HIGHLIGHTED TOPIC | Mechanism of Beneficial Effects of Physical Activity on Atherosclerosis and Coronary Heart Disease

Role of endothelial progenitor cells in the beneficial effects of physical exercise on atherosclerosis and coronary artery disease

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Lenk K, Uhlemann M, Schuler G, Adams V. Role of endothelial progenitor cells in the beneficial effects of physical exercise on atherosclerosis and coronary artery disease. J Appl Physiol 111: 321–328, 2011. First published February 4, 2011; doi:10.1152/japplphysiol.01464.2010.—In clinical trials as well as in several animal experiments it is evident that physical exercise is a powerful tool to positively influence the development and/or progression of atherosclerosis and coronary artery disease (CAD). The main target of physical exercise seems to be the maintenance of an intact endothelial cell layer. Since the discovery that endothelial progenitor cells (EPCs) are present in the circulation and the knowledge that exercise, either as a single exercise bout or an exercise training program, have the potency to mobilize EPCs from the bone marrow, the contribution of the EPCs for the preservation or repair of the endothelial cell layer is still under debate. Either the EPCs differentiate into mature endothelial cells, or they stimulate via a paracrine mechanism mature endothelial cells to proliferate. It is still unclear, if the exercise-induced mobilization of EPCs is casually related to the improvement of endothelial function. This review will discuss the role of endothelial progenitor cells in the beneficial effects of physical exercise on atherosclerosis and coronary artery disease.

CORONARY ARTERY DISEASE (CAD) is still the main cause of mortality in many countries and results from the development of atherosclerosis, which is considered as a gradual process of endothelial dysfunction and inflammation (42, 48). Large epidemiological studies in healthy subjects (8, 38, 39, 52, 53, 56, 57) and in patients with documented cardiovascular disease (CVD; 39, 56) have demonstrated an inverse, graded, independent, and robust association between fitness status and mortality. Furthermore, based on many clinical and experimental studies, it is evident that exercise training is a very potent tool in secondary prevention (reviewed in Ref. 45). A primary target of the exercise intervention seems to be the impaired endothelial function (20, 65). At the molecular level the bioavailability of nitric oxide (NO) is the central key component regulating endothelial function. The concentration of NO is either upregulated by an increased activity of endothelial nitric oxide synthase (eNOS; 23) or by a reduced production of reactive oxygen species (ROS; 3).

Another feature often observed in patients with CAD is the vasoconstrictive reaction of the vessel after the application of acetylcholine (4). This is due to a damage of the endothelial cell layer, whereby acetylcholine can react directly with the smooth muscle cells and trigger a vasoconstrictive response. EPCs are immature cells capable of differentiating into mature endothelial cells and thereby repairing the damaged endothelial cell layer (81). After the first description and isolation of EPCs by Asahara et al. in 1997 (5), experimental and human studies demonstrated that physical exercise has the potency to mobilize EPCs (2, 44) and that these cells can contribute to the maintenance and integrity of the endothelial cell layer (37). The importance of EPCs is supported by the observation that EPC number and function correlate with the number of cardiovascular risk factors (87) and even predict cardiovascular events and death (90). The aim of this review is to provide a critical review of the current literature linking physical exercise, development of atherosclerosis/CAD, and EPCs.

ENDOTHELIAL PROGENITOR CELLS

Adult bone marrow (BM) is a rich reservoir of tissue-specific stem and progenitor cells. Among these, a scarce population of cells described as EPCs can be mobilized by various stimuli into the circulation to contribute to the neoangiogenic process or to the repair of the damaged endothelial cell layer. In patients with atherosclerosis or cardiovascular risk factors, repair of endothelial damage by progenitor cells appears to be attenuated due to a decrease in their number and...
a reduced regenerative capacity (25). At the molecular level, this has been predominantly attributed to cellular senescence and impairment of proliferation (25, 41). In addition, it is conceivable that inflammatory cytokines, which are circulating in high concentration in particular in patients with end-stage chronic heart failure (CHF), exert myelosuppressive effects and, thereby, preclude the release of EPCs that are urgently needed for vascular regeneration (19, 78). Alternatively, the accelerated vascular damage might lead to an exhaustion of competent circulating EPCs in the blood and the BM in patients with CVD (35).

Definition and Detection

Even 14 years after the first description of EPCs by Asahara and colleagues (5), no unique marker set for the characterization of an EPC has been reported. There is still an ongoing debate whether these cells represent a structurally and functionally homogeneous cell population (27, 54).

Another critical point in the definition of the cells involved in repairing damaged endothelium is the nomenclature for the cells used in different studies. Some names are based purely on cell surface markers like EPCs (5), CEPs (circulating endothelial precursors; 21), CACs (circulating angiogenic cells; 64), whereas other names deviated from properties observed in cell culture like CFU-ECs (colony forming unit endothelial cells; 95), or early and late outgrowth EPCs (25). This makes it rather difficult to compare results obtained in different studies.

Although the BM has been shown to be a principal source of EPCs, cells with endothelial cell-like properties have also been isolated from adipose and cardiac muscle tissue (82). Currently, EPCs are defined and quantified by three different methods. In one method, mononuclear cells are isolated by density gradient centrifugation and plated on fibronectin- or gelatin-coated tissue culture plates. After 4 to 7 days in culture, the cells have the ability to take up acetylated low density lipoprotein (acLDL) and to bind lectin. In the case that acLDL and lectin are labeled with different fluorescent dyes, double positive cells can be quantified either under the microscope or by flow cytometry. The disadvantage of this simple method is, that under nearly identical culture conditions especially monocytes can differentiate into macrophages (17) expressing proteins normally expressed by endothelial cells. Therefore, it is not possible to discriminate between EPCs and a macrophage displaying endothelial markers (64, 71, 96). The second method uses specific antibodies labeled with different fluorescent dyes directed against specific cell surface marker. For subsequent quantification the cells are analyzed by flow cytometry. In most cases a combination of a hematopoietic stem cell marker, like CD34, CD117 (cKit), or CD133, with a marker for endothelial cells, like vascular endothelial growth factor receptor 2 (KDR) or VEGFR-2, are used to identify EPCs (summarized in Refs. 1, 28, 81). The combination of CD34+/KDR+ is the most often used set for quantification of EPCs in the current literature. Nevertheless, whether cells identified in this way are true EPCs has to be questioned, since it could recently be demonstrated that CD34+CD133+/VEGFR-2+ cells were devoid of vessel forming activity (10).

Finally EPCs can be counted by colony forming cell assays (25, 30). These procedures claim to avoid contamination for early adherent cells such as differentiated monocytic or possi-

ble mature endothelial cells (73). After replating non-attached cells onto fibronectin in endothelial specific medium, typical clusters of round cells centrally with spindle-shaped cells sprouting at the periphery appear. Some but not all of the cells inside the colony-forming unit are indeed double positive for acLDL and lectin staining (25). For quantitative analysis the number of colonies finally appearing on the culture plate are counted (colony forming units assay; 25). Nevertheless, one has to be aware that colonies formed by this procedure have to be considered as a mixed population of cells originating mainly from the lymphocyte/monocyte lineage (66, 83).

In summary, all of the above described methods have their individual uncertainties to quantify EPCs. Nevertheless, the approach most often used to enumerate circulating EPCs is flow cytometry using specific antibodies against specific cell surface markers.

PHYSICAL EXERCISE AND MOBILIZATION OF ENDOTHELIAL PROGENITOR CELLS

Stem cells within the bone marrow usually exist in a quiescent state and specific signals stimulate them to differentiate and to be mobilized into the systemic circulation. Beside humoral factors like cytokines (6, 33, 77), hormones (6, 11, 77), chemokines (18, 93), and drugs (13, 49, 88), exercise training has been shown to increase the number of circulating EPCs in healthy individuals and patients with CVD (2, 69). In addition to influencing the amount of circulating EPCs, exercise training also has the potency to positively affect the functional capacity of EPCs. This beneficial effect on migratory capacity could be demonstrated in mice (44) as well as in patients with CHF (69, 84, 84) and myocardial infarction (9).

Despite the observation that physical exercise increases the concentration of circulating EPCs and their functional capacity some questions still remain to be answered.

Single Exercise Bout vs. Exercise Training Program

As pointed out above, several studies clearly demonstrated that an acute bout of exercise either in patients with CAD (2) or healthy subjects (58, 86, 94) resulted in the mobilization of EPCs from the BM. Along with the mobilization of EPCs, metabolic genes increase transiently after an acute bout and returning to near pre-exercise levels 24 h after the end of exercise (60, 61). In addition to the induction of the peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) hypoxia-inducible factor-1 (HIF-1) is activated after a single exercise bout (50). HIF-1 activates the transcription of vascular endothelial growth factor (VEGF), stromal cell-derived factor-1 (SDF-1), and erythropoietin (EPO), which are important molecules inducing EPC mobilization (6, 24).

Beside these humoral factors it is reasonable to assume that reactive oxygen species generated by the NAD(P)H oxidase are essential for hypoxia or EPO-induced mobilization of EPCs (72). The exposure of C57Bl6 wild-type mice or Nox2 knockout (Nox2−/−) mice to hypoxia or EPO resulted in a significant mobilization in the wild-type animals but failed in the Nox2−/− mice. The importance of Nox2-based NAD(P)H oxidase for the mobilization of stem/progenitor cells was also evident in a hindlimb ischemia model (80). In this model the induction of a hindlimb ischemia in C57Bl6 mice resulted in a significant increase of Nox2 expression in BM, which was
associated with an increase in circulating stem/progenitor cells. Mice lacking Nox2 showed a significant reduction of ischemia-induced flow recovery, ROS levels in BM cells, as well as EPC mobilization from the BM. This defect in Nox2 deficient mice could be rescued by the transplantation of BM from wild-type mice.

In contrast to a single exercise bout the mechanisms by which an exercise program over a longer time period mobilizes EPCs is different. The central role of the eNOS/NO system for exercise-mediated increase in EPCs was first documented in animal experiments using eNOS knockout mice (44). Voluntary running in a running wheel over a period of 28 days resulted in a significant upregulation of EPCs, defined as Sca-1+/KDR+ cells, only in wild-type mice, but was significantly blunted in eNOS knockout mice. The same effect could be mimicked in wild-type mice treated with the specific NOS inhibitor L-NAME (44). Mechanistically the following scenario is discussed: increased shear stress activates the eNOS via a PI3K/Akt dependent step (16) leading to a higher concentration of NO. NO in turn activates matrix metalloproteinase 9 (MMP-9) within the BM (32), leading to enhanced mobilization of progenitor cells (Fig. 1). As soon as the EPCs are circulating the most important factors for cell homing and tissue engraftment of the mobilized cells are the local concentration of SDF-1α and its cell receptor CXCR4 (7). This notion is further supported by the observation that mice lacking CXCR4 die in utero due to defects in vascular development (76).

On the basis of the remarks above it is obvious that a single exercise bout as well as an exercise program over a longer time period elevates circulating EPCs. Is it therefore acceptable to assume that a single bout of exercise is sufficient to induce an EPC-mediated vascular protection? For this discussion it is important to realize that the amount of circulating progenitors after a single exercise bout declines immediately back to baseline values (2, 55). Regular physical activity on the other hand leads to elevated circulating EPC levels over a longer time period (69). Therefore, it is reasonable to assume that for keeping the endothelial cell layer intact a single exercise bout is not sufficient compared with a continuous exercise training program.

**Type of Exercise—Resistance vs. Aerobic Endurance Training**

Most studies investigating the effect of exercise training on the mobilization of EPCs from the BM used aerobic endurance exercise training as intervention strategy (2, 44, 69, 70, 74, 75, 84). No data are available so far on the influence of a pure resistance training on EPC concentration and function.

**Duration and Intensity of the Exercise**

To our knowledge there is only one study available analyzing the effect of duration and intensity of a single exercise bout on EPC mobilization (43). In this prospective crossover study, healthy volunteers performed different protocols of running exercise; intensive running [10 or 30 min at 100% of the velocity of the individual anaerobic threshold (IAT)] and moderate running (10 or 30 min at 80% of the velocity of the IAT). Analysis by flow cytometry to detect circulating EPCs

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**Fig. 1.** A model for exercise-induced mobilization of endothelial progenitor cells (EPCs) from the bone marrow. Exercise will activate endothelial nitric oxide synthase (eNOS), resulting in an increase of nitric oxide (NO), and stimulates the expression of circulating factors like VEGF, SDF-1, and Epo. Subsequently, MMP-9 is activated leading to a mobilization of EPCs from the bone marrow. In addition, exercise can induce the expression of EPC cell surface receptors CXCR4 and VLA4, both important for homing of the EPCs.
clearly demonstrated that intensive and moderate exercising for 30 min, but not for 10 min, increased circulating levels of EPCs. Studies analyzing the effect of duration and intensity in patients with CAD are not available at the moment.

REGENERATION OF THE VASCULAR ENDOTHELIAL CELL LAYER

As outlined above, homeostasis of the vascular endothelial cell layer is essential for maintaining proper vessel function. The concept of endothelial cell repair by resident endothelial cells surrounding the damaged cell area (31, 79) has to be extended by the identification of circulating EPCs (5). It is believed that following mobilization into the circulation, EPCs are recruited to the damaged endothelium in response to secretion of SDF-1 (7) or VEGF (46) and after homing and differentiation into mature endothelial cells, these cells are able to repair the damaged endothelial cell layer (Fig. 2).

Beside EPCs liberated from the BM and transported to the damaged endothelial cell layer via the circulation, it recently became evident that EPCs also reside in the vessel wall (30). Murine (68) as well as human studies (15, 30) provide evidence that vascular progenitor cells exist in the vessel wall of arteries and veins. At least based on cell surface marker the blood derived EPCs (CD34+/KDR+) differ from the resident vascular progenitor cells (RPC) isolated from the human fetal arterial wall [CD105+/CD34- and fetal liver kinase (Flk1+)]; 15]. Nevertheless, this provides a new conceptual framework for determining both the origin and function of EPCs in maintaining vessel integrity (Fig. 2).

What evidence do we find in the current literature to support the model that circulating endothelial progenitor cells promote the repair of the vascular cell layer or even angiogenesis?

Direct Incorporation of EPCs into the Damaged Endothelial Cell Layer

Asahara and others (5, 24, 77) provided the first evidence that EPCs may contribute to neoangiogenesis. In mice and rabbit models of ischemia, it was obvious that CD34 and Sca-1 enriched cell populations promoted the formation of new blood vessels, leading to the recovery of the ischemic tissue (5, 34, 77).

In one of the first studies, Kalka and colleagues (34) injected fluorescent labeled EPCs into nude mice with hindlimb ischemia. Subsequent histological evaluation revealed an incorporation of the labeled injected cells into the endothelial cell layer in conjunction with an improved limb vascularization. These results could be confirmed by others using different techniques for cell transplantation as well as different experimental models. For example an analysis of vein (92) or allograft (29) atherosclerosis in a chimeric mouse model with bone marrow from Tie2-LacZ mice revealed, that at least one-third of the endothelial cells of the grafts are derived from circulating progenitor cells.

Beside EPCs mobilized from the BM, non-bone marrow-derived EPCs also have the ability to contribute to vascular repair. For example systemically applied spleen-derived EPCs home to the site of vascular injury, resulting in enhanced reendothelialization (89).

Fig. 2. Potential role of EPCs and resident progenitor cells (RPC) in maintaining an intact endothelial cell layer. Circulating progenitor cells bind to mature endothelial cells (EC) via specific cell surface marker. After binding of EPCs to EC two scenarios are possible as to how EPC contributes to the maintenance of an intact endothelial cell layer. A: after binding EPCs differentiate into mature EC and fill in the gap. B: after binding to ECs EPCs secret growth differentiation factors, thereby stimulating mature endothelial cells to proliferate and thereby closing the gap in the endothelial cell layer.
Beside all the reports showing an involvement of EPCs in vascular reendothelialization, several studies are available in the current literature unable to corroborate these results. The first hint that BM-derived EPCs do not contribute substantially to endothelial cell repair comes from a letter to the editor by Hillebrands and coworkers (26). The authors reported that although BM-derived EPCs can add to endothelial cell replacement in their transplant atherosclerosis model, this contribution is only marginal (1–3%) and therefore may not be relevant. This observation could be confirmed by several other groups (59, 63, 97). Using a chimeric mouse model Ziegelhoeffer and colleagues (97) investigated whether BM-derived cells incorporate in vessels due to hindlimb ischemia or tumor growth. Seven or 21 days after surgery the authors failed to colocalize cells carrying the green fluorescent protein (GFP) with endothelial or smooth muscle cell markers. Since they detected some GFP-positive cells around growing collateral arteries they suggested that in the adult organism BM-derived cells do incorporate into vessel walls, but may function as supporting cells. As discussed by Perry and coworkers (59), most of the above mentioned experiments were performed mainly on acute injury to demonstrate the contribution of EPCs to vascular biology. Using transplantation of GFP-labeled BM cells into radiated eNOS knockout mice, which exhibit a dysfunctional endothelium due to chronic hypertension, no evidence for green-labeled endothelial cells by immunohistological methods could be provided (59). The authors concluded that BM-derived EPCs do not participate in endothelial homeostasis and thus do not repair chronic, systemic endothelial dysfunction.

Taken together, based on reports in the current literature it is unclear at the moment if EPCs participate in a significant manner in endothelial cell repair by replacing directly the damaged endothelial cells.

### EPCs AND PARACRINE MECHANISMS

Emerging evidence in the literature suggest that paracrine signals are essential players in various processes of tissue repair (22, 36, 40). Indeed, it is well known that EPCs isolated from the peripheral blood have the capacity to release a number of proangiogenic factors (22, 64). Recently, the characterization of the EPC secretome has been addressed (62), but further studies are necessary to clarify the activation and the downstream signals. The discovery that cell-free administration of paracrine factors secreted by cultured EPCs achieve angiogenic effects equivalent to cell therapy supports the idea that EPCs act via the secretion of paracrine factors (12). In addition, the paracrine secretion model of EPCs is promoted by the observation that injected EPCs are only detectable for a short time, whereas the positive effect is visible for a much longer time period. For example, Ma and colleagues (15) injected culture-derived EPC labeled with superparamagnetic iron oxide into balloon-injured rabbit carotid arteries. Using magnetic resonance (MR) imaging they detected a reduced stenosis for up to 15 wk but the local MR signal, indicating EPC integration, was only present for 2 wk.

Taken together it seems reasonable to assume that the positive effect observed by EPCs with respect to vascular repair is at least to a great extent due to the secretion of paracrine factors. These factors will subsequently stimulate endothelial cells to proliferate, thereby maintaining an intact endothelial cell layer.

### RELATIONSHIP BETWEEN TRAINING-INDUCED EPC MOBILIZATION AND ENDOTHELIAL FUNCTION

One of the biggest challenges of the near future is to answer the question whether the training-induced mobilization of EPCs and the training-induced improvement in endothelial cell function...
function are closely related, or if both observations are independent from each other.

Several studies in different patient populations demonstrated that the number of circulating EPCs is associated with vascular endothelial function and therefore can be used as surrogate marker for vascular function (14, 47, 67). For example, Liao and colleagues (47) investigated in Type 2 diabetic patients the impact of metformin on EPC count and endothelial function. They could clearly demonstrate that in multivariate regression analysis, the EPC number correlated significantly with changes of FMD before and after treatment. With respect to exercise-induced mobilization of EPC and the improvement in endothelial function, at least two reports are available (74, 91). Stein and colleagues (74) analyzed the impact of aerobic exercise training (12 wk) on endothelial function and EPC content in patients with coronary artery disease. They reported a significant correlation between the improvement of flow-mediated brachial reactivity (ΔFMD) and the relative increase of EPCs. In addition they also detected a positive correlation between the changes in nitric oxide and EPCs. This positive correlation between exercise-induced changes in FMD and EPCs could be confirmed in healthy older men (91).

CONCLUSION

Taken together, we have solid evidence in the current literature that physical exercise has the potency to modulate endothelial function as well as to increase the amount of circulating EPCs and to improve their function. Also the involvement of NO, ROS, and MMP-9 for the mobilization of EPCs from the BM seems to be accepted. Much more uncertain is the mechanism how EPCs contribute to the repair of a damaged endothelial cell layer and finally to a better endothelial function. At the moment two scenarios are discussed in the literature. First, EPCs home at the damaged endothelial cell layer via specific receptors and differentiate toward a mature endothelial cell, thereby closing the gap in the cell layer. Second, the circulating EPCs bind for a limited time period to the damaged endothelial cell area and stimulate the proliferation of mature endothelial cells via the secretion of defined growth factors. At least based on correlation analysis, the modulation of EPCs by physical exercise seems to influence endothelial function positively (Fig. 3). Nevertheless, direct proof for this is still missing, and future experiments have to show that an exercise-induced mobilization of EPCs is necessary and relevant for an improvement of endothelial function in various diseases.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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