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REVIEW
Epidemiology and prevention

Endothelial progenitor cell mobilization based on exercise volume in patients with cardiovascular disease and healthy individuals: a systematic review and meta-analysis

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Endothelial progenitor cells (EPCs) play a vital role in protecting endothelial dysfunction and cardiovascular disease (CVD). Physical exercise stimulates the mobilization of EPCs, and along with vascular endothelial growth factor (VEGF), promotes EPC differentiation, and contributes to vasculogenesis. The present meta-analysis examines the exercise-induced EPC mobilization and has an impact on VEGF in patients with CVD and healthy individuals. Database research was conducted (PubMed, EMBASE, Cochrane Library of Controlled Trials) by using an appropriate algorithm to indicate the exercise-induced EPC mobilization studies. Eligibility criteria included EPC measurements following exercise in patients with CVD and healthy individuals. A continuous random effect model meta-analysis (PROSPERO-CRD42019128122) was used to calculate mean differences in EPCs (between baseline and post-exercise values or between an experimental and control group). A total of 1460 participants (36 studies) were identified. Data are presented as standard mean difference (Std.MD) and 95% confidence interval (95% CI). Aerobic training stimulates the mobilization of EPCs and increases VEGF in patients with CVD (EPCs: Std.MD: 1.23, 95% CI: 0.70–1.76; VEGF: Std.MD: 0.76, 95% CI:0.16–1.35) and healthy individuals (EPCs: Std.MD: 1.11, 95% CI:0.53–1.69; VEGF: Std.MD: 0.75, 95% CI: 0.01-1.48). Acute aerobic exercise (Std.MD: 1.40, 95% CI: 1.00–1.80) and resistance exercise (Std.MD: 0.46, 95%CI: 0.10–0.82) enhance EPC numbers in healthy individuals. Combined aerobic and resistance training increases EPC mobilization (Std.MD:1.84, 95% CI: 1.03–2.64) in patients with CVD. Adequate exercise volume (>60% VO_{2max} >30 min; $P = 0.00001$) yields desirable results. Our meta-analysis supports the findings of the literature. Exercise volume is required to obtain clinically significant results. Continuous exercise training of high-to-moderate intensity with adequate duration as well as combined training with aerobic and resistance exercise stimulates EPC mobilization and increases VEGF in patients with CVD and healthy individuals.

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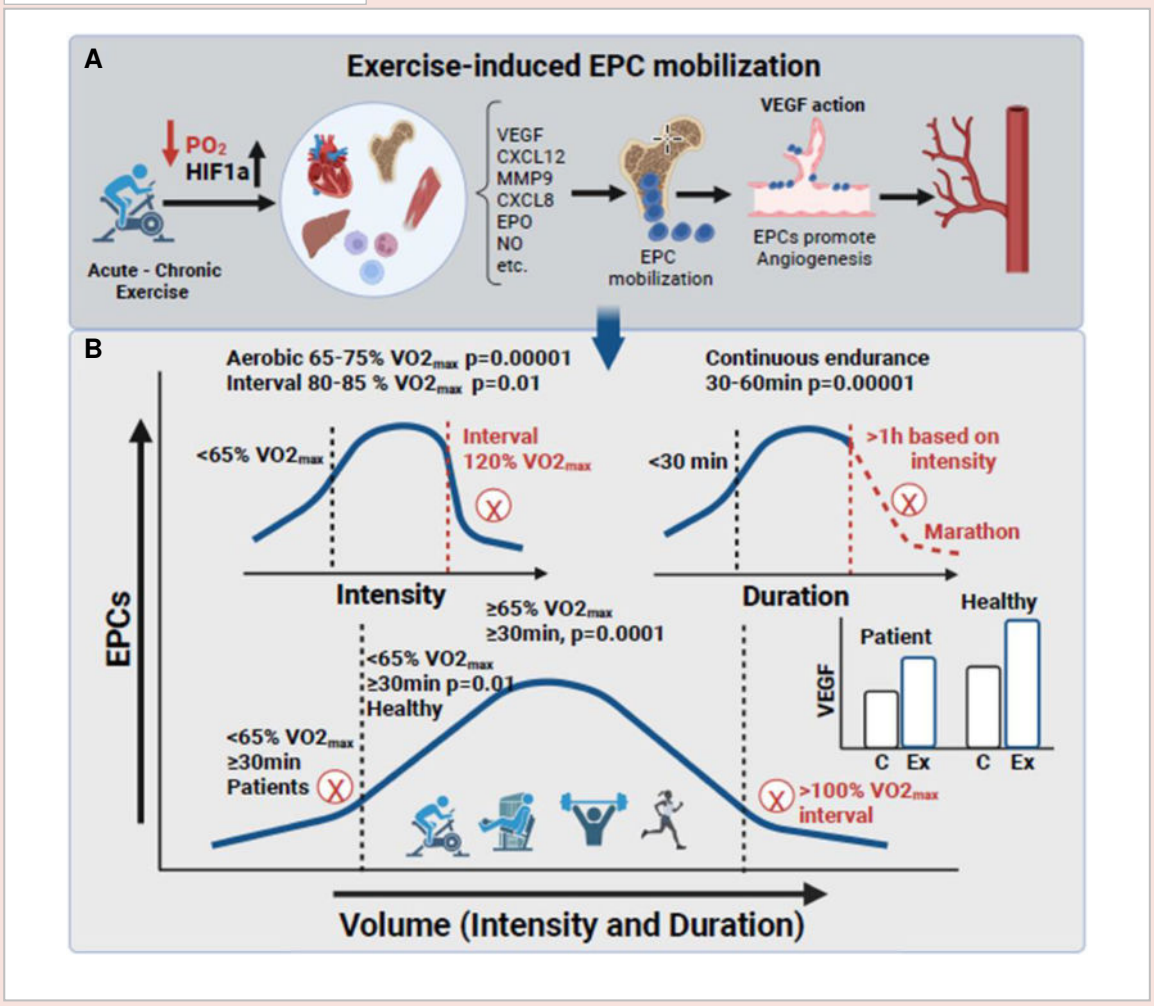
Graphical Abstract

Key question(s)	Key finding(s)	Take-home message
-What kind of exercise provides clinically significant results and promotes EPC mobilization? -What is the proper volume of exercise or training stimulus to induce health-related effects on EPC figures?	Adequate volume of exercise based on intensity and duration, appears to be the proper stimulus to induce VEGF action and EPC mobilization which promote angiogenesis.	-Any kind of exercise may bring about the desirable health-related effects. -Exercise volume is vital and should be considered to achieve better results in cardiac rehabilitation.



(*A*) Mechanism of the exercise-induced EPC mobilization. (*B*) EPC response based on duration, intensity, and volume of exercise. Note the insufficiency to induce a significantly detectable response with low exercise stimulus and an inhibited response when a cardiac patient or a healthy individual overpasses its higher physiological limits.

Keywords Endothelial progenitor cells (EPCs) • Vascular endothelial growth factor (VEGF) • Exercise volume • Acute exercise • Exercise training • Cardiovascular disease (CVD)

Introduction

Endothelial progenitor cells (EPCs) have been proposed over the last two decades as a prognostic index and therapeutic tool for diseases stemmed from endothelial dysfunction such as coronary artery disease (CAD),[1] heart failure,[2] pulmonary arterial hypertension,[3] and peripheral arterial disease.[4] EPCs play a key role in normal endothelial function and along with the vascular endothelial growth factor (VEGF) are involved in the repair of injured endothelium and in the vital process of vasculogenesis facilitated by exercise.[5] Vasculogenesis is promoted by the differentiation of progenitor cells derived from hemangioblasts and implies the *de novo* formation of a primitive capillary network.[6]

This has led researchers to study the association of EPCs in patients with cardiovascular disease (CVD) and observe the clinical value of exercise-induced benefits.[7]

Regular exercise induces beneficial changes in the lipoprotein profile of patients with CVD.[8,9] It enhances perfusion and improves endothelium-dependent vasorelaxation and endothelial function.[10] An adequate volume of exercise (based on duration and intensity) brings about the health-related beneficial effects and promotes cardiovascular health.[11,12] High intensity, within tolerable physiological limits, seems to induce favourable effects on vascular function in patients with CVD.[12,13] Certain studies have observed EPC dysfunction due to systemic and localized inflammatory responses and oxidative stress in patients with CVD.[14,15] Exercise produces an antioptotic effect on EPCs and improves their numbers. This process relies on the exercise-induced increase of nitic oxide bioavailability that regulates VEGF activation, a main component and mediator of EPC up-regulation which significantly contributes to the effects of exercise on EPC differentiation. Indeed, the properly prescribed, tailored individualized exercise stimulus promotes the mobilization of EPCs[16] which step into ischaemic regions, regenerate vessels and form entirely new ones by cell division and differentiation into endothelial cells.[1]

Numerous studies have evaluated the endothelial function of EPC increase and have highlighted the health-related importance of exercise.[5,7] This area, however, requires further analysis. Two previous meta-analyses with cardiovascular patients reported that aerobic and combined (aerobic and resistance) training of moderate intensity enhanced EPC levels[17] and improved endothelial function and repair.[18] Recently, however, two systematic reviews reported EPC mobilization in patients with CVD[19] and healthy populations[20] without taking into consideration the volume of exercise (i.e. intensity and duration). Even more, assessment of the EPC response to exercise in trained participants that perform exercise of strenuous intensity and/or duration such as in the case of athletes was not included in the above-mentioned studies. Thus, more evidence is required to define the exercise-induced response to EPC numbers. For instance, the type of exercise to induce EPC mobilization has not been clarified yet the required volume of exercise as well as the proper dose of intensity and duration remain a challenge. Moreover, whether aerobic continuous and interval exercise provoke a degree of EPC mobilization or not remains to be seen. The effects of resistance and combined exercise also require thorough analysis. Thus, the aim of the present systematic review and meta-analysis is to reveal the impact of various types of exercise on EPC numbers and examine the required exercise intensity and duration (i.e. volume). This will primarily examine EPC mobilization and observe the VEGF responses in patients with CVD and healthy individuals.

Methods

Search strategy

The current systematic review and meta-analysis was registered with PROSPERO (CRD 42019128122), following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. We conducted a search in PubMed, Embase, and Cochrane Library of Controlled Trials up until September 2019 to identify publications relevant to our research question. Weekly alerts were also received and updated up until September 2022. We have also searched for eligible publications in selected clinical journals, reviews, as well as in the reference lists of papers suitable to be included in our systematic review.

Selection criteria

The studies that were included met the criteria of EPC measurements following acute exercise and training intervention in patients with CVD and healthy individuals. Acute exercise was comprised continuous and resistance exercise, and training interventions were comprised aerobic (continuous and interval), resistance, and combined (i.e. aerobic and resistance or interval and resistance) training. Due to the absence of a standard definition of EPCs in the literature, studies were also eligible when EPC identification mainly included the following surface markers: $CD34^+$, $CD45^-$, $CD133^+$, and $VEGFR_2^+$ (or KDR^+). No other eligibility criteria such as language and date of publication were set. It should be recognized that although age and CVD may affect the outlined results, the benefits of observing the functional reaction of all ages in a unique physiological response and the number of obtained data may counterbalance the confounding effects. Based on the selective criteria, reviews, conference proceedings, and unpublished trials were excluded.

The searching procedure (see Supplementary material online, Search Algorithm) and the selection of the eligible publications were performed independently by two investigators (G.M. and P.C.D.). Conflicts between them were resolved by a third investigator (S.P.T.).

Outcome measure and data extraction

The outcome measure was EPC mobilization; EPCs absolute (cells/mL) or EPCs%. Data have been extracted for (i) the design of the studies, (ii) the participants' characteristics, (iii) the duration and intensity of exercise as well as the type of exercise, (iv) the time of blood drawn, and (v) the number of EPCs before and after the intervention. The exercise-induced increase of VEGF was also reported, as a secondary outcome. The results of most of the studies in the quantitative analysis were presented with standard error (SE); the latter was converted into standard deviation (SD) using the formula: $SD = SE \times \sqrt{n}$. The estimation of sample mean ± SD proposed by Wan et al.[21] was used to convert the data reported in median and range. When ambiguous or unclarified data were indicated e.g. unit of EPC measurement was reported in fold increase or when data could not be extracted from figures, we communicated with the corresponding authors. Response from corresponding authors was realized via 2–3 e-mails so that the study would not be excluded from the quantitative analysis.

Statistical analysis

The Revman 5.3 statistical analysis (The Nordic Cochrane Centre, Copenhagen, Denmark) software was adopted for quantitative analysis. A continuous random effect model meta-analysis was used to calculate mean differences in EPCs between baseline and post-exercise values or between an experimental (exercise) group and a control (non-exercise) group. The standardized mean difference (Std.MD) was used due to deviations in the units of measurements in several eligible studies. The mean values of the Std.MD of the outcome measure were plotted with associated error bars and presented in the forest plot. The percentages of the peak oxygen uptake (VO_{2peak}) and maximal oxygen uptake (VO_{2max}) were used to prescribe the exercise intensity. When studies used percentages of peak heart rate (HR_{peak}, patients) or maximal heart rate (HR_{max}, healthy individuals) they were converted into VO_{2peak} or VO_{2max} using the formula $VO_{2max} = 404.56–0.648$ (HR). The data that were not provided in the main texts or tables were extracted from the relevant figures using the *WebPlotDigitizer*.[22] Data of studies that included more than one intervention group or different exercise protocols or different combinations of EPC identification markers were analysed as independent samples. When studies presented EPC assessment following exercise in patients with CVD as well as in healthy participants, they were also independently analysed. Heterogeneity of the included studies was assessed by the I^2 test (range: absolute homogeneity 0–100% highest heterogeneity). The GRADE (Grading of Recommendations, Assessment, Development, and Evaluation) analysis was adopted to assess the quality of the evidence on exercise response to EPC mobilization and VEGF increase for each meta-analysis. The level of significance to report a change in the outcome measure was set at $P \leq 0.05$.

Risk of bias and quality of reporting data

To evaluate the included randomized controlled trials (RCT), an assessment of the risk of bias was conducted with the 'Cochrane Collaboration's tool'[23] whereas the included observational studies were analysed using the risk of bias via the 13-item of Research Triangle Institute item bank tool.[24] The process was independently performed by two reviewers (G.M. and P.C.D.; disagreements were resolved by S.P.T.). The CONSORT (Consolidated Standards of Reporting Trials) 25-item checklist was used to measure and

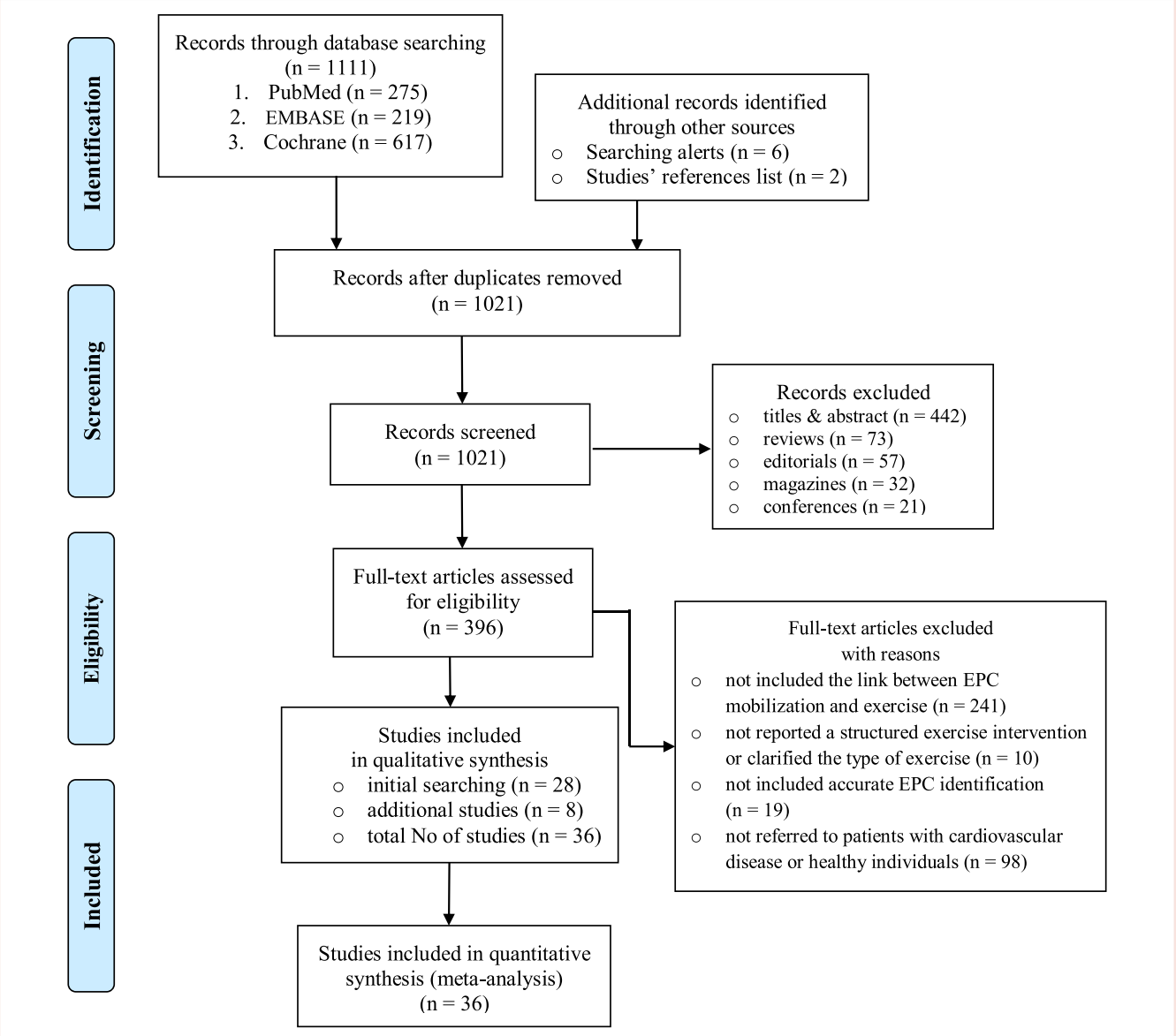


Figure 1 PRISMA flow chart of the study.

report a score for each RCT, regarding the quality of the reported results. Given that seven out of the 25-item CONSORT checklist was not applicable for controlled trials (CTs) and single group studies (SGS) (i.e. blinding of participants and researchers), a modified, 18-item, checklist was used to report a score for the latter studies (CTs and SGS). In addition, the 22-item STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) checklist was adopted to assess the quality of the reporting data of the cross-sectional studies (CSS) and provide a score for each study.

Results

A total of 36 eligible studies with 1460 participants (1203 in intervention and 257 in control groups) were identified. Twenty-eight studies that met the inclusion criteria were revealed after the removal of duplicates and the exclusion of articles based on the abstract and title (*Figure 1*). The reference lists of these studies and the searching alerts resulted in the identification of eight additional articles,[25–30] two of which[16,31] were added manually. Details of full-text articles which were reviewed, but excluded from different reasons, are provided in *Figure 1*.

Characteristics of the included studies

Characteristics and results of the included studies can be found in the Supplementary material online, *Table S1*. From these eligible studies, 12 were RCTs (33%), 3-CTs (8%), 10-SGS (28%), and 11-CSS (31%). A total of 16 studies were conducted in patients with CVD,[16,28–42] (CAD,[28,32,37,39] heart failure,[16,29,31,33–36,38,40] peripheral arterial obstructive disease,[42] and myocardial infarction[30,41]) seventeen in healthy individuals[25–27,43–56] and three in athletes.[57–59] Thirteen studies examined the effects of acute exercise protocols while most of the studies ($n = 23$) used exercise training interventions. Concerning the exercise regime, seven studies used acute continuous aerobic moderate intensity,[25,43–47,58] two used acute resistance exercise,[27,56] one study was conducted after a marathon race (~4 h),[59] 19 studies used

continuous training of moderate intensity,[29,30,32–38,40–42,48,50–55] and two combined resistance exercise either with continuous or with interval training.[28,31] The rest of the studies assessed two modes of exercise; one study used acute continuous and interval exercise,[16] one acute continuous and resistance exercise,[26] another used acute continuous exercise and a marathon race (~4 h),[57] and two studies were conducted with both aerobic continuous and interval training.[39,49] Exercise protocols and intervention programmes are presented separately for each population in *Table 1*. The average age of participants ranged from 18 to 80 years and sex distribution was predominantly male (79%).

Risk of bias and assessment results

A summary of the risk of bias assessment is illustrated in the included RCTs (see Supplementary material online, *Figure S1A*) and the observational studies (CTs, SGS, CSS; see Supplementary material online, *Figure S1B*). A detailed description of the risk of bias assessment for all the eligible studies in the current systematic review is presented in the Supplementary material online, *Tables S2* and *S3*.

The evaluation of the reporting data showed a mean score of 14 out of 25 for the RCTs, 14 out of 18 for the CTs, and 13 out of 18 for the SGS (see Supplementary material online, *Table S4*). The CSS displayed a mean score of 13 out of 22 (see Supplementary material online, *Table S5*). The score represents the number of items on the checklist that were reported satisfactorily in each study (a high or a low score represents a high or a low adherence to reporting guidelines).

Reporting the outcomes

Continuous and combined exercise training increased EPC numbers in patients with CVD ($P < 0.00001$; *Figure 2A*, $P < 0.00001$; *Figure 2B*). Although EPCs increased after a single trial of interval training in patients with chronic heart failure (CHF) (31 min, 80% VO_{2peak}; $P = 0.005$), limited reports on the impact of interval training reduced the significant outcome (overall effect $P = 0.01$; *Figure 2C*).

In healthy individuals, EPCs increased in response to acute ($P < 0.00001$; *Figure 3A*), resistance exercise ($P = 0.01$; *Figure 3B*), and continuous training ($P = 0.0002$; *Figure 3C*). Fifteen studies (5 with acute exercise and 10 with training interventions) revealed an increase in VEGF serum levels in patients with CVD ($n = 7$, $P = 0.01$; *Figure 4A*) and healthy individuals including three studies in athletes ($n = 8$, $P = 0.03$; *Figure 4B*).

The volume of exercise based on long-duration (≥ 30 min/session) and low-intensity (< 65% VO_{2peak}) failed to increase EPC numbers in patients with CVD, ($P = 0.17$; *Figure 5A*). However, when long-duration exercise training performed at high intensity (≥ 65% VO_{2peak}) it stimulated the mobilization of EPCs ($P = 0.001$; *Figure 5B*). Acute exercise of short duration (< 30 min/session) and high intensity (≥ 65% VO_{2max}) in healthy individuals revealed increased in EPC numbers ($P = 0.04$; *Figure 6A*). Furthermore, acute exercise of long-duration accompanied by either low or high intensity (< 65% or ≥ 65% VO_{2max}) showed significant results in EPC mobilization in healthy individuals, ($P = 0.00001$; *Figure 6B* and *6C*). Finally, the Funnel plot assessment by Egger's test indicated no publication bias (see Supplementary material online, *Figure S2*).

GRADE analysis

Due to the inclusion of several exercise interventions in patients with different types of CVD and healthy individuals and the large number of eligible studies, a high heterogeneity was observed in some of the meta-analyses. The GRADE analysis implements an effective approach and was adopted to assess the quality of evidence and underline the importance of the recommendations in the results. The rating of the quality of the studies and the overall effect of the meta-analyses were determined through consideration of five domains: risk of bias, inconsistency, indirectness, imprecision, and publication bias (see Supplementary material online, *Table S6*). The results mainly indicated a moderate and low level of the quality of the evidence (*Table 2*).

Qualitative perspective

To perform a meta-analysis with interval exercise training in healthy individuals only two studies[49,60] were identified. In one study, the interval exercise load was out of limits (120% peak work rate, 6 weeks, 5 sessions/week, 30–40 min/session)[60] and thus cannot be included in the analysis. In the other study,[49] the participants performed interval training within acceptable limits (80% VO_{2max}, 6 weeks, 5 sessions/week, 30 min/session). Therefore, the analysis was omitted due to the incomparable exercise intensity (120% peak work with restricted EPC response vs. 80% VO_{2max} with positive EPC response).

Discussion

The aim of the current review was to systematically examine the exercise-induced EPC mobilization in patients with CVD and healthy individuals. The meta-analysis revealed that the volume of exercise brings about desirable effects when it reaches an adequate level. Continuous aerobic training promotes EPC mobilization in both patients and healthy individuals (*Figures 2A* and *3C*). Acute aerobic exercise and resistance exercise increase circulating EPC numbers in healthy individuals (*Figure 3A* and *3B*) and high intensity reveals even better results.[26,43]

Table 1 Exercise characteristics of the eligible studies using patients with cardiovascular disease, healthy individuals, and athletes

	Patients	Healthy individuals	Athletes	Total
No of studies (%)	16 (45%)	17 (47%)	3 (8%)	36
Age (range in yrs)	49–73	18–80	32–57	18–80
Duration (range)				
min/day	30–53 min/day	10–60 min/day	5–245 min	5–245 min
days/wk	3–7 days/wk	4–7 days/wk		3–7 days/wk
wks	4–24 wks	10 days to 12 wks		10 days to 24 wks
Intensity (range) %				
VO_{2max}	50–85	45–80	75–100	45–100
% 1-RM	60–75	55–80		55–80

yrs, years; min, minutes; wk, week; wks, weeks; VO_{2max}, maximal oxygen uptake; 1-RM, 1-repetition maximum.

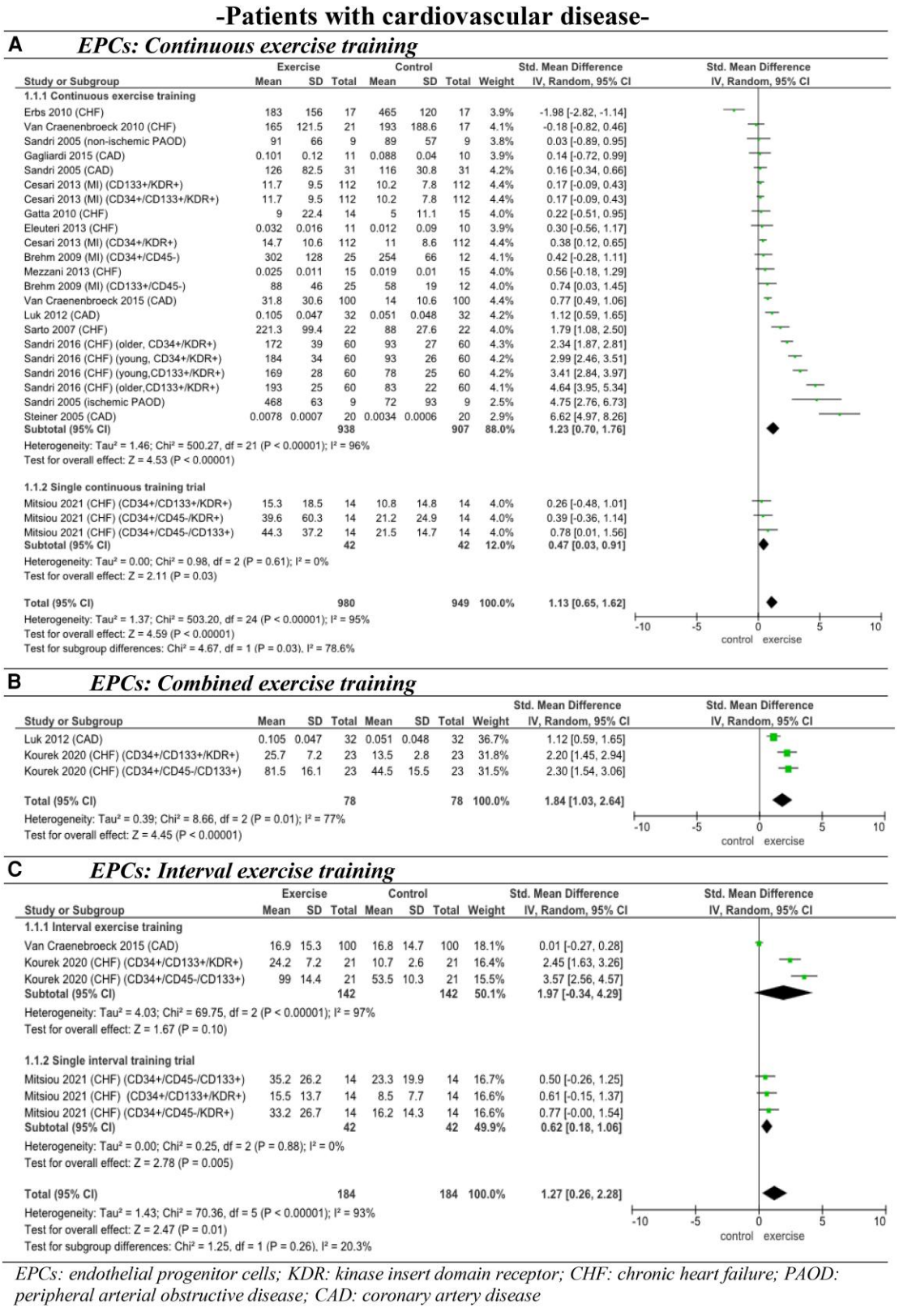


Figure 2 Change in endothelial progenitor cell (EPC) numbers in patients with cardiovascular disease before vs. after (*A*) continuous exercise training, (*B*) combined exercise training, and (*C*) interval exercise training. Average values of EPCs are represented as cells/mL and cells%. Squares represent the mean difference between intervention and control post-intervention with 95% confidence intervals, size of the square proportional to the weight of the study; pooled estimates from the meta-analysis are depicted as solid black diamonds.

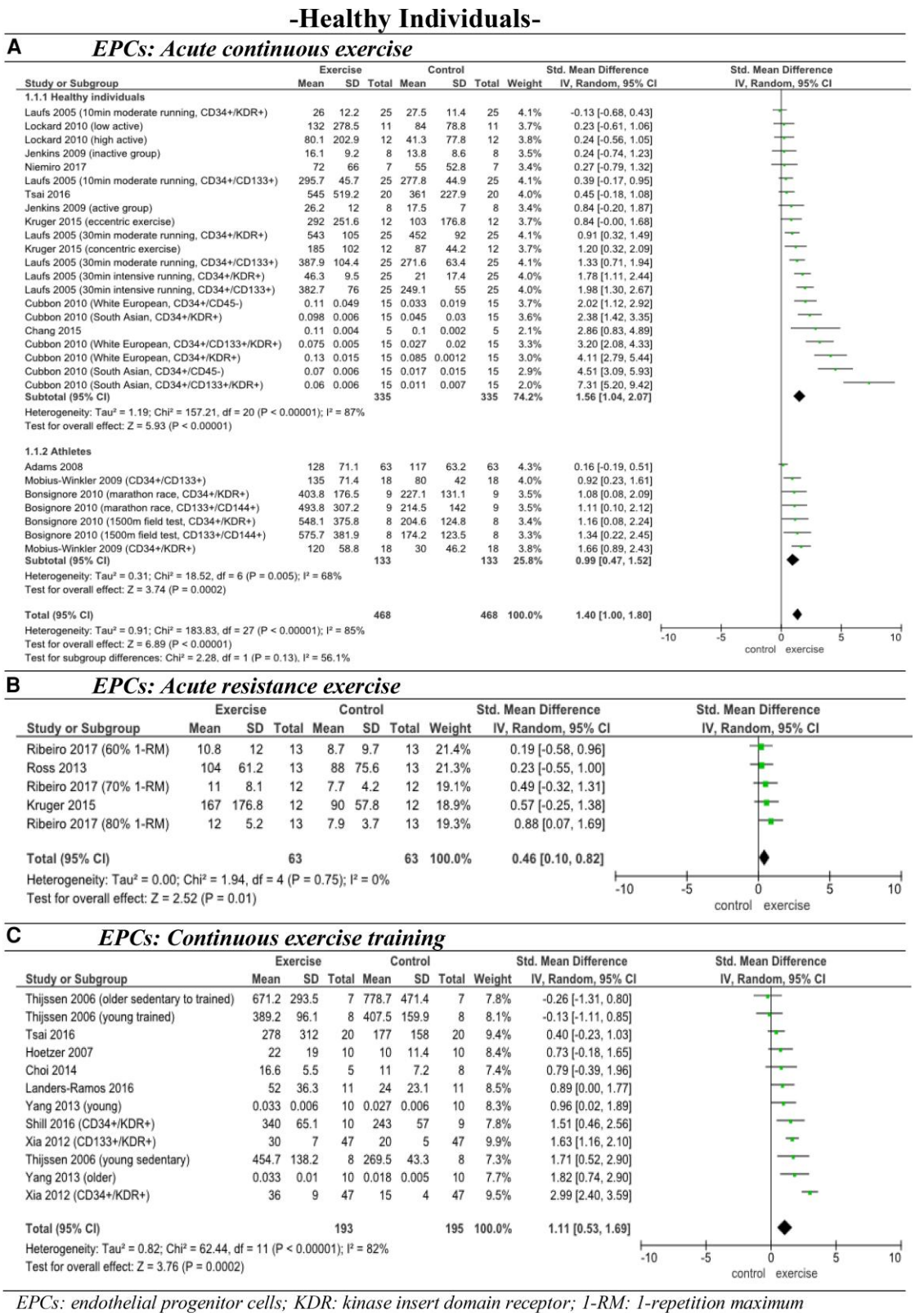


Figure 3 Change in endothelial progenitor cell (EPC) numbers in healthy individuals before vs. after (*A*) acute continuous exercise, (*B*) acute resistance exercise, and (*C*) continuous exercise training. Average values of EPCs are represented as cells/mL and cells%.[5] Squares represent the mean difference between intervention and control post-intervention with 95% confidence intervals, size of the square proportional to the weight of the study; pooled estimates from the meta-analysis are depicted as solid black diamonds.

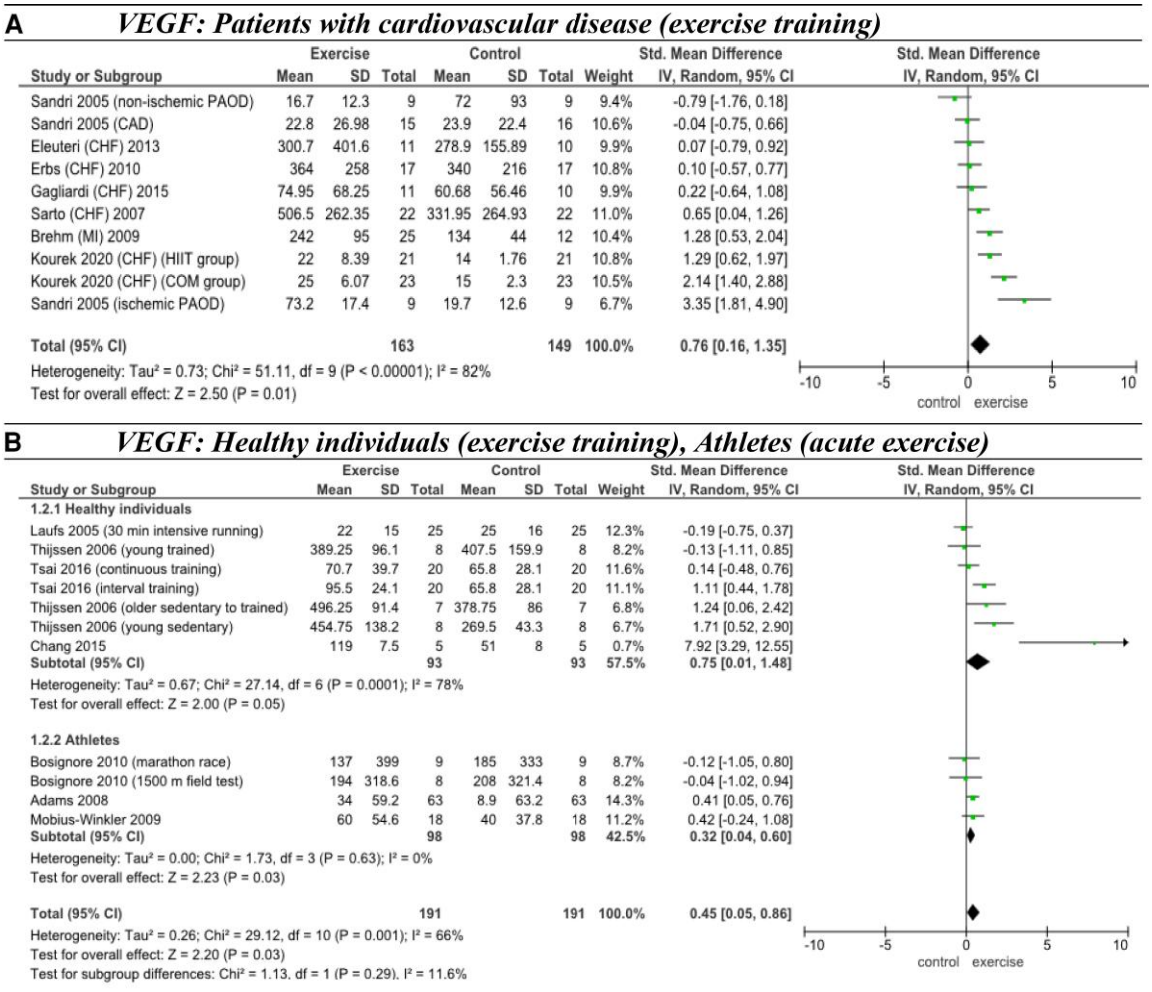


Figure 4 Change in vascular endothelial growth factor (VEGF) numbers before vs. after exercise in (*A*) patients with cardiovascular disease and (*B*) healthy individuals and athletes. Average values of VEGF are represented as pg/mL. Squares represent the mean difference between intervention and control post-intervention with 95% confidence intervals, size of the square proportional to the weight of the study; pooled estimates from the meta-analysis are depicted as solid black diamonds.

Despite the favourable results concerning interval training based on a limited number of studies (*Figure* 2C), more research is required. Different types of training (i.e. cycling, walking, running, or resistance) may bring about comparable results. The combined training of resistance exercise to either aerobic interval or continuous training, however, significantly affects EPC mobilization in patients with CVD (*Figure* 2B). Along with the exercise-induced mobilization of EPCs, the augmented VEGF action facilitates the constructive vasculogenic process (*Figure 4*).

The exercise-induced EPC mobilization

Exercise volume

Intensity and duration are the key exercise stress parameters that define training stimulus, dose response, and total volume of the performed workload. When running at moderate intensity (68% VO_{2max}),[43] circulating EPC numbers increased after 30 min but not after 10 min. Exercise duration is important, but intensity makes a difference. Athletic running of 5 min at high intensity (100% VO_{2max}),[57] for example, was enough to increase EPC numbers. It seems, therefore, that when a fit individual has the capacity to perform at high intensity, the impact of exercise volume on EPC mobilization is mainly based on intensity and less on duration but generally there must be a balance. Only high intensity with low volume as compared to high volume (4 min vs. 16 min intervals) yielded desirable results in cardiac patients.[12] Moreover, a total volume of activity in bouts ≥10 min failed to be beneficial in older men.[61]

In addition, the individual status of healthy participants (trained or untrained) or patients should be taken into consideration. EPC mobilization following continuous aerobic exercise training is even better when bouts of 30 min or more are performed at an intensity of 60–70% VO_{2max} as part of long (12 weeks)[51] or even short-term (4–6 weeks)[30,49,54] intervention programmes. Indeed, aerobic exercise training of longer duration ranging from 30 to 40 min promotes EPC mobilization as confirmed in patients with CAD.[37] Patients with CVD may not be able to endure high intensity. Thus, the duration of exercise can increase training volume and affect the elevation of EPC mobilization. The volume of exercise,

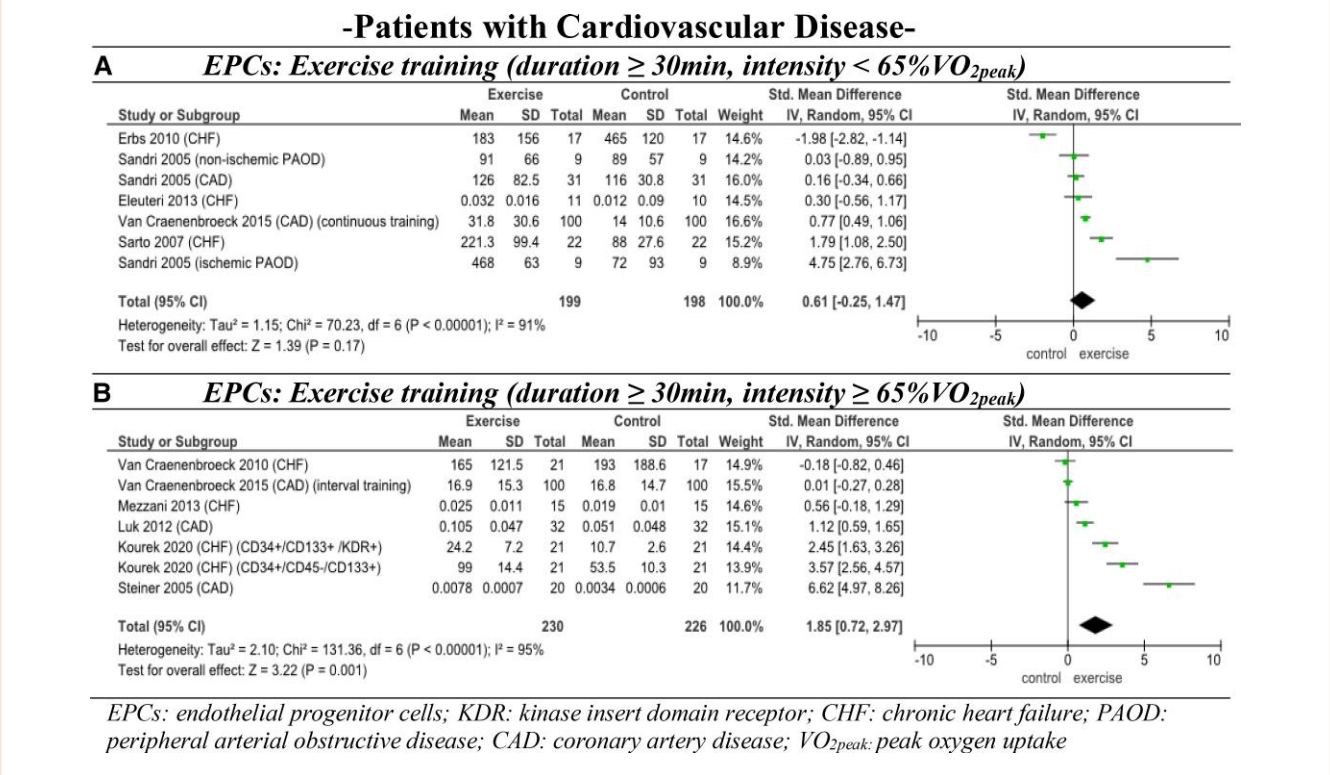


Figure 5 Change in endothelial progenitor cell (EPC) numbers in patients with cardiovascular disease before vs. after exercise training of (*A*) duration ≥ 30 min, intensity < 65%VO_{2peak} and (*B*) duration ≥ 30 min, intensity ≥ 65%VO_{2peak}. Average values of EPCs are represented as cells/mL and cells%. Squares represent the mean difference between intervention and control post-intervention with 95% confidence intervals, size of the square proportional to the weight of the study; pooled estimates from the meta-analysis are depicted as solid black diamonds.

therefore, based on duration and intensity acts as a physiological trigger which induces the desirable effects on EPC mobilization.

Continuous training

A study using continuous training with short duration and high intensity[34] revealed similar results in EPC mobilization as compared to studies that performed continuous training of moderate duration and intensity.[29,33,38] In addition, following a 3-week short-term intensive training intervention of higher frequency (i.e. higher volume; 6 days/week, 30 min/session, 2 sessions/day, 90% of HR_{peak} ~80% VO_{2peak})[34] EPCs increased in patients with CHF. Moreover, 30 days of short-term exercise training (3–4 days/week, mean sessions 14 ± 4.7) with intensity reaching 70% VO_{2peak} stimulated the EPC mobilization in patients with myocardial infarction.[30] In contrast, exercise training of moderate duration and intensity (12 weeks, 3 sessions/week) has not been associated with increased circulating EPCs in patients with CAD.[37] These findings suggest that the exercise-induced EPC-enhanced activity requires a higher exercise volume based either on intensity, duration, or frequency.

Interval training

Exercise training research in various laboratories may respond differently for many reasons (i.e. excessive exercise, poor physical condition, etc.). Van Craenenbroeck et al.[39] in a pioneer study showed that a training programme adjusted to 3-min intervals (3 sessions/week, 38 min/session) with an intensity reaching 90–95% HR_{max} (~85% VO_{2peak}) was not effective to promote EPC mobilization in patients with CAD. Whereas, Kurek et al.[31] revealed that exercise training with a longer 4-min interval (4 reps, 80% VO_{2peak}, active recovery 50% VO_{2peak}, 3 sessions/week, 31 min/session) increased EPC numbers in patients with CHF. Thus, to balance high volume,[12] more interval training research is required to resolve discrepancies (see limits in the Graphical abstract).

Resistance and combined training

Resistance exercise for muscular endurance (upper and lower limbs) was an effective means to increase circulating EPCs (55–80% 1-RM) in trained men[56] and women.[27] This is further supported by Kruger et al.[26] who found that acute bouts of resistance exercise (70% 1-RM) are associated with increased EPC numbers. Concomitantly, the high intensity of such resistance exercise (80% 1-RM)[27] led to better EPC mobilization. Favourable results in EPC numbers were also attributed during combined training with aerobic and resistance exercise (8–12 weeks, 3 sessions/week, 40–50 min/session, ~75% VO_{2peak}, 60–80% of 1-rep max, 1-RM) increased EPC numbers in CHF[31] and CAD[28] patients. It seems that the total volume of the multi-component variation of aerobic and resistance exercise stimulus was sufficient to promote EPC mobilization and when resistance exercise was combined with interval training even better results were revealed in CHF patients.[31] The impact of exercise volume on EPC mobilization, therefore, remains to be further verified in studies performing various personalized exercise interventions of high intensity in both patients with CVD and healthy individuals.

VEGF action and EPC mobilization

VEGF, an endothelial cell survival cytokine, protects endothelial cells against apoptosis and promotes angiogenesis. The beneficial effects of exercise on endothelial protection are related to intensity and the overall metabolic stress demands of adequate training volume which promote

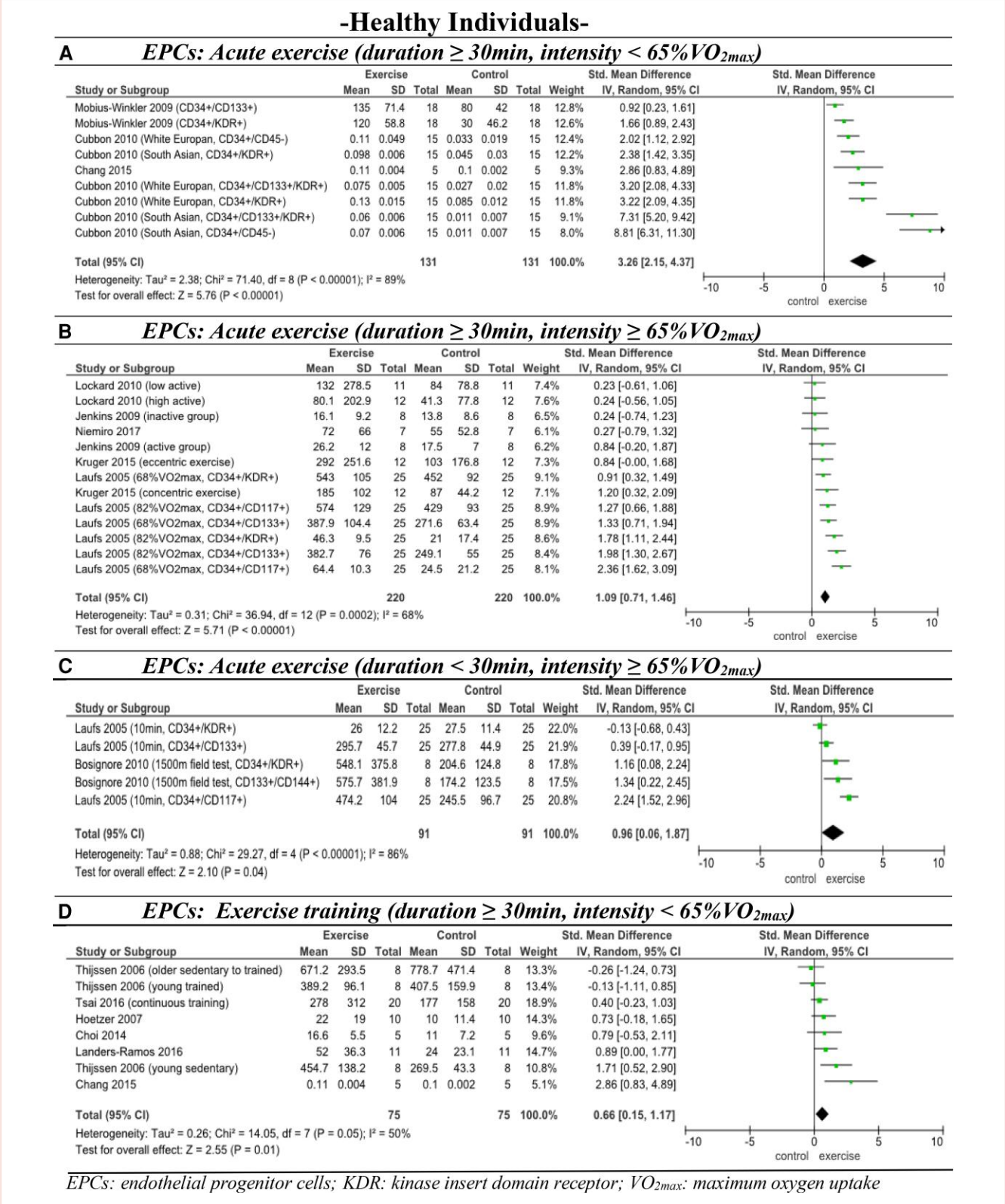


Figure 6 Change in endothelial progenitor cell (EPC) numbers in healthy individuals before vs. after acute exercise of (*A*) duration ≥ 30 min, intensity < 65%VO_{2max}, (*B*) duration ≥ 30 min, intensity ≥ 65%VO_{2max}, (*C*) duration < 30 min, intensity ≥ 65%VO_{2max}, and training regime of (*D*) duration ≥30 min, intensity <65%VO_{2max}. Average values of EPCs represented as cells/mL and cells%. Squares represent the mean difference between intervention and control post-intervention with 95% confidence intervals, size of the square proportional to the weight of the study; pooled estimates from the meta-analysis are depicted as solid black diamonds.

Table 2 GRADE analysis of the exercise-induced EPC mobilization and change in VEGF

Outcomes	No. of participants (studies)	Quality of the evidence (GRADE)	Relative effect (95% CI)
Patients with cardiovascular disease			
EPC mobilization			
Continuous exercise training	1929 (25 studies)	⊕⊕◯◯ LOW due to inconsistency	Std.MD 1.13 (0.65–1.62)
Interval exercise training	368 (6 studies)	⊕⊕◯◯LOW due to inconsistency	Std.MD 1.27 (0.26–2.28)
Combined exercise training	156 (3 studies)	⊕⊕⊕◯ MODERATE due to inconsistency	Std.MD 1.84 (1.03–2.64)
Exercise training (≥ 30 min, ≥ 65%VO_{2peak})	456 (7 studies)	⊕⊕⊕◯ MODERATE due to inconsistency	Std.MD 1.85 (0.72–2.97)
Change in VEGF			
Exercise training	312 (10 studies)	⊕⊕⊕◯ MODERATE due to inconsistency	Std.MD 0.76 (0.16–1.35)
Healthy individuals			
EPC mobilization			
Acute continuous exercise	936 (27 studies)	⊕⊕◯◯ LOW due to inconsistency	Std.MD 1.40 (1–1.80)
Continuous exercise training	388 (12 studies)	⊕⊕◯◯ LOW due to inconsistency	Std.MD 1.11 (0.53–1.69)
Acute resistance exercise	126 (5 studies)	⊕⊕◯◯ MODERATE due to inconsistency	Std.MD 0.46 (0.10–0.82)
Exercise training (≥ 30 min, < 65%VO_{2max})	262 (9 studies)	⊕⊕◯◯ VERY LOW due to inconsistency	Std.MD 3.26 (2.15–4.37)
Exercise training (≥ 30 min, ≥ 65%VO_{2max})	440 (13 studies)	⊕⊕◯◯ LOW	Std.MD 1.09 (0.71–1.46)
Acute exercise (< 30 min, ≥ 65%VO_{2max})	182 (5 studies)	⊕⊕⊕◯ VERY LOW due to inconsistency	Std.MD 0.96 (0.06–1.87)
Exercise training (≥ 30 min, < 65%VO_{2max})	150 (8 studies)	⊕⊕◯◯◯ LOW	Std.MD 0.66 (0.15–1.17)
Change in VEGF			
Exercise training	382 (11 studies)	⊕⊕⊕⊕ HIGH	Std.MD 0.45 (0.05–0.86)

EPC, endothelial progenitor cell; VEGF, vascular endothelial growth factor.
GRADE Working Group grades of evidence.
High quality: Further research is very unlikely to change our confidence in the estimate of the effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of the effect and may change the estimate.
Low quality: Further research is likely to have an important impact on our confidence in the estimate of the effect and is likely to change the estimate.
Very low quality: We have very little confidence in the effect estimate. The true effect is likely to be substantially different from the estimate of effect.

VEGF action (see *Figure 4*, $P < 0.01$; patients, $P < 0.003$; healthy individuals and athletes) and circulating angiogenic factors. VEGF, a major component of EPC mobilization, induces the release of nitric oxide and is upregulated during ischaemia. It also engages in directing EPCs to the site of injury while facilitating angiogenesis.[62] The exercise-induced hypoxia along with the increased nitric oxide synthase, the activation of VEGF and parallel mechanisms (shear stress, interleukin-8 activation, increased expression of matrix metallopeptidase-9, and activation of stroma cell-derived factor-1) contribute to the process of bone marrow EPC mobilization into the peripheral circulation.

Clinical perspective of exercise volume

Clinical management in cardiac rehabilitation[8] based on exercise volume[11,12] can provide better results in CVD patients. The present meta-analysis indicates that exercise volume of higher (80–85% VO_2max) or lower intensity (65–75% VO_2max) with 30–60 min duration is a prerequisite to reach the required workload and stimulate exercise-induced mobilization of EPCs (see the Graphical abstract). Among these critical limits, the increase or decrease of exercise intensity may be alternatively counterbalanced by an increase or decrease in exercise duration to reach the metabolic demands and promote EPC mobilization. Similar work-output of two exercise protocols either with higher intensity (80% VO_{2peak}) and shorter duration (~28 min) or with moderate intensity (50% VO_{2peak}) and longer duration (~50 min) improves mobilization of EPCs in patients with CHF.[16] In most studies where exercise preceded[35,43] or exceeded[59,60] the required intensity or duration, no significant changes were observed in EPC numbers. Exercise volume below the lower critical limits (65% VO_{2max}, 30 min) does not activate the molecular mechanisms of EPC mobilization. On the other hand, the exercise of strenuous intensity (>100% VO_2max)[60] or prolonged duration (>1 h)[59] may distress the physiological process of EPC mobilization. Thus, exercise volume[11,12] within the above-mentioned limits of intensity and duration appears to be mandatory and should be thoroughly applied to reach the required workload. Based on this approach, however, further research tailored to the patient's individual needs is required.

Strengths and limitations

To our knowledge, the present study is the first systematic review and meta-analysis that examines the effects of all types of exercise on EPC mobilization in patients with CVD and healthy individuals, taking into consideration the volume (i.e. intensity and duration) of exercise-induced metabolic stress under different conditions irrespective of age, sex, and health status. Common limitations concerning the lack of standardized identification markers and nomenclatures[63] (see Supplementary material online, *Table S7*) along with the heterogeneity among studies, and methodological assessment of EPCs (flow cytometry, cell culture) were observed. Most of the studies were conducted in men (79%) and the results were mainly applied to males. In addition, data pooling, different EPC units, data extraction from figures, and unclear exercise description (e.g. intensity, duration, frequency) of interventions were also encountered in our study. Nevertheless, a random effect model meta-analysis was applied to the above-mentioned heterogeneities.

Further study recommendations

The general outline of the critical factor of exercise volume in the present meta-analysis challenges researchers to study intensity and duration in detail and set more accurately upper and lower limits of

exercise volume in patients with CVD. Such an approach should be studied further since it can be tailored for patients with chronic disease and healthy individuals not only for EPCs but also for various biomarkers. Interval exercise seems to be promising but requires an individualized approach tailored to the patient's needs. In addition, it should be noted that only continuous aerobic exercise was assessed thoroughly by an adequate number of studies. More studies with different types of exercise (combined, interval, resistance training) may bring about more evidence and indicate the degree of EPC mobilization. Further RCT studies may define the dose response and the balance between intensity and duration, taking into consideration the volume of exercise when planning effective protocols and programmes.

Conclusions

In summary, our study supports what has been published in the relevant literature. The role of EPCs mobilization can be facilitated through exercise to regenerate injured endothelium and promote angiogenesis. Exercise training as a physical, non-pharmacological intervention, increases the number of EPCs, promotes VEGF action, improves endothelial function, and may be used as a complementary therapeutic approach in patients with CVD. Presently, aerobic continuous exercise (~3–5 times/wk, 40–60 min/session at 65–75% VO_{2max}) appears to be the standard mode to promote EPC mobilization in both populations of patients with CVD and healthy individuals. Intensity and duration (i.e. volume of exercise), however, play a dominant role in regulating the clinical outcome of the intervention. High-intensity interval training (~30 min/session at 80–85% VO_{2max}) brings desirable results. The meta-analysis supports a dose response relationship of EPC mobilization when the required exercise volume is adjusted within critical limits. However, the exact levels of intensity and duration remain to be examined further.

Lead author biography

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Authors' contributions

Substantial contributions to the conception or design of the work (G.M., S.P.T., and S.N.); acquisition of the data (all authors); data extraction (G.M., S.P.T., and P.C.D.); statistical analyses (G.M., P.C.D., I.S.); drafting the work (all authors); revising the manuscript critically (all authors); final approval of the version to be published (all authors). The corresponding author attests that all listed authors meet authorship criteria.

Data availability

All required links or identifiers for our data are present throughout the manuscript as described.

Supplementary material

Supplementary material is available at *European Heart Journal Open* online.

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