatier et al., 2009] and inflammatory cytokines [Godo & Shimokawa, 2017]) could have provided further information on the response to multicomponent exercise training. Finally, the present study used the rating of perceived exertion to monitor exercise intensity during the exercise sessions. Future studies assessing cardiorespiratory fitness capacity (e.g., cardiopulmonary exercise testing) and dynamic muscular strength (e.g., 1-repetition maximum [1-RM]), at baseline and after the intervention, and providing an objective monitorization of the exercise load are clearly necessary among this population. Further randomized controlled studies are needed to confirm the positive effects of multicomponent exercise training on the levels of circulating indicators that modulate vascular competence in adults with cardiovascular risk factors.

Conclusions

Multicomponent exercise training improves the circulating levels of proangiogenic cells (endothelial progenitor cells, EPCs) without increasing the levels of circulating endothelial cells (CECs)—a marker of endothelial damage. Thus, multicomponent exercise training seems to be an efficient tool to modulate

vascular competence and improve endothelial health among adults with cardiovascular risk factors.

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Author Contribution. All authors contributed to the study conception and design. The conceptualization was developed by Suiane Cavalcante and Fernando Ribeiro; Data collection, Suiane Cavalcante, Manuel Teixeira, Marisol Gouveia; Data analysis, Suiane Cavalcante; Resources, Ana Duarte, Miriam Ferreira, Maria I. Simões, Maria Conceição, Mariana Costa, Ilda P. Ribeiro, Ana

Cristina Gonçalves, José Oliveira and Fernando Ribeiro; Writing (original draft preparation), Suiane Cavalcante; Writing (review and editing), Manuel Teixeira, Marisol Gouveia, Ana Duarte, Miriam Ferreira, Maria I. Simões, Maria Conceição, Mariana Costa, Ilda P. Ribeiro, Ana Cristina Gonçalves, José Oliveira and Fernando Ribeiro. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declarations

Conflict of interest. S. Cavalcante, M. Teixeira, M. Gouveia, A. Duarte, M. Ferreira, M.I. Simões, M. Conceição, M. Costa, I.P. Ribeiro, A.C. Gonçalves, J. Oliveira and F. Ribeiro declare that they have no competing interests. The present study was approved by the local ethics committee (ARS Centro Ref. 174211) and was conducted under ethical standards. All participants provided informed consent.

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Chapter IV

General Discussion

This thesis aimed to analyse the acute and chronic effects of exercise on the levels of EPCs (as an indicator of endothelial repair) and CECs (as an indicator of endothelial damage) to describe the potential vascular repair capacity of adults with CVRFs and patients with CVDs, specifically HF patients.

Acute Effects Of Exercise On Potential Vascular Repair Capacity

The studies 1 and 2 demonstrated that single exercise sessions can enhance vascular repair capacity by augmenting the levels of EPCs without elevating endothelial damage in adults with CVRFs and patients with HF.

Based on the results, 45 min of multicomponent exercise did not alter the levels of EPCs, while 30 min of multicomponent exercise and approximately 9 min of CPET led to an increase in EPC levels. It could be suggested that the duration of single exercise sessions may not exhibit a dose-dependent relationship concerning exercise-induced EPCs, as previously proposed for intensity (Ribeiro et al., 2017). Therefore, a complex relationship between exercise duration and intensity may elucidate our results, irrespective of the exercise mode (i.e., multicomponent, or aerobic exercise). Although it may seem intuitive to draw this conclusion, we emphasize that generalizing these results to clinical practice based solely on the exercise session duration is significantly limited. We emphasize that Original Study 1 examined patients with CVRFs, while Original Study 2 investigated patients with HF using different modes (multicomponent and aerobic maximal exercise, respectively) at different intensities (light-moderate intensity vs. graduated intensity until reaching maximal effort – CPET, respectively). Indeed, exercise modality (Ferentinos et al., 2022), intensity (Ribeiro et al., 2017), and the presence of established CVD (Kaźmierski et al., 2015) may influence the capacity to mobilize EPCs in adults and should be considered in future studies.

A recent meta-analysis suggested that the cutoff point for exercise session duration is < 30 min at moderate to high intensities ($\geq 65\% \text{VO}_{2 \text{ max}}$) and ≥ 30 min at mild to very high intensities ($< 65\% \text{VO}_{2 \text{ max}}$ or $\geq 65\% \text{VO}_{2 \text{ max}}$) for effective upregulation of exercise-induced EPCs, irrespective of the mode (Mitsiou et al., 2022). The data from study 1 aligns with the findings regarding the 30-minute exercise session but not with the 45-minute exercise session. Indeed, the aforementioned cutoff point was based on studies that examined aerobic,

resistance, or combined exercises (i.e., aerobic combined with resistance exercises), which limits the extension of this cutoff to multicomponent exercises (i.e., strength, cardiorespiratory endurance, flexibility, and balance exercises). Indeed, the principles of exercise training (frequency, intensity, time, type – FITT) are utilized to structure and adapt exercise programs based on the vascular responses exhibited by individuals (Königstein et al., 2023), suggesting a significant influence of exercise characteristics on vascular adaptations.

Specifically concerning the mobilization of EPCs under different exercise durations (e.g., 30 min and 45 min), multicomponent exercise may influence the endothelium in specific patterns and result in variations in EPC levels after single exercise sessions, which were not discerned due to blood collection at a single time point. This may explain the lack of alterations in the levels of EPCs after 45 min of multicomponent exercise. Indeed, previous studies have found fluctuations in the levels of EPCs after single exercise (Chang et al., 2015; Kaźmierski et al., 2015). We emphasize that this hypothesis must be confirmed with further research by collecting blood samples at different time points after exercise to identify a possible delay in the mobilization of EPCs after longer durations (e.g., 45 min) of multicomponent exercises. In terms of clinical practice, based on these data, we can suggest that a multicomponent exercise session, particularly a 30-minute exercise session, is an effective alternative for an acute increase in vascular repair capacity by increasing the circulating levels of EPCs in adults with CVRFs. Indeed, within the context of Predictive, Preventive, Personalized Medicine (PPPM), the fluctuation in circulating EPC levels can be suggested as a potential indicator to predict and prevent the development of CVDs (Golubnitschaja & Costigliola, 2012; Kou et al., 2020). In this context, multicomponent exercise can serve as an ally in PPPM by increasing the levels of EPCs, particularly regarding the acute effects of a 30-minute exercise session.

Furthermore, the absence of exacerbation in endothelial damage (evaluated by CEC levels) after 30-minute and 45-minute sessions may be considered a positive aspect, particularly for this population. Although it was not an objective of the present thesis, these patients may potentially benefit from the acute effects of exercise through other vascular adaptations. For instance, mechanisms related to neural, metabolic, and humoral factors, both in microvascular and macrovascular circulation, could contribute to improvements

in arterial structure and function (Königstein et al., 2023). The potential to sustain beneficial vascular adaptations without exacerbating endothelial damage appears to be advantageous, particularly for this population. Further studies are warranted to investigate acute vascular adaptations resulting from 30- and 45-minute sessions of multicomponent exercise in adults with CVRFs to substantiate this hypothesis.

Data from original study 2 suggests that maximal exercise (i.e., CPET) enhances EPCs levels, albeit possibly transiently, with no exacerbation of endothelial damage in patients with HF. This may offer insights into the ramifications of high-intensity exercise regimens on vascular responses. In fact, the adoption of HIIT has recently introduced as a "novel prehabilitation concept" aimed at augmenting EPCs to reduce postoperative complications among patients undergoing incontinence surgery with abdominal laparotomy (Bauer et al., 2022). According to previous guidelines, HIIT can be included between 1 and 3 days per week in the training routine of patients with HF, typically alternating with MICON training (Ambrosetti et al., 2021; Keteyian et al., 2014; Squires et al., 2018; Taylor et al., 2023). In patients with HF, the exercise prescription should be tailored to attain health goals aligned with the patient's clinical profile and physical fitness, ensuring patient safety and efficacy.

Indeed, the significance of a single exercise session (acute effects of exercise) appears to extend well beyond its immediate impact, suggesting potential long-term, cumulative effects within an exercise training program (chronic effects of exercise). Additional information regarding the acute effects of CPET could prove beneficial in clinical practice. For instance, a diminished ability to mobilize circulating EPCs after CPET served as a predictive factor for post-operative complications, including cardiac events, dysrhythmias, and congestive HF, in patients undergoing major thoracic surgery (Schier et al., 2014).

We emphasize that the original study 2 included patients with HFpEF, HFmrEF and HFrEF. Although exercise intolerance is a common symptom in both HFpEF and HFrEF patients (Taylor et al., 2023), their pathophysiology is distinct from each other (Tsigkou et al., 2023). The differences have been attributed to the presence of CVRFs that lead to a state of systemic inflammation, which is associated with an increase in coronary microvascular dysfunction in patients with HFpEF (Cornuault et al., 2022; Tsigkou et al., 2023). Differences in risk

factors and phenotypic presentations among categories of HF may result in distinct mechanisms and molecular expressions (Lee et al., 2009; Tsigkou et al., 2023). Thus, although the effects of a single maximal exercise have yielded controversial results in patients with HF, irrespective of HF phenotyping (Gevaert et al., 2019; Kourek et al., 2020; Van Craenenbroeck et al., 2011), we cannot disregard the possibility of the influence of different vascular health states related to HF phenotype on the capacity for mobilizing EPCs to peripheral blood. Consequently, the generalization of these findings to the entire HF population is restricted, and future studies should account for specific HF phenotypes.

Additional data (not presented in the published versions of original studies 1 and 2) demonstrated an increase in vascular repair potential after the single exercise session through the EPCs/CECs ratio following the 30-minute exercise session (1.6 ± 0.4 % to 2.9 ± 1.6 %, $P = 0.009$), while no change (1.3 ± 0.4 % to 1.1 ± 4.1 %, $P = 0.20$) was observed after the 45-minute exercise session in individuals with CVRFS. The EPCs/CECs ratio did not change after a single maximal exercise session in patients with HF (1.1 ± 0.3 % to 1.2 ± 0.5 %, $P = 0.50$). Indeed, contrasting to 45-minute exercise session and a maximal exercise session, the reduction in the levels of CECs after the 30-minute session of multicomponent exercise has resulted in a higher ratio of EPCs/CECs.

In general, the original studies examining the acute effects of exercise (studies 1 and 2) suggested that single exercise sessions might upregulate exercise-induced EPCs without exacerbating endothelial damage in adults with CVRFS and patients with HF. However, given the disparity in protocols and sample populations tested, these findings cannot be extrapolated to the broader population of patients undergoing primary and secondary prevention of CVDs. For practical applications to be effective, the attributes of exercise (e.g., mode, duration, and intensity) and the presence of CVDs should be regarded as potential variables that could impact the mobilization capacity of EPCs. Moreover, methodological considerations, such as the timing of blood collection post-single exercise session, are recommended to mitigate the risk of false-negative outcomes and to elucidate the influence of various characteristics of single exercise sessions (e.g., differing durations) on the fluctuation of EPCs and CECs following single exercise stimuli.

Chronic effects of exercise on Potential Vascular Repair Capacity

The original study 3 demonstrated that 18 weeks of multicomponent exercise increased the levels of EPCs without altering the levels of CECs in individuals with CVRFs. Consistent with our findings, a recent meta-analysis (Mitsiou et al., 2022) observed beneficial effects of structured exercise training in the circulating EPC levels in healthy adults. However, the meta-analysis only included studies investigating the impact of aerobic, resistance, and combined exercise programs. There were no studies on multicomponent exercise. Multicomponent exercise has been shown to enhance muscle strength, walking efficiency, and aerobic capacity in older adults (Labata-Lezaun et al., 2023). The upregulation of EPC in response to physical activity may enhance vascular health through endothelial repair and angiogenesis processes, resulting in improved blood distribution, perfusion within exercised muscles and improvements in cardiovascular health (Asahara et al., 1997; Ross et al., 2023; Volaklis et al., 2013; Yan et al., 2022). Consequently, the increase in EPC levels likely serves as a supporting mechanism contributing to the beneficial effects of multicomponent exercise on bodily functionality and aerobic capacity in older adults.

Chronic vascular adaptations appear to depend on various factors, including the individual's fitness level and the type of muscle fiber (Königstein et al., 2023). In fact, physically active individuals exhibit higher vascular density compared to their age-matched non-exercising peers (Brodal et al., 1977; Königstein et al., 2023). The rise in capillary density among physically active individuals may be attributed to the heightened mobilization of EPCs in response to exercise training (Mitsiou et al., 2022; Volaklis et al., 2013). While the significant role of EPCs in vessel formation has been questioned owing to their diminished concentration in peripheral circulation (Ross et al., 2023), an extensive body of literature has underscored the potential of EPCs as a resource for enhancing vascular repair and angiogenesis. This potential could contribute to improvements in perfusion and blood flow in clinical settings (Asahara et al., 1997; Khakoo & Finkel, 2005; Salybekov et al., 2022; Schier et al., 2014; Yan et al., 2022).

Indeed, the duration and intensity of exercise have been suggested to significantly influence the mobilization capacity of EPCs (Mitsiou et al., 2022) and

vascular processes associated with vessel formation (Ross et al., 2023). Moreover, molecular mechanisms leading to the enhanced mobilization capacity of EPCs (Volaklis et al., 2013), such as the upregulation of nitric oxide and VEGF, are also linked to increased angiogenesis processes and vascular density (Ross et al., 2023). These associations imply a comprehensive enhancement in the vasculature, encompassing both repair capacity and vessel formation in response to exercise training. Hence, increased attention directed towards determining the optimal training duration and intensity may have a positive impact not only on vascular repair capacity but also on the development of the vascular system. This includes enhancing cardiovascular function and exercise capacity, as indicated by improved oxidative capacity and increased $\text{VO}_{2\text{ max}}$ (Ross et al., 2023). Therefore, all these factors must be taken into consideration when aiming for the effectiveness of preventive measures in CVD prevention.

Original Study 3 found no change in the EPCs/CECs ratio after an 18-week light-to-moderate intensity multicomponent exercise training program among this population ($1.3 \pm 1.0\%$ to $1.6 \pm 0.4\%$, $P = 0.18$; data not shown in the published version). We emphasize the need for prospective studies to evaluate the impact of the EPCs/CECs ratio in predicting clinical outcomes in future research. Furthermore, the impact of single exercise sessions and exercise training on the potential for vascular repair through the use of the EPCs/CECs ratio warrants further investigation.

In general, our data pave the way for new research aimed at a more comprehensive analysis of the effects of single exercise (acute effects) and its implications for an exercise training program (chronic effects). The acute adaptive responses of endothelial function following a single exercise session were significantly associated with the adaptive responses of endothelial function after a 2-week exercise training period in healthy young men (Dawson et al., 2018). The identification of potential predictive capabilities stemming from the acute response of single exercise on the levels of EPCs and CECs would bolster the development of an optimal exercise training regimen to enhance specific vascular responses. Such advancements could carry significant prognostic implications within the realm of PPPM. Clearly, further research in this domain is warranted.

Limitations

This thesis has some limitations that warrant attention:

i) The original studies 1 and 2 examined populations with varying clinical profiles (CVRFs and patients with HF), which could influence the ability to mobilize EPCs and release CECs in response to acute exercise to different extents. Hence, the findings regarding the acute effects of exercise must be interpreted within the context of each specific sample tested; ii) The original study 2 included patients with HFpEF, HFmrEF, and HFrEF. We cannot discount the possibility of different responses to a CPET based on the HF phenotype. However, this hypothesis requires confirmation through future studies; iii) In the original study 3, the absence of a control group limits the assessment of the effects of exercise training on the mobilization of EPCs and CECs levels in adults with CVRFs; iv) The singular time-point blood collection constrained the assessment of potential fluctuations in EPCs and CECs following the exercise interventions in all three original studies, thereby increasing the risk of false-negative results; v) Since we did not discard the initial blood collection, we cannot dismiss the possibility of a potential overestimation of CECs levels attributed to endothelial damage resulting from needle injection during all blood collections; vi) The original studies in this thesis involved a small number of participants, which restricts the robustness of statistical analysis and may introduce bias into the results.

Despite the limitations of the present thesis, these data represent initial steps for future studies with larger samples aimed at elucidating the effects of exercise on vascular repair potential in adults with CVRFs and patients with CVDs. Along these lines, in addition to the recognized benefits of exercise on cardiovascular health, identifying the effects of acute and chronic exercise based on its characteristics on enhancing vascular repair potential may play a crucial role in the development of optimal personalized exercise prescriptions for CVD prevention.

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Chapter V

Conclusions and Future Perspectives

Conclusions

In conclusion, both single exercise sessions and an exercise training program showed potential to increase the levels of EPCs without significantly altering the level of endothelial damage, thereby increasing the potential for vascular repair in individuals with CVRFs and patients with HF. However, it is important to note that the exercise session duration may influence the mobilization of EPCs following an acute bout of exercise.

Future Perspectives

Future studies are suggested to address the following aspects:

- I. Testing larger sample sizes to enhance statistical power regarding the acute and chronic effects of exercise on the circulating levels of EPCs and CECs in adults with CVRFs and patients with HF.
- II. Discriminating and analyzing patients with HF based on their phenotype.
- III. In terms of the chronic effects of multicomponent exercise in primary prevention of CVDs, including a control group that abstains from participating in an exercise training program would enable a more precise analysis of the impacts of multicomponent exercise training on vascular competence in adults with CVRFs.
- IV. Conducting blood collection over time following single exercise sessions and exercise training may offer further insights into possible delayed patterns in circulating EPCs and CECs and also help mitigate the risk of false-negative results.
- V. Analyzing the mechanisms related to the mobilization of EPCs [e.g., VEGF, stromal cell-derived factor-1 α (SDF-1 α), and erythropoietin (EPO)] would provide more insights into the impact of acute and chronic exercise on vascular repair capacity.