

Mobilization of Endothelial Progenitor Cells with Exercise in Healthy Individuals: a Systematic Review

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Physical exercise mobilizes endothelial progenitor cells (EPCs) to peripheral blood. However, this effect seems to depend on exercise characteristics, such as duration and intensity.

The aim of this systematic review was to verify the impact of a single bout of aerobic exercise on the mobilization of EPCs in healthy individuals, and the potential mechanisms involved.

The bibliographic search was conducted on the following electronic databases in May 2011: SciELO, LILACS, Cochrane, ClinicalTrials.gov, SPORTDiscus and Medline. Of the 178 articles initially identified, 12 met the inclusion criteria and were classified regarding quality according to the PEDro scale.

The magnitude and duration of the EPC mobilization response were higher after long/ultralong duration exercises, and they are correlated with vascular endothelial growth factor (VEGF) plasma levels. The EPC mobilization peak in response to a maximal or submaximal single bout of exercise lasting up to one hour occurs immediately after the exercise or within the first hour after it. One possible mechanism is nitric oxide (NO) bioavailability. The individuals' age and exercise intensity seem to interfere with the EPC mobilization response.

Long/ultralong duration exercises promote more pronounced EPC mobilization as compared with maximal or submaximal exercises. The mechanisms involve VEGF release in long/ultralong duration exercises and NO bioavailability in maximal or submaximal exercises lasting less than one hour.

Introduction

Endothelial progenitor cells (EPCs), described in 1997 by Asahara et al¹, constitute a heterogeneous population of circulating cells in peripheral blood¹. Their origin is found in multiple precursors, such as hemangioblasts¹, non-

hematopoietic precursors, monocytic cells², or tissue-resident stem cells³. Endothelial progenitor cells play an important role in vascular repair and new vessel formation, because of their capacity to proliferate, migrate, differentiate *in vivo* and *in vitro* into endothelial cells¹, and incorporate into the preexisting endothelium^{2,4}. Thus, phenotypically, they have morphofunctional characteristics of both hematopoietic and mature endothelial cells⁵.

Endothelial progenitor cells are rare, representing approximately 0.01% to 0.0001% of the mononuclear fraction in peripheral blood⁶. However, several stimuli, such as physical exercise, can mobilize them from bone marrow, temporarily increasing their number in peripheral circulation⁷⁻⁹. In the peripheral circulation, EPCs secrete pro-angiogenic factors, such as vascular endothelial growth factor (VEGF)¹⁰ and granulocyte colony stimulating factor (G-CSF)¹¹, which are capable of paracrine stimulation of neovasculation and angiogenesis¹.

The regular practice of physical exercise contributes to an approximate 30% reduction in mortality due to cardiovascular diseases¹². Because the reduction in classical cardiometabolic risk factors, such as hyperlipidemia, hypertension and insulin resistance, explains only approximately 40% of the exercise-induced reduction in mortality¹³, variables directly related to the endothelium could explain why and how physical exercise prevents and decreases the progression of disease, and reduces cardiovascular mortality¹⁴.

One of the possible mechanisms involved in that process is EPC mobilization to peripheral blood⁹. However, different exercise types, durations and intensities can promote distinct responses, and, thus, alter the bioavailability and functionality of EPCs. Thus, the impact of a bout of aerobic exercise on EPC mobilization in healthy individuals and the possible mechanisms involved in the process were systematically reviewed.

Methods

Selection of the studies

Bibliographic search was performed in May 2011 by two independent examiners (JFRS and NGR) by use of the following electronic databases: SciELO, Cochrane, LILACS, ClinicalTrials.gov, SPORTDiscus, and Medline. The studies were assessed by use of all or part of the following descriptors in English or their Portuguese correspondents: ("*endothelial progenitor*" OR *CD34+KDR+* OR *CD34+VEGFR2+* OR *sca-1_{flk-1}* OR *CD133+VEGFR2+* OR *CD133+KDR+* OR *AC133+VEGFR2+* OR *AC133+KDR+* OR

Keywords

Exercise; endothelial progenitor cells; cell mobilization.

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“circulating angiogenic cells” OR “blood derived progenitor cells” OR “circulating progenitor”) AND (exercise OR “aerobic fitness” OR training OR “physical activity” OR “physical fitness” OR “sports activities” OR “sports medicine” OR marathon OR athletes OR cyclists OR runners OR ergometer OR endurance OR treadmill).

From all electronic databases searched, 178 studies were identified. After applying the terms *NOT* disease and *NOT* review to the search, 56 studies were selected in the *Medline* database, four in the *ClinicalTrials.gov* database, two in the *SciELO* database, two in the *Cochrane* database, one in the *SPORTDiscus* database, and none in the *LILACS* database. Of those studies, two were found in two different databases (*Medline* and *Cochrane*), and one was found in three different databases (*Medline*, *ClinicalTrials.gov*, and *SPORTDiscus*).

Through manual analysis, studies with the following characteristics were excluded: children or adolescents as participants (n = 02); participants affected by disease, inflammation, or metabolic change, and those on drugs (n = 27); studies assessing neither the intervention nor the outcome of interest, that is, if the intervention was not one single physical exercise bout, or the cell population analyzed did not correspond to the EPC profile, according to inclusion criteria (n = 19); and review studies (n = 01) (Figure 1).

When the title and abstract of the study suggested its potential eligibility for inclusion, a copy of the complete text was obtained and the study was classified according to the following five inclusion criteria: 1. year of publication: from 1997, date of the first publication about EPCs; 2. study designs: cohort, cross-sectional, clinical trials, controlled clinical trials, and randomized controlled clinical trials; 3. study population: healthy adults (humans or animals); 4. intervention: one aerobic physical exercise bout; and 5. outcome of interest: quantitative assessment of EPCs, characterized as positive for CD133 (AC133) or CD34 (sca-1) and VEGFR2 (KDR or flk-1), or endothelial cell colony forming units (EC-CFUs)¹⁵.

The quality of the studies was assessed by use of the PEDro scale¹⁶, according to which studies with a score equal to or higher than four are classified as of high quality. The criteria adopted were as follows: 1. well-defined inclusion criteria; 2. random allocation into groups; 3. description of the demographic characteristics and size of the sample (\geq eight individuals); 4. blind (blind participants or examiners or result analyses); 5. sufficient method (when the exercise protocol was described in details or the cell analysis was performed by use of at least one quantitative or qualitative method); 6. measurements (were obtained in more than 85% of the individuals initially allocated into groups for at least one key-result); 7. statistical analysis (at least one result of the statistical

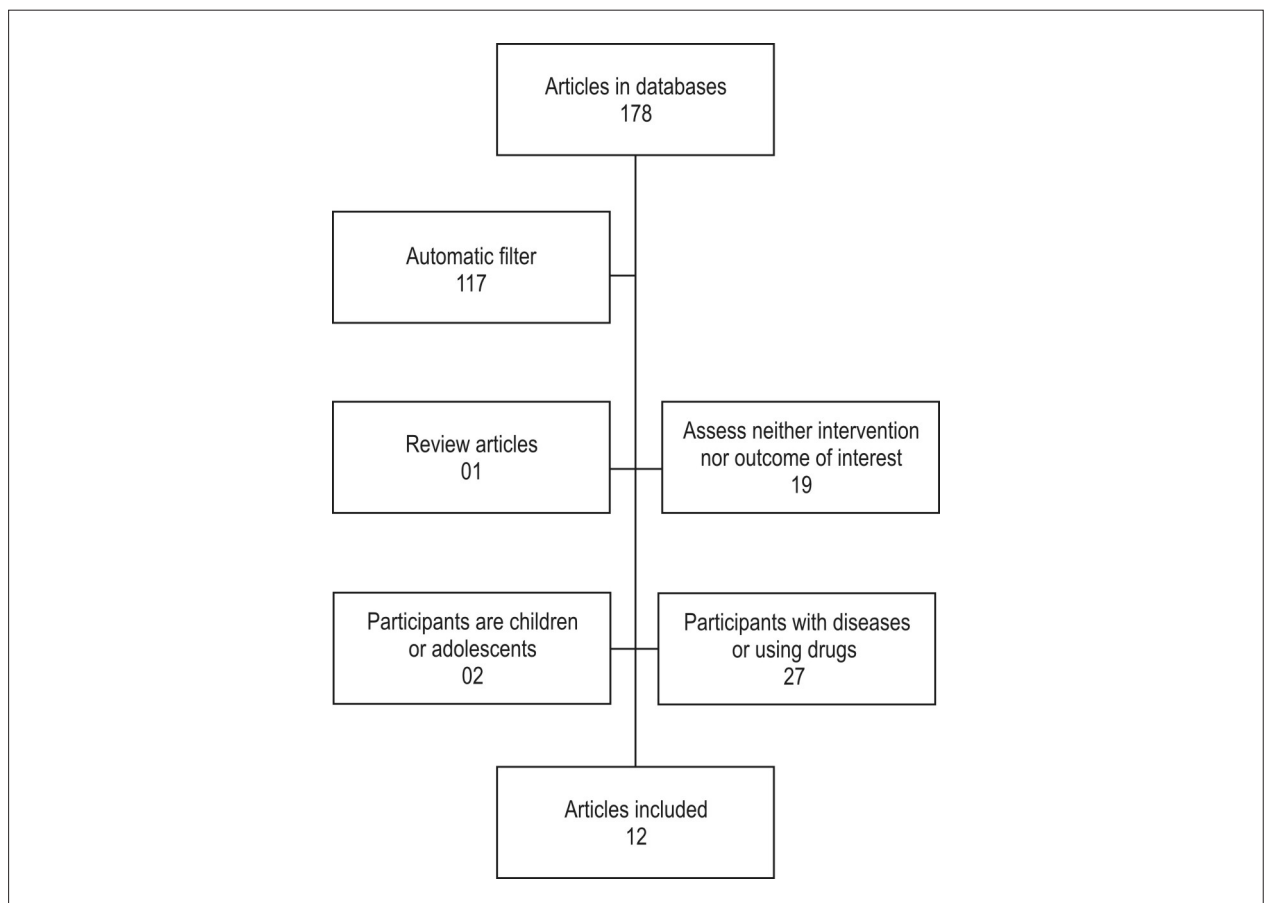


Figure 1 - Flow chart of study selection

comparisons between groups or moments was described); 8. regression analysis (to assess the association between the intervention and outcome variables) (Table 1).

Data extraction

The following data were obtained from each publication: name of the first author and year of publication; population data, demography and size of the sample; blood sample collection time; variables studied and the method through which they were analyzed; the protocol, intensity and volume of exercise; and the results of the study, with correlation between intervention and outcome.

Characteristics of the participants

The exercise training level of the participants ranged from non-active or sedentary^{17,18} to athletes who finished a 246-km foot race in up to 36 hours¹⁹. Thus, in the present systematic review, a classification based on the volume of exercise training of the participants of all studies was elaborated to standardize and classify the individuals into only three levels of physical fitness as follows: sedentary group, participants whose volume of aerobic training was lower than or equal to once a week, with 20-minute bouts each; active group, those that practiced aerobic exercise twice or three times a week, with 20-minute bouts each; trained group, participants whose volume of aerobic training was greater than or equal to four times a week, with 30-minute bouts each.

Table 1 – Methodological quality of the studies

Study	Inclusion criteria	Random allocation	Sample	Blind	Methods	Measures	Statistical analysis	Regression analysis	Result
Adams et al., 2008 ⁷	+	-	68 trained ♂, 57±6 years	+	+	+	+	-	6/8
Bonsignore et al., 2010 ⁸	+	-	Marathon (n = 9,44 ±12 years); 1,500-m foot race (n = 8,43 ±11 years)	+	+	+	+	+	7/8
Cubbon et al., 2010 ²⁶	+	-	15 sedentary Europeans, 28±1 year; 15 sedentary South-Asians, 30±1 years	+	+	+	+	+	7/8
Goussetis et al., 2009 ¹⁹	+	-	10 trained ♂, 43±1 years; 10 sedentary ♂, 42±10 years	-	+	+	+	-	5/8
Jenkins et al., 2009 ¹⁷	+	-	8 trained ♂, 25±4 years; 8 active ♂, 25±3 years	+	+	+	+	-	6/8
Laufs et al., 2005 ²⁷	+	+	25 trained ♂, 28±7 years	+	+	+	+	-	7/8
Lockard et al., 2010 ¹⁸	+	-	12 trained ♂, 62±2 years; 11 active ♂, 65±2 years	-	+	+	+	-	5/8
Möbius-Winkler et al., 2009 ²²	+	-	18 trained ♂, 32±2 years	-	+	+	+	+	6/8
Thijssen et al., 2006 ²¹	+	-	8 sedentary elderly (67-76 years); 8 sedentary youngsters (19-28 years); 8 trained youngsters (18-28 years)	-	+	+	+	+	6/8
Thorell et al., 2009 ²⁵	+	-	11 trained individuals (9 ♀ and 2 ♂), 31±7 years	-	+	+	+	-	5/8
Van Craenenbroeck et al., 2008 ²³	+	-	11 active individuals, 24±1 years; 14 active individuals, 36±9 years	-	+	+	+	-	5/8
Yang et al., 2007 ²⁴	+	-	16 sedentary ♂, 25±3 years	+	+	+	+	+	7/8

(+) – applied by the study; (-) – not applied by the study.

Exercise characteristics

The response of EPCs to exercise can vary according to the type of protocol used^{7,8,20}. Thus, the following data were obtained from each study: intensity, volume, duration, and frequency of physical exercise training; percentage values of maximal heart rate, maximal oxygen consumption (VO_2) and anaerobic threshold of participants; authors' description of the exercise protocol used, enabling the classification of the exercises into the following three categories: 1. long/ultralong duration exercise (half-marathon, marathon or ultramarathon training); 2. maximal intensity exercise, usually progressive and reaching maximal effort and/or maximal VO_2 in 5 to 20 minutes. e.g. typical examples are the conventional exercise test or cardiopulmonary exercise test (CPET); and 3. Submaximal intensity exercise, usually using a percentage of the anaerobic threshold and/or maximal VO_2 as reference, and with maximal duration of one hour. In the present study, exercise intensity ranged from 80% to 100% of the anaerobic threshold or exceeded 75% of the maximal VO_2 .

Results

The 12 studies included were classified as of high quality and were as follows: seven cross-sectional studies; four longitudinal studies; and one study with both designs. In all studies included, the methodological procedures adopted in dimensioning and selecting the samples, the measurements of the variables, and the ethical aspects were sufficiently described. The composition of the samples (total of 286 participants) varied in age, from 18 to 80 years, and physical condition, from sedentary to trained (tab. 1).

Most studies reported significant effects of different types of exercises on the mobilization of EPCs to peripheral blood. However, neither the volume of training nor the physical condition of the participants has influenced the number of EPCs prior to exercise^{17,18,21}. In addition, after one single bout of submaximal exercise, the number of EPCs increased in the blood stream of the participants with a higher volume of training¹⁷, suggesting that EPC mobilization requires an acute disturbing stimulus in the organism.

Tables 2a-2c show specific information about the studies included, which were classified as: 1. long/ultralong duration exercise (n = 4 studies, tab. 2a); 2. maximal intensity exercise (n = 4 studies, tab. 2b); 3. submaximal intensity exercise (n = 5 studies, tab. 2c).

Long/ultralong duration exercise

An increase in the number of CD34+/KDR+ cells or EC-CFUs after long/ultralong duration exercise (marathon/ultra-distance running) was observed in three of the four studies assessed (tab. 2a). An elevation in inflammatory markers, mainly interleukin-6, was observed after exercise in the studies assessed^{8,19,22}.

In trained individuals, the number of CD34+/KDR+ cells increased approximately twice at the end of a marathon (p < 0.005), while the number of circulating angiogenic cells (CAC) increased approximately three times (8.5 ± 1.9 vs. 30.2 ± 4.6 cells/ 1×10^6 mononuclear cells; p < 0.0001), with

a return to baseline levels on the next morning⁸ (10.9 ± 1.8 cells/ 1×10^6 mononuclear cells). In addition, in trained men, the number of EC-CFUs increased by 11-fold (44.5 ± 2.5 vs. 494.5 ± 27.9 /mL) at the end of an ultra-distance foot race (246 km) in up to 36 hours, and remained increased for 48 hours (428.5 ± 31.5 /mL; p < 00001) after the end of the race¹⁹. In addition, in trained runners, the number of CD34+/VEGFR2+ cells did not change after a marathon⁷ (117.0 ± 8.0 vs. 128.0 ± 9.0 cells/mL; p = 0.33). One exercise bout on the cycle ergometer at 70% of the anaerobic threshold revealed a 5.5-fold increase in CD34+/KDR+ cells (p < 0.001) and a 3.5-fold increase in CD133+/KDR+ cells from 210 minutes of exercise (p < 0.001) in a time-dependent way, with return to baseline levels after 24 hours²². The VEGF-C levels increased on the day following a marathon and were greater than those of maximal exercise (p < 0.05)⁸. Ten minutes after exercising on the cycle ergometer²², VEGF plasma levels showed a 1.9-fold maximal increase (79.8 ± 15.4 pg/mL vs. 132.6 ± 32.1 pg/mL; p < 0.05), which significantly correlated with the number of CD133+/KDR+ cells (r = 0.67; p = 0.0045)²². However, Adams et al⁷ have reported a reduction in VEGF plasma levels after a marathon (48.9 ± 8.0 vs. 34.0 ± 7.5 pg/mL; p < 0.05), which did not correlate with the number of CD34+/VEGFR2+ cells.

Maximal intensity exercise

Four studies assessed the effect of one single bout of maximal exercise on the number of EPCs^{8,21,23,24} (tab. 2b).

Yang et al²⁴ have reported a significant increase in CD34+/KDR+ cells (p < 0.05) and UEA-1 lectin-FITC+/Dil-acLDL+ cells (p < 0.05) 30 minutes after a CPET on the treadmill. In trained individuals, the number of CD34+/KDR+ cells has increased three times (p < 0.01) after the end of a 1,500-m foot race⁸. The levels of CAC have also increased significantly after a 1,500-m foot race⁸ (p < 0.005). Similar results have been shown by Van Craenenbroeck et al²³, who have reported a 76% increase in CD34+/KDR+ cells in young individuals aged 24 ± 1 years (15.4 ± 10.7 vs. 27.2 ± 13.7 cells/mL; p = 0.01) and a 69% increase in adults aged 36 ± 9 years (30.9 ± 14.6 vs. 52.5 ± 42.6 cells/mL; p = 0.03); however, no significant change was observed in the percentage of EC-CFUs (11.9 ± 10.9 vs. 9.0 ± 8.3 ; p = 0.2). Only Thijssen et al²¹ have reported no change in the number of EPCs after CPET in trained and sedentary young and elderly individuals (p > 0.05).

Most studies have shown no significant change in VEGF plasma levels after one single bout of maximal exercise^{21,23,24}, however, Bonsignore et al⁸ have reported an increase in VEGF-C plasma levels (7.3 ± 1.8 vs. 8.8 ± 1.7 pg/mL; p < 0,001) after a 1,500-m foot race (tab. 2b).

According to the study by Yang et al²⁴, an increase in nitric oxide (NO) plasma levels has been observed 30 minutes after a treadmill CPET (p < 0.05). In addition, linear regression analysis has revealed a positive correlation (r = 0.70; p < 0.05) between EPC increase and NO levels²⁴.

Submaximal intensity exercise lasting less than one hour

Of the five studies^{17,18,25-27} assessing the effect of one bout of submaximal exercise on the number of EPCs or of EC-CFUs

Table 2a – Results of studies using long/ultralong duration exercise protocols

Study	Blood collection	Identification of EPC	Exercise protocol	Variables/ Methods	Results	Correlation (intervention/ outcome)
Adams et al., 2008 ⁷	Before and immediately after the marathon	Cytometry: CD34+/VEGFR2+	Dusseldorf Marathon 2006, duration \cong 4h	Plasma VEGF and EGF/ ELISA	No change in the number of CD34+/VEGFR2+ cells and \downarrow VEGF after the marathon	No correlation
Bonsignore et al., 2010 ⁸	2-3 days before, immediately after, and 18-20h after the marathon	Cytometry: CD34+/KDR+; Culture: CAC	Palermo International Marathon 2005, duration \cong 3h30min	Plasma VEGF-A, VEGF-C and VEGF-D/ ELISA	\uparrow CD34+/KDR+ (\cong 2x), \uparrow CAC (3x) immediately after and \uparrow VEGF-C (18-20h after)	–
Goussetis et al., 2009 ¹⁹	Before, immediately after and 48h after the foot race	Culture: EC-CFU	246-km ultradistance race (Spartathlon), duration \cong 32h8min	–	\uparrow EC-CFU (11x) immediately after and 48h after the race	–
Möbius-Winkler et al., 2009 ²²	Before, during (5, 10, 15, 30, 60, 90, 120, 150, 180, 210, 240min) and after exercise (30, 60, 120, 1,440min)	Cytometry: CD34+/KDR+ and CD133+/KDR+	Cycle ergometer test at 70% of the AT for 4h	Plasma VEGF/ ELISA	\uparrow CD34+/KDR+ (5,5x), \uparrow CD133+/KDR+ (3,5x) and \uparrow VEGF after 10min	Correlation between CD133+/KDR+ cells and VEGF ($r = 0.67$)

EPC – endothelial progenitor cells; (+) – positive; CD34 – cluster of differentiation 34; VEGFR2 – receptor 2 of the vascular endothelial growth factor; VEGF – vascular endothelial growth factor (types A, C, D); EGF – epidermal growth factor; ELISA – enzyme-linked immunosorbent assay; KDR – kinase insert domain receptor of the vascular endothelial growth factor; CAC – circulating angiogenic cell; EC-CFU – endothelial cell colony forming unit; CD 133 – cluster of differentiation 133; AT – anaerobic threshold.

Table 2b – Results of studies using maximal intensity exercise protocols

Study	Blood collection	Identification of EPC	Exercise protocol	Variables/ Methods	Results	Correlation (intervention/ outcome)
Bonsignore et al., 2010 ⁸	Before and after the race (3-5min)	Cytometry: CD34+/KDR+; Culture: CAC	1,500-m foot race (duration: 5min \pm 35s); maximal heart rate 178 \pm 6 bpm	Plasma VEGF- A, VEGF-C and VEGF-D/ ELISA	\uparrow CD34+/KDR+ (\cong 3x), \uparrow CAC and \uparrow VEGF-C	–
Thijssen et al., 2006 ²¹	Before and 10min after exercise	Cytometry: CD45-/CD34+/VEGFR2+	Maximal CPET (cycle ergometer) Youngsters: 20W /min Elderly: 10W/min	Plasma VEGF/ ELISA	No change in the number of CD34+/VEGFR2+ cells in youngsters and elderly (trained or sedentary); no change in VEGF in youngsters and elderly (trained or sedentary)	No correlation
Van Craenenbroeck et al., 2008 ²³	Before and 10min after exercise peak	Cytometry: CD34+/KDR+; Culture: EC-CFU	Maximal CPET (cycle ergometer) at 40W and 20W/min increment	Plasma nitrate-nitrite/ Colorimetry; VEGF/ ELISA	\uparrow CD34+/KDR+ after exercise in both groups	No correlation
Yang et al., 2007 ²⁴	Before and 30min after exercise	Cytometry: CD34+/KDR+; Culture: UEA-1 lectin-FITC +/- Dil-acLDL+	Maximal CPET (treadmill - modified Bruce protocol, duration: 9.6 \pm 2.2 min and 10.2 MET)	Plasma nitrate-nitrite and culture/ Colorimetry; VEGF/ ELISA	\uparrow CD34+/KDR+, \uparrow UEA-1 lectin-FITC+/Dil-acLDL+ and \uparrow plasma nitrate-nitrite after exercise	Positive correlation between EPC and plasma nitrate-nitrite ($r = 0.70$)

EPC – endothelial progenitor cells; (+) – positive; CD34 – cluster of differentiation 34; VEGFR2 – receptor 2 of the vascular endothelial growth factor; VEGF – vascular endothelial growth factor (types A, C, D); ELISA – enzyme-linked immunosorbent assay; KDR – kinase insert domain receptor of the vascular endothelial growth factor; CAC – circulating angiogenic cell; EC-CFU – endothelial cell colony forming unit; UEA-1-FITC – Ulex Europaeus agglutinin type 1- conjugated to fluorescein isothiocyanate; Dil-acLDL – 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine-labelled acetylated low-density lipoprotein; CPET – cardiopulmonary exercise test; MET – metabolic equivalent; W – watts; bpm – beats per minute; CD45 – cluster of differentiation 45.

Table 2c – Results of the studies using submaximal intensity exercise protocols lasting less than one hour

Study	Blood collection	Identification of EPC	Exercise protocol	Variables/ Methods	Results	Correlation (intervention/ outcome)
Cubbon et al., 2010 ²⁶	Before and 20min after exercise	Cytometry: CD34+/KDR+; CD133+/CD34+/KDR+	Cycle ergometer test at 80% of the AT for 30min	eNOS blockade (L-NMMA)	↑CD34+/VEGFR2+, ↑CD133/CD34+/KDR after exercise; ↓CD34+/KDR+; ↓CD133+/CD34+/KDR+ after L-NMMA infusion	–
Jenkins et al., 2009 ¹⁷	Before and 30min after exercise	Culture: EC-CFU	Treadmill test at 75%-80% of VO ₂ max. for 30min	NOi /fluorescent dye; oxidative stress/ RT-PCR	↑EC-CFU in the trained group after exercise; ↓NADPH-ox (gp9 ^{1phox}) in both groups after exercise; ↑NOi and ↓NADPH-ox (p47 ^{phox}) in the active group, after exercise	–
Laufs et al., 2005 ²⁷	Before and 10min, 30min, 2h, 6h and 24h after exercise	Cytometry: CD34+/KDR+; Culture: EC-CFU	Foot races: 1) ↑ intensity: 30min, 100% of the AT; 2) Moderate intensity: 30min, 80% of the AT; 3) Short and moderate intensity: 10min, 80% of the AT	Plasma VEGF / ELISA	↑CD34+/VEGFR2+ 30min after high- and moderate-intensity foot races; ↑CFU 30min after high-intensity foot race	No correlation
Lockard et al., 2010 ¹⁸	Before and 30min after exercise	Cytometry: CD34+/VEGFR2+; Culture: EC-CFU	Treadmill test (75% ± 5% of VO ₂ max.) for 30min	–	No change in the number of CD34+/VEGFR2+ cells and EC-CFU before and after exercise	–
Thorell et al., 2009 ²⁵	Before and 1h, 24h and 48h after exercise	Culture: late outgrowth endothelial cell colonies	Spinning exercise at 80% of maximal heart rate for 1h	Plasma VEGF/ ELISA	↑number of late colonies (2x) 1h after exercise	Correlation between the number of colonies and VEGF levels ($r = 0.903$)

EPC – endothelial progenitor cells; (+) – positive; CD34 – cluster of differentiation 34; VEGFR2 – receptor 2 of the vascular endothelial growth factor; VEGF – vascular endothelial growth factor (types A, C, D); ELISA – enzyme-linked immunosorbent assay; KDR – kinase insert domain receptor of the vascular endothelial growth factor; EC-CFU – endothelial cell colony forming unit; CD 133 – cluster of differentiation 133; AT – anaerobic threshold; eNOS – endothelial nitric oxide synthase; L-NMMA – N5-[imino(methylamino)methyl]-L-ornithine citrate; NOi – intracellular nitric oxide; NADPH oxidase – nicotinamide adenine dinucleotide phosphate oxidase; RT-PCR –real-time polymerase chain reaction; VO₂ max. – maximal oxygen consumption.

(tab. 2c), three studies^{17,18,27} have used a protocol based on a 400-m foot race or a treadmill race for 30 minutes and exercise intensity between 68% and 82% of maximal VO₂.

Although Lockard et al¹⁸ have found no alteration in the number of CD34+/VEGFR2+ cells ($p > 0.05$) and EC-CFUs ($p > 0.05$) 30 minutes after an exercise with intensity of $75\% \pm 5\%$ of maximal VO₂, most studies do not seem to support that finding. Jenkins et al¹⁷ have shown that, in trained men, EC-CFUs increased significantly 30 minutes after submaximal exercise ($p = 0.02$). Moderate or intense exercise (80% and 100% of the anaerobic threshold, respectively) of same duration has increased the number of circulating EPCs (CD34+/VEGFR2+ and UEA-1 lectin-FITC+/Dil-acLDL+ cells), with a peak response between 10 and 30 minutes after the end of the exercise bout ($p < 0.01$). The number of EC-CFUs has also increased 30 minutes

after high-intensity running²⁷ ($p < 0.05$). In addition, one cycle ergometer exercise bout, lasting 30 minutes and at 80% of the anaerobic threshold²⁶, caused an increase in the number of CD34+/KDR+ and CD133+/CD34+/KDR+ cells in European Caucasians ($85.4 \pm 1.3\%$ and $73.9 \pm 9.1\%$; respectively) and in Asians ($53.2 \pm 6.9\%$ and $48.3 \pm 8.8\%$; respectively) 20 minutes after the end of the exercise bout. In another study²⁵, one single bout of spinning exercise, with mean duration of 44.3 ± 3.4 minutes and heart rate ranging from 77% to 95% of maximal heart rate, could double the number of late outgrowth endothelial cell colonies, another type of EPC colony. In addition, VEGF levels have shown a significant correlation with the number of late outgrowth endothelial cell colonies ($r = 0.903$)²⁵. The effects of the single bout of spinning exercise have decreased over time with return to baseline levels after 48 hours.

In trained men, exercise has acutely reduced the messenger RNA (mRNA) levels of NADPH oxidase (gp91^{phox}) in EC-CFUs¹⁷ ($p = 0.02$). Active men showed an increased production of intracellular NO ($p = 0.004$) and a reduced expression of the p47^{phox} subunit of NADPH oxidase ($p < 0.05$), but with no difference for other oxidative stress markers¹⁷ ($p > 0.05$). The infusion of L-NMMA, an inhibitor of the endothelial nitric oxide synthase (eNOS), has reduced the number of EPCs (CD34+/KDR+: -3.3% vs. 68.4%; $p < 0.001$; CD133+/CD34+/KDR+: 0.7% vs. 71.4%; $p < 0.001$). In addition, linear regression analysis has shown a positive correlation between flow-mediated vasodilation and CD34+/KDR+ ($r = 0.41$; $p = 0.02$) and CD133+/CD34+/KDR+ cells ($r = 0.39$; $p < 0.04$)²⁶.

Discussion

Recent studies^{15,24} have shown that the exercise-induced improvement in endothelial function is mainly due to the mobilization of EPCs to the peripheral circulation, where they participate in the processes of neovascularization and endothelial repair¹⁵. Thus, physical exercise is an important method to promote health for cardiovascular system in both healthy individuals²⁸ and those with cardiovascular risk factors^{29,30}.

Long/ultralong duration exercise

Marathons, half-marathons, and ultramarathons are models of induced inflammation that stimulate the increase in the circulating levels of pro- and anti-inflammatory mediators³¹, promoting the release, migration and differentiation of bone marrow stem cells³². That type of exercise also increases the plasma levels of cytokines and endothelial activation markers, providing an opportunity to assess the physiological mechanisms related to vascular repair, before an irreversible tissue damage has occurred³³.

Long/ultralong duration exercises have shown to significantly increase the concentration of progenitor cells, especially the endothelial ones, and leukocytes^{8,19,22}. Interleucin-6 plasma levels have increased during and after long/ultralong duration exercises, evidencing their inflammatory character^{8,19}.

In addition, the highest VEGF plasma levels have been observed after that type of exercise. Elevated VEGF levels have been believed to associate with the presence of tissue hypoxia, favoring the stimulus of EPC mobilization and endothelial repair. Thus, that increase in VEGF seems to demonstrate a mechanism of physiological adaptation to long/ultralong duration exercise, suggesting the existence of a positive correlation between exercise intensity and the release of growth factors⁸.

Exercise duration, on its turn, seems to be associated with the permanence of the exercise effects on EPC. Protocols of extreme exercises¹⁹ have shown longer-lasting responses (up to 48 hours after the end of the exercise bout) than marathons do (up to 24 hours after the end of the exercise bout)⁸.

Finally, the difference in age between the populations studied is believed to explain the controversial results obtained, because age influences the number and function of EPCs³⁴.

Maximal intensity exercise

Maximal intensity exercises are characterized by a large increase in vascular shear stress³⁵, which can be considered a subacute effect of physical exercise²⁸. Most results have shown that one single maximal exercise bout seems to increase the number of EPCs^{8,23,24}, NO plasma levels²⁴, and VEGF-C plasma levels⁸. However, a positive correlation has been found only between the increase in the number of EPCs and NO levels²⁴. In addition, the magnitude of the increment used in exercise protocols seems to contribute to more significant results²⁴.

Some studies using similar conditions of exercise and population profile^{21,23,24} have shown very different results for the number of EPCs at baseline condition. At least part of that heterogeneity is due to the absence of a standardized protocol to quantify, identify and analyze EPCs. Thus, comparing and consolidating the results of the different studies is difficult, and that constitutes the greatest methodological challenge to knowledge advance in that area.

Submaximal intensity exercise lasting less than one hour

Successive submaximal intensity exercise bouts comprise the training; thus, the study of the physiological responses to that type of exercise can contribute to the understanding of the global process of endothelial adaptation to physical training³⁶.

In young individuals, submaximal intensity exercise has caused a significant increase in EC-CFUs, EPCs and late outgrowth endothelial cell colonies. In addition, circulating EPC levels have not varied in response to a greater intensity exercise of same duration²⁷. However, a strenuous protocol, combining submaximal intensity and longer duration (approximately one hour), has shown a longer-lasting effect of exercise on EPCs, observed up to 48 hours after the end of exercise bout. In addition, that protocol has strongly correlated with VEGF plasma levels ($r = 0.903$)²⁵.

Because of the large interindividual variability found, no significant difference in the number of EPCs or EC-CFUs has been observed after exercise in elderly athletes¹⁸.

Studies are controversial regarding the influence of physical condition on EPCs. Trained youngsters¹⁷ have shown a significant increase in EC-CFUs. However, an increase in CD34+/KDR+ or CD34+/VEGFR2+ cells has been observed in both sedentary²⁶ and trained individuals²⁷.

The major mechanism involved in EPC mobilization after performing a submaximal exercise seems to be associated with NO bioavailability. In active men, exercise has caused a reduction in the expression of NADPH oxidase, an important cause of oxidative stress on the cardiovascular system, and an increase in intracellular NO levels in EC-CFUs¹⁷. In sedentary individuals, exercise has caused a significant reduction in the mobilization of CD34+/VEGFR2+ cells after eNOS blockade with L-NMMA infusion²⁶. Those data suggest that NADPH oxidase might be one of the mechanisms involved in the inhibition of intracellular NO production in EC-CFUs¹⁷.

So far, some studies^{9,26,35} have shown one of the possible mechanisms by which acutely exercise-induced NO production contributes to increase EPCs. In those studies, the authors have proposed that exercise promotes an increase in

endothelial shear stress, elevating intracellular calcium levels, and, consequently, activating eNOS. The activation of that enzyme, on its turn, promotes NO increase, metalloproteinase 9 activation, and the release of the soluble kit ligand, which is crucial for the mobilization of EPCs from the bone marrow to peripheral circulation²⁶.

Despite the fact that an ischemic tissue is by definition a pathological tissue, and, thus, lacks in healthy individuals, long and ultralong duration exercises are models of physiological ischemia, because they cause changes in the anaerobic glycolytic metabolism, in addition to generating oxidative stress²³. Oxygen reactive species are essential for hypoxia³⁷. Hypoxia inducible factor-1 is activated after single exercise bouts³⁸, stimulating the gene expression of important EPC mobilizing molecules, such as VEGF¹⁰. In the presence of VEGF, eNOS activation and NO increase occur, thus mobilizing EPCs from bone marrow²⁶. In addition, NO is also the key molecule in shorter-duration exercises, and the increase in its availability seems to depend especially on two mechanisms: in the first, the activation of protein kinase 3 mediated by the increase in shear stress activates eNOS, which increases NO production³⁹. In the second mechanism, exercise reduces NADPH expression, increasing NO bioavailability, and, consequently, EPC mobilization from the bone marrow occurs. Considering that, one can suggest that single exercise bouts of different intensities and durations mobilize EPCs to peripheral blood through different mechanisms; however, NO seems to be a molecule common to both mobilization pathways.

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Conclusion

The mobilization of endothelial progenitor cells was more marked in long/ultralong duration exercises and seems to be associated with the vascular endothelial growth factor plasma levels. Maximal exercises and submaximal exercises lasting less than one hour cause a lower magnitude increase in the number of circulating endothelial progenitor cells, and their main mechanism of mobilization seems to be nitric oxide bioavailability.

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Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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