




Review

Effect of Physical Exercise on the Release of Microparticles with Angiogenic Potential

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Abstract: Cellular communication has a fundamental role in both human physiological and pathological states and various mechanisms are involved in the crosstalk between organs. Among these, microparticles (MPs) have an important involvement. MPs are a subtype of extracellular vesicles produced by a variety of cells following activation or apoptosis. They are normally present in physiological conditions, but their concentration varies in pathological states such as cardiovascular disease, diabetes mellitus, or cancer. Acute and chronic physical exercise are able to modify MPs amounts as well. Among various actions, exercise-responsive MPs affect angiogenesis, the process through which new blood vessels grow from pre-existing vessels. Usually, the neo vascular growth has functional role; but an aberrant neovascularization accompanies several oncogenic, ischemic, or inflammatory diseases. In addition, angiogenesis is one of the key adaptations to physical exercise and training. In the present review, we report evidence regarding the effect of various typologies of exercise on circulating MPs that are able to affect angiogenesis.

Keywords: microparticles; microvesicles; extracellular vesicles; molecular markers; cell–cell communication; physical exercise; physical activity; angiogenesis; secretome; paracrine signaling; cellular crosstalk

1. Introduction

The capacity to communicate between cells, tissues, and organs is a fundamental requisite to ensure appropriate physiological functions [1]. One of the most important mechanisms governed by cell communication and signaling is represented by the formation of vasculature. It is well known that vascular formation and growth are processes strictly regulated by growth factors and bioactive molecules. Hypoxia represents an important stimulus for angiogenesis: it activates hypoxia-inducible factors (HIFs) that upregulate various angiogenic genes, including vascular endothelial growth factor (VEGF) [2]. On the other hand, other different factors (e.g., thrombospondin-1, statins) are involved in the inhibition of angiogenesis [3].

Neovascular growth can be a physiological (e.g., in injured tissues [4]) or a pathological process, as it occurs in diabetic retinopathy, maculopathy, in tumors, and in many other disorders. Physiological angiogenesis represents the results of an accurate balance between pro-angiogenic and anti-angiogenic signals [5]. In pathological conditions, this balance is compromised and the new vessels do not provide a functional vascular perfusion [6].

Small extracellular vesicles, named microparticles (MPs), can be involved in angiogenic processes [7]. MPs are defined as heterogeneous small membranous vesicular structures derived from different cell types [8]. They are lacking nucleus and ranging in size from 0.1 to 1 μm . The most

abundant population of MPs derives from platelets, but also other different cell types release these vesicles into the circulation such as endothelial cells [9], leucocytes [10] erythrocytes [11], epithelial cells [12], muscle cells, and various tumor cell lines [12]. Therefore, MPs express surface protein and antigens deriving by their parental cell as well as cytoplasmic and nuclear content (e.g., proteins, mRNAs, microRNAs, small-interfering RNAs, long non-coding RNAs), that are signature of their cellular origin [13]. MPs are released in response to cellular activation or apoptosis, elicited by a variety of stimuli, such as inflammation, oxidative stress, or mechanical/hemodynamic fluctuations depending on the involved parental cell [14].

Physical exercise can induce modifications in MP concentration [15]. In fact, MP concentration is modified by stimuli such hypoxia and shear stress [16], conditions elicited to a different extent by physical exercise. It has been demonstrated that MPs produced in response to physical exercise have a stimulating effect on new vessels growth [17].

The purpose of this review is to summarize the effects of different patterns of physical exercise on the concentration of circulating MPs with a known angiogenic potential.

2. Vasculogenesis, Arteriogenesis, and Angiogenesis

Vessels can be formed mainly by three processes: vasculogenesis, arteriogenesis, and angiogenesis. Vasculogenesis is the formation of new vessels that occurs in the embryo as well in the fetal annexes involved in the transportation of maternal nutrients: the placental villi and the yolk sac [18]; it derives from the differentiation of mesodermal cells into angioblasts, that proliferate, migrate, and couple to produce a primitive tube-like vessel. Consequently, vessels continue to develop as angioblasts differentiate in endothelial cells, form a vascular lumen, and deposit the basal lamina [19]. Various growth factors are involved in the molecular signaling of vasculogenesis: the fibroblast growth factor (FGF)-2, involved in mesodermal induction as well as in the induction of the angioblasts from the mesoderm [20], VEGFs family, that plays a pivotal role for its stimulating effects, [21], and finally the transforming growth factor- β family (TGF- β s) that contributes to vasculogenesis, with a dose dependent effect (low doses stimulate, while high doses inhibit endothelial cell growth [22]).

Arteriogenesis is the process whereby small pre-existing arterioles are transformed in functional arteries. One of the major stimuli inducing arteriogenesis is represented by shear stress, a mechanical signal able to activate transcription factors such as early growth response protein-1 (erg-1), that in turn lead to gene expression of chemokines like monocyte chemoattractant protein-1 (MCP-1), and adhesion molecules like intercellular adhesion molecule-1 (ICAM-1). These molecules are important for the docking of monocytes on endothelial cells, a fundamental step in arteriogenesis. Moreover, also growth factors such as VEGF, FGF, and TGF- β are involved in this process [23,24].

Lastly, angiogenesis is the process in which new blood vessels rising from pre-existing vessels, and it can occur mainly through mechanisms such as sprouting and intussusception (also known as vessel splitting or non-sprouting angiogenesis) [25]. Angiogenesis is a fundamental process during embryonic, fetal, and adult life and plays a pivotal role in many physiological process (e.g., as in skeletal growth, in wound healing, in pregnancy); however, angiogenesis represents also an important hallmark of pathological processes such as cancer and several noncommunicable diseases. Angiogenesis is governed by a precise balance between angiogenic growth factors and inhibitors. VEGF-A and its tyrosine kinase receptors represent the main and best characterized signaling pathway involved in developmental angiogenesis [26]. However, other molecules play a key role in endothelial cell proliferation and migration. They are represented by FGFs, platelet-derived growth factor (PDGF), and epidermal growth factor (EGF) [27]. Therefore, angiogenesis is a complex process controlled by several molecules and can have useful as well as detrimental effects on human health, and for this reason the various underlying mechanisms are object of great interest.

3. Extracellular Vesicles Classification

Human cells are able to release various types of membrane vesicles in response to different stimuli, generally called extracellular vesicles (EVs). EVs include exosomes, MPs (also called microvesicles), and apoptotic bodies [28] (Table 1).

Table 1. General characteristics of extracellular vesicles (EVs).

EVs Types	Size	Surface Markers	Mechanism of Production	Content
Exosomes	50–100 nm	Phosphatidylserine (PS, Annexin V ⁺), Lysosomal-Associated Membrane Protein 1 (LAMP1), Tumor Susceptibility 101 (TSG101), Granulophysin (CD63), Target of antiproliferative antibody 1 (CD81), Tetraspanin-29 (CD9)	Fusion of late endosomes/multivesicular bodies (MVBs) with the plasma membrane	Proteins, RNAs, miRNAs
Microparticles	100 nm–1 μm	PS * (Annexin V ⁺) markers of parental cell	Budding/blebbing of the plasma membrane	Proteins, RNAs, miRNAs
Apoptotic bodies	500 nm–3 μm	PS (Annexin V ⁺)	Blebbing of cells undergoing apoptosis	Proteins, RNAs, miRNAs, DNA, organelles

* PS = phosphatidylserine.

Exosomes are vesicles surrounded by a double layer of phospholipids and their size ranges between 50 and 100 nm in diameter. They are released in both physiological condition and upon activation by exocytosis of multivesicular bodies (MVBs) [29]. Exosomes can communicate with other cells either through a direct contact between surface proteins or by vesicles–cell membrane fusion or by endocytosis. They expose phosphatidylserine (PS) on the external membrane and various markers such as LAMP1, TSG101, CD63, CD81, and CD9 [30]. The main functions of exosomes include antigen presentation and immunostimulatory activity. In addition, exosomes are able to horizontally transfer mRNA and miRNA as well as oncogenic receptors [31].

MPs, formerly described as “platelet dust”, are now well known cell–cell communicators and they act as signaling molecules in physiological homeostatic processes or as a consequence of pathological condition including diabetes, chronic kidney disease, rheumatoid arthritis, multiple sclerosis, and cardiovascular diseases [32]. MPs are found in most body fluids such as plasma, saliva, urine, and cerebrospinal fluid. They represent a heterogeneous population of vesicular-like structures composed by a phospholipid bilayer and their sizes range from 100 nm to 1 μm in diameter; however precise cut-off values remain to be established. MPs found in bloodstream may originate from various vascular cells (e.g., platelets, monocytes, erythrocytes, granulocytes) [33]. However, among all circulating MPs, platelet-derived MPs (Plt-MPs) are the most abundant population, representing 70–90% of the total [34]. They are produced by budding of the plasma membrane, in a process called ectocytosis. After their release, MPs carry proteins that are signature of their cell of origin, and transport various enzymes, RNA, and miRNA [35]. MPs generally expose PS on their outer leaflet (Annexin V⁺), however some evidence shows that MPs can also be PS negative [36]. In addition, a subpopulation of various phenotypes of MPs expose tissue factor [37]. The release of MPs is induced upon cell activation or apoptosis.

Apoptotic bodies are EVs larger than MPs and exosomes. Their sizes range from 500 nm to 3 μm and they expose PS; however, their outer membrane differs from MPs due to its permeability [38]. Apoptotic bodies are released by membrane blebbing of cells undergoing apoptosis and they may horizontally transfer their content, such as cell organelles, fragmented DNA, and oncogenes.

4. Methods of Detection of Microparticles

Flow cytometry (FC) is one of the most common methods of detection, quantification, and size evaluation of MPs [39]. In addition, FC allows the phenotypic characterization of MPs using fluorescent antibodies against the diverse antigens expressed by the cell of origin [40]. However, FC has some limitations. For example, MPs with a size ranging from 0.1 to 0.4 μm are too undetected by most cytometers [41]. In addition, the type of instrument, settings, and sample preparation procedures can cause a high level of variability of the results [42].

The nanoparticle tracking analysis (NTA), on the other hand, is able to detect vesicles smaller than those recognized by FC. Such type of analysis correlates the speed of movements, tracked using a microscope, with the size of MPs [43]. Nevertheless, NTA is unable to discriminate vesicles derived from cells from other particles of small dimensions such as high-density lipoprotein (HDL) [44]. Fluorescent nanoparticle tracking analysis (F-NTA), diversely from NTA, is based on the fluorescence of the particles [45].

Other techniques to analyze the EVs are based on image analyses obtained by optical and electron microscopy. Optical microscopy permits to assess the size and morphological features of MPs and of apoptotic bodies, while cannot provide information on their phenotype. This kind of information can, anyway, be obtained by fluorescent microscopy labeling MPs with fluorescent probes that recognize specific surface markers [41]. However, this approach does not permit to specify the size of the EVs [41]. An accurate morphological analysis of MPs is obtained by transmission electron microscopy (TEM), that represents the most used method to assess the composition, morphology, size, and membrane structure of MPs [34]. However, TEM requires a considerable amount of time for both preparation and analysis of the sample.

Western blotting (WB) is a commonly used technique that allows to identify the cellular origin of MPs [46]; anyway, this methodological approach presents some important limitations: indeed, it requires a large amount of MPs to be performed and the analysis needs to be completed with complementary methods such as NTA or TEM [47,48].

Finally, another method for MPs detection is the enzyme-linked immunosorbent assay (ELISA). This is also a quantitative analysis, that measures MPs indirectly: indeed, such method relies on the binding of MPs to specific conjugate-antibodies adsorbed to a well plate [49]. ELISA permits the specification of MPs subtypes, but fresh plasma is indispensable, because the freezing-thawing process leads to an increase of annexin-binding MPs [50].

However, is important to note that most of the studies analyzing MPs in exercise settings utilize the flow cytometric approach [17,51–56].

5. Production Mechanisms of MPs

It is well known that MPs can be released upon activation or apoptotic stimuli; however, most cells constitutively shed MPs by ectocytosis and significant concentrations of these vesicles can be detected in the plasma [57]. The common mechanism for the production of MPs is represented by a change in the asymmetry of the phospholipids composing the plasma membrane. The lipid bilayer of normal cells presents an asymmetrical composition of phospholipids [58]. PS and phosphatidylethanolamine (PE) are located in the internal layer of the plasma membrane whereas the external layer is composed of phosphatidylinositol (PI), phosphatidylcholine (PC), sphingomyelin (SM), and other glycolipids. In order to allow MPs formation, this precise distribution is disrupted. Such changes in membrane architecture are controlled by three enzymes: flippase, floppase, and scramblase [59]. Flippase is involved in the maintenance of the phospholipid asymmetry between the membrane layers. Floppase controls the ATP-dependent translocation of PS to the external layer in response to cellular activation. Finally, scramblase is responsible for the membrane randomization allowing phospholipids to flow down their concentration gradients. In resting cells, only flippase is active, contributing to the internalization of negatively charged phospholipids (e.g., PS and PE) and to the maintenance of the physiological membrane asymmetry. Nevertheless, various events providing cellular activation are

able to increase intracellular calcium concentration, causing the flippase inhibition and the activation of floppase, scramblase, and other calcium-sensitive enzymes such as calpain and gelsolin, resulting in MPs release [60].

An important mediator of apoptotic MPs production is Rho-associated kinase (ROCK I), a protein kinase activated via caspase-mediated cleavage. ROCK I may allow the phosphorylation of myosin light-chains, triggering cytoskeletal rearrangement and formation of apoptotic MPs through the coupling of actin-myosin filaments to the plasma membrane [61].

6. MPs and Angiogenesis

Vesicle-like structures have recently emerged as important mediators for cellular communication in both physiological and pathological processes [30]; in particular, MPs released by various cell types may play an important role in angiogenesis [62,63], by modulating the production of proangiogenic factors through changes in the secretome of endothelial cells [64,65]. Like exosomes, MPs can communicate with other cells through ligands on their membrane that interact with specific receptors of the target cells; alternatively, MPs can fuse with the plasma membrane of the target cells, delivering its cytoplasmic content (e.g., proteins, miRNAs, RNAs). In this way, MPs may elicit the synthesis of proangiogenic or antiangiogenic factors and affect the main processes involved in formation of new vessels, such as endothelial cells adhesion, migration, and proliferation [66].

Endothelial cells-derived MPs (E-MPs) are clearly involved in the formation of blood vessels. A pioneer *in vitro* study highlighted that vesicles released by endothelial cells can transport integrin- β 1 and active matrix metalloproteinases-2 and 9 (MMP-2 and MMP-9), thus inducing endothelial cells invasion and tube-like structure [67]. In addition, it has been demonstrated that upon stimulation with the proinflammatory interleukin-3, endothelial cells release vesicles containing pro-angiogenic factors such as miR-126-3p and Stat5 [68]. Moreover, another study shows that endothelial MPs are able to transfer miR-126 from endothelial cells to vascular smooth muscle cells, corroborating the angiogenic properties of endothelial MPs [69].

Additionally, Plt-MPs are involved in angiogenesis. These vesicles can promote angiogenic effects by transferring various cytokines, such as PDGF, VEGF, FGF-2, and activating key proteins such as PI3k, ERK, and src kinase [70]. Various studies found that the angiogenic potential of the platelet-derived MPs can have both functional positive and negative effects: when Plt-MPs were infused in a rat model of myocardial ischemia, indeed, they increased the quantity of functional capillaries [70]; on the other hand, MPs shed by platelets can favor angiogenesis involved in tumor progression, upregulating important angiogenic factors, such as VEGF, HGF, and interleukin-8 [71].

7. Angiogenesis Induced by Physical Exercise

Physical activity has beneficial effects on human health [72–75], and regular physical exercise improves health in both physiological and pathological conditions [76–79]. Numerous evidence demonstrates the inverse relationship between regular exercise and cardiovascular diseases, hypertension, stroke, type 2 diabetes mellitus, metabolic syndrome, obesity, osteoporosis, and various types of cancer [76,80–83]. Depending on the type of physical exercise, we can observe different effects on the organism: endurance (or aerobic) exercise is characterized by a high number of muscular contractions, relative low load, and an important involvement of the cardiorespiratory system, whereas resistance exercise is represented by low number of muscular contraction but with relative higher loads and thus it relies mainly on the neuromuscular system [84]. These two types of exercise can be considered two extremes in a line, and between these two, numerous types of exercise exist, based on the modulation of the exercise variables (e.g., intensity, volume, density, rest). Each type of exercise can increase different responses and adaptations, triggering diverse cellular pathways.

Cardiovascular endurance training is the strongest stimulus for exercise-induced angiogenesis; this results in an improved capillary to fiber ratio in the skeletal muscle to provide an enhanced blood flow and oxygen delivery to the exercising muscles [85–89]. Endurance exercise stimulates

the angiogenesis activating several pathways (Figure 1). It induces an important reduction of the partial pressure of oxygen that leads to a local tissue hypoxia. This modification in oxygen levels decreases the hydroxylation of HIF-1 α through the inhibition of prolyl hydroxylase domain enzymes (PHD); in this way, HIF-1 α becomes more stable and translocates into the nucleus, where it forms an active complex with HIF-1 β [90] and activates genes involved in angiogenesis such as VEGF [84]. Another key regulator of VEGF synthesis is the peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1 α), which is modulated by aerobic exercise in several ways: intense endurance exercise produces an energy deficit and an increase of the AMP/ATP ratio that activates the AMP-activated protein kinase (AMPK) responsible for the PGC-1 α phosphorylation. Moreover, an acute bout of exercise increases the NAD⁺/NADH ratio that promotes the sirtuin (specifically SIRT1) activity and PGC-1 α deacetylation [91]. Finally, aerobic exercise affects also the estrogen-related receptor gamma (ERR gamma) that controls the skeletal muscle vascularization by modifying the VEGF expression [92].

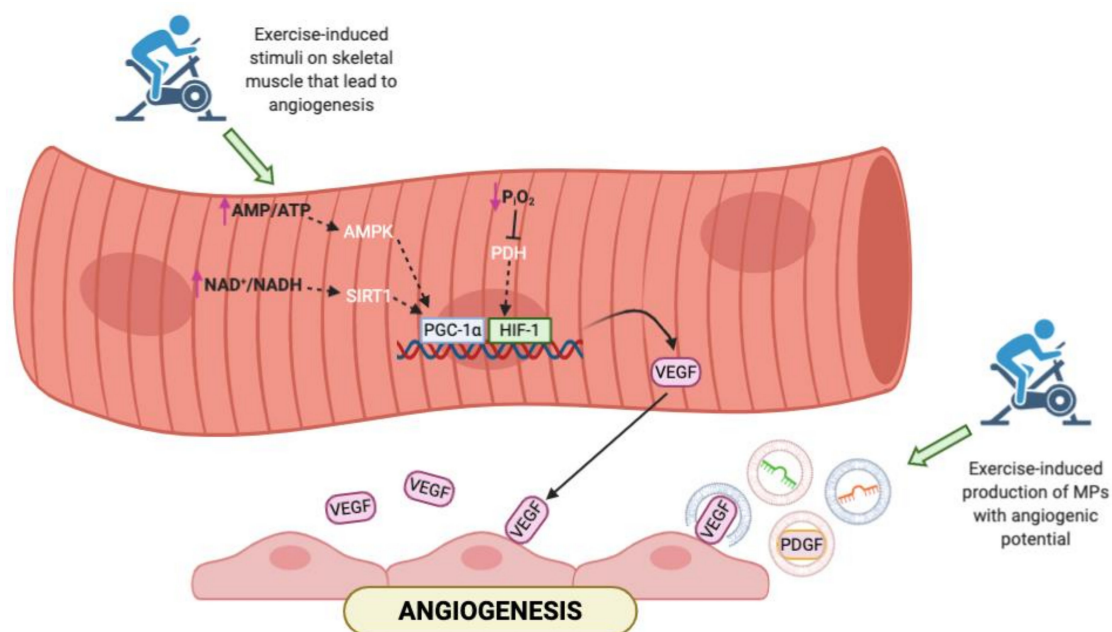


Figure 1. Representation of the main pathways activated in skeletal muscle in response to exercise. In addition to the canonical molecular pathways, aerobic exercise may affect the release of microparticles (MPs) that carry soluble factors involved in angiogenesis. AMP = adenosine monophosphate; ATP = adenosine triphosphate; NAD = nicotinamide adenine dinucleotide; NADH = reduced nicotinamide adenine dinucleotide; PGC-1 α = peroxisome proliferator-activated receptor gamma coactivator 1-alpha; HIF = hypoxia-inducible factor; VEGF = vascular endothelial growth factor; PDGF = platelet-derived growth factor.

In the last years it was evidenced that physical exercise induces modification of the MPs release into the bloodstream [93]. These EVs carry various molecules important for the tissues' crosstalk during and post exercise [94,95] and can be also a cargo of soluble factors involved in endothelial cell proliferation, migration, and adhesion. This results in the generation of new vessels in response to exercise.

8. MPs and Physical Exercise

Physical exercise, particularly endurance exercise, is known to stimulate shear stress due to its hemodynamic effects [96]. Intraluminal shear stress has a beneficial effect on vasculature because it is able to ameliorate vascular dilation through mechanisms mediated by nitric oxide [97]. In addition, exercise-induced shear stress can modulate the production of both E-MPs and Plt-MPs. For example, it was seen that high shear stress can reduce the concentration of E-MP while low shear stress is prone to increase such concentration [98]. Moreover, both high and low shear stress can elicit the production of MPs from platelets [99] (Figure 2).

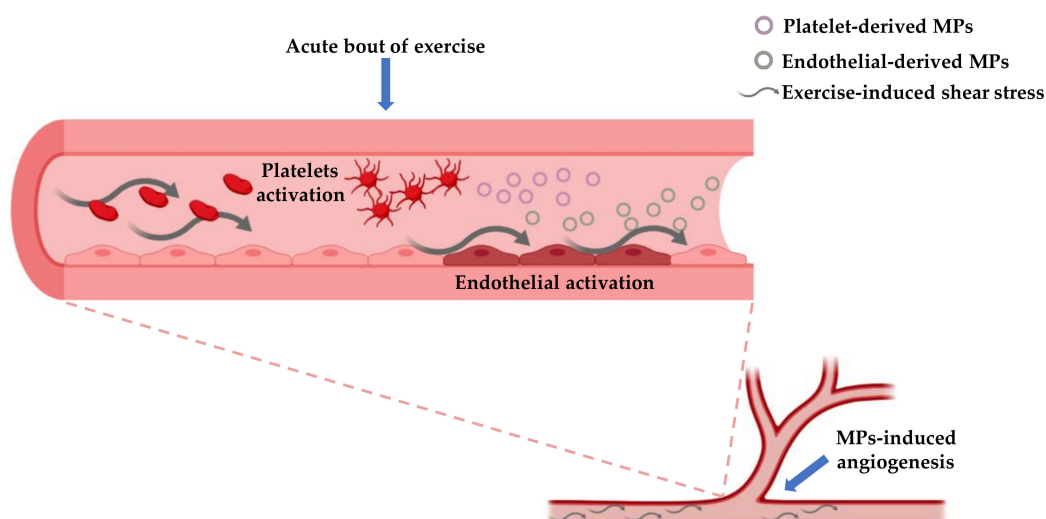


Figure 2. This figure shows the possible mechanisms involved in the production of platelet-derived MPs and endothelial-derived MPs and their role in stimulating angiogenesis. Both platelets and endothelial cells are activated in response to mechanical and chemical stimuli provided by exercise, that in turn elicit the MPs production by ectocytosis. The branching point refers to the site in which MPs stimulate neo-vascularization.

Acute exercise leads to an augmentation of the sympathetic activity, increasing both epinephrine and norepinephrine concentrations [100]. In this regard, studies report that norepinephrine is able to stimulate Plt-MPs release [101]. Along with hormonal response, acute exercise stimulates the secretion of many cytokines from skeletal muscle, named myokines [102]. Among these myokines, IL-6 is released in the bloodstream and elicits multiorgan effects [103]. It has been reported that IL-6 is also able to stimulate Plt-MPs formation [104], thus exercise can be a potent stimulus to enhance such mechanism.

The following sections provide an overview of various studies (summarized in Table 2) investigating the effects of physical exercise and training on MPs concentration, with an emphasis on endothelial and platelet-derived MPs, due to their specific involvement in angiogenesis [17,105–107].

Table 2. Summary of studies that evaluate MPs concentration in relation with physical exercise/exercise training in various populations.

Reference	Participants	Exercise Protocol	MPs Antigen Expression	MPs Collection Time Point	Effects of Exercise on MPs
Bittencourt CRO et al. (2017) [52]	Twenty-five professional half-marathon runners	No experimental protocols, blood was analyzed at rest and compared with that of healthy controls	CD42 ⁺ /CD31 ⁺ (Plt-MPs)CD51 ⁺ (E-MPs)	At rest, after 12 h of fasting	MPs concentration did not differ between runners and controls
Boyle JL et al. (2013) [53]	Eleven healthy men (25 ± 2 year)	5 days of reduced physical activity	CD31 ⁺ /CD42b ⁻ (apoptotic E-MPs)CD62E ⁺ (activated E-MPs)	At baseline, days 1, 3, 5 of reduced physical activity	↑ CD31 ⁺ /CD42b ⁻ after 5 days↔ CD62E ⁺ after 5 days
Brahmer A et al. (2019) [108]	Twenty-one healthy male athletes (28.7 ± 4.2)	Incremental cycling test until exhaustion	EVs array including different subtypes	Before, during, and after the exercise test	Among others, ↑ E-MPs and ↑ Plt-MPs
Durrer C et al. (2015) [15]	Overweight/obese males and females	20 min cycling just above VT (HICE) vs. 10 × 1 min at ≈90% peak aerobic power (HIIE) vs. control	CD62E ⁺ (activated E-MPs)CD31 ⁺ /CD42b ⁻ (apoptotic E-MPs)	≈18 h after the three conditions	↓ CD62E ⁺ , CD31 ⁺ /CD42b ⁻ in men after both HICE and HIIE↑ CD62E ⁺ in women after HICE↔ CD31 ⁺ /CD42b ⁻ in women after both conditions
Guiraud T et al. (2013) [109]	Nineteen males with coronary heart disease (62 ± 11 year)	2 × 10 min of cycling (15 s at 100% PPO/15 s passive rest) (HIIE) vs. isocaloric MICE session (at 70% PPO)	CD31 ⁺ /CD42b ⁻ (apoptotic E-MPs)CD62E ⁺ (activated E-MPs)CD42b ⁺ (Plt-MPs)	10 min before protocols and 20 min, 24 h, and 72 h after protocols	↔ all types of MPs after both exercise protocols
Highton PJ et al. (2019) [110]	Fifteen men (22.9 ± 3.3 year)	1 h of running (70% VO _{2max})	TF ⁺ Plt-MPS and TF ⁺ neutrophil MPs	Before exercise, immediately after exercise, 1:30 h post exercise	↓ TF ⁺ Platelet and TF ⁺ neutrophil MPs after exercise
Kim JS et al. (2015) [111]	Twenty-one male and female subjects (52.1 ± 1.4)	40 min of aerobic exercise at 65% HR _{max} (3 days/week for 6 months)	CD31 ⁺ /CD42a ⁻ (apoptotic E-MPs)CD62E ⁺ (activated E-MPs)	Pre and post exercise intervention	↓ CD31 ⁺ /CD42a ⁻ ↓ CD62E ⁺
Kirk RJ et al. (2013) [112]	Seven healthy males (22 ± 3.2 year)	10 × 15 s at 120% PPO of cycling	CD105 ⁺ (E-MPs)CD106 ⁺ (E-MPs)	Immediately before and after exercise, 90 min and 180 min post-exercise	↑ CD105 ⁺ , CD106 ⁺ 90 min post-exercise
Kirk RJ et al. (2019) [55]	Eleven women with PCOS (28.00 ± 6.72) and 10 control women (24.26 ± 6.18)	1 h of running on a treadmill at 60% VO _{2max} (3 days/week for 8 weeks)	CD105 ⁺ (E-MPs)CD106 ⁺ (E-MPs)	Pre, at 4 weeks of the exercise program (mid), and post exercise program	↓ CD105 ⁺ in women with PCOS post exercise↔ CD106 ⁺ in either groups post exercise
Lansford KA et al. (2015) [51]	Sixteen healthy men (24.5 ± 0.8 year) and 10 healthy women (22.4 ± 0.52 year)	Stationary cycling at 60–70% VO _{2peak} until reaching a total energy expenditure of 598 kcal	CD62E ⁺ (activated E-MPs)CD34 ⁺ (hematopoietic cell derived MPs)CD31 ⁺ /CD42b ⁻ (apoptotic E-MPs)	At baseline and within 5 min of completing the exercise protocol	↑ CD62E ⁺ in men only↔ CD31 ⁺ /CD42b ⁻ ↑ CD34 ⁺ in women only
Rafiei H et al. (2019) [54]	Fifteen overweight/obese women (48.9 ± 10.7)	2 weeks (10 sessions) of progressive HIIT or MICT on a cycle ergometer	CD62E ⁺ (activated E-MPs)CD31 ⁺ /CD42b ⁻ (apoptotic E-MPs)	Pre and post exercise protocols	↓ CD62 ⁺ for both HIIT and MICT↔ CD31 ⁺ /CD42b ⁻ for both HIIT and MICT

Table 2. Cont.

Reference	Participants	Exercise Protocol	MPs Antigen Expression	MPs Collection Time Point	Effects of Exercise on MPs
Rakobowchuk M et al. (2017) [56]	Twelve healthy men (29.2 ± 6.1 year)	45 min of eccentric or concentric cycling	CD62E ⁺ (activated E-MPs)CD41 ⁺ (Plt-MPs) CD14 ⁺ (monocyte/macrophage derived -MPs), CD61 ⁺	Pre and post exercise protocols	↑ CD41 ⁺ after both exercise modalities↔ CD62E ⁺
Rigamonti AE et al. (2020) [113]	Fifteen obese (21.2 ± 8.8) and eight normal weight subjects (26.2 ± 7.2) male and female	Moderate intensity (60% VO _{2max}) treadmill to exhaustion	(Plt-MPs), CD62E ⁺ (activated E-MPs), CD105 ⁺ (E-MPs), SCGA ⁺ (skeletal muscle derived MPs), FABP ⁺ (adipocyte derived MPs) CD144 ⁺ (E-MPs), CD62E ⁺ (activated E-MPs), CD31 ⁺ CD62P ⁺ (Plt-MPs), CD42b ⁺ (Plt-MPs)	1 h before, at the end, 3 h, and 24 h after exercise	Changes were tissue, sex, and BMI specific, ↓ CD61 ⁺ immediately and 24 h post exercise
Schwarz V et al. (2017) [114]	Ninety marathon runners (49 ± 6 year)	Marathon run (i.e., 42 km run)	CD14 ⁺ (monocyte derived MPs), CD45 ⁺ (leukocyte derived MPs)	Three days before, immediately after, within 2 days after the marathon run	↑ CD144 ⁺ , CD62E ⁺ , CD31 ⁺ ↑ CD62P ⁺ , CD42b ⁺ ↓ CD14 ⁺ , CD45 ⁺
Serviente C et al. (2019) [115]	Thirty-six healthy active women (40–65 year)	30 min of treadmill exercise at 60–64% VO _{2peak}	CD62E ⁺ (activated E-MPs) CD31 ⁺ /CD42b ⁻ (apoptotic E-MPs)	At baseline, and 30 min after the exercise protocol	↓ CD62E ⁺ ↓ CD31 ⁺ /CD42b ⁻
Shill DD et al. (2018) [116]	Ten healthy men (23.6 ± 3.27) and 10 healthy women (23.6 ± 5.58)	HIIE (10 × 1 min ≈95% VO _{2max} /75 s active recovery) vs. MICE (energy-matched continuous bout at 65% VO _{2max})	CD34 ⁺ (hematopoietic cell derived MPs) CD62E ⁺ (activated E-MPs)	At baseline, half exercise, immediately after exercise, 30, 60, 90, 120 min after exercise	Changes in CD62E ⁺ and CD34 ⁺ were intensity dependent, and sex specific
Whal P et al. (2014) [117]	Twelve men triathletes/cyclists (24.7 ± 3.4)	130 min at 55% PPO vs. 4 × 4 min at 95% PPO vs. 4 × 30 s all-out	CD31 ⁺ /CD42b ⁻ (apoptotic E-MPs)	Before exercise, and 0, 30, 60, 180 min post-exercise	↓ CD31 ⁺ /CD42b ⁻ after all three exercise protocols
Wilhelm EN et al. (2016) [17]	Nine healthy young men (25 ± 1 year)	1 h of moderate (46 ± 2% VO _{2max}) vs. heavy (67 ± 2% VO _{2max}) intensity semirecumbent cycling	CD62E ⁺ (apoptotic E-MPs) CD41 ⁺ (Plt-MPs)	At baseline, 30, 60, 80, 100, 120, 150, 180, and 240 min of the protocol	↔ CD62E ⁺ for both MI and HI↑ CD41 ⁺ at 30 and 60 min of HI exercise

↑ = increased concentration, ↓ = decreased concentration, ↔ unaltered concentration; E-MPs = endothelial cell-derived MPs; Plt-MPs = platelets derived MPs; TF⁺ = tissue factor positive; VT = ventilatory threshold; HICE = high-intensity continuous exercise; HIIE = high-intensity interval exercise; HIIT = high-intensity interval training; MICT = moderate-intensity continuous training; PPO = peak power output.

8.1. The Effect of Physical Exercise on MPs with Angiogenic Potential in Athletes

Endurance athletes have the characteristics to present greater adaptation of the cardiorespiratory and neuromuscular systems resulting in the increase of the delivery and the consumption of oxygen [118]. Two of the sports that highly rely on these adaptations are marathon and half-marathon. Bittencourt et al. compared the amounts of E-MPs and Plt-MPs in a group of half-marathon runners with a control group of gender-matched healthy controls showing that CD51⁺ E-MPs and CD42⁺/CD31⁺ Plt-MPs did not significantly differ between the two groups [52]. This data suggests that athletes exposed to chronic endurance training can benefit from exercise effects but that such type of training does not lead to further decrease in MPs compared with a healthy non-athlete population. On the other hand, E-MPs and Plt-MPs significantly increase following a marathon run and return to baseline values within 2 days; conversely, leukocyte and monocyte-derived MPs decrease after such run [114].

Like long distance running, road cycling and triathlon are two sports heavily relying on cardiorespiratory endurance [119]. In triathletes/cyclists, high-volume (2 h at 55% of peak power output) and high intensity (four sets of minutes at 90–95% of peak power output interspersed by 3 min of active recovery or four sets of 30 s at maximal effort separated by 7:30 of active recovery) trials on cycle ergometer, significantly decrease CD31⁺/CD42b⁻ apoptotic E-MPs 60 and 180 min after the termination of the trials [117]. It seems that such decrease was due to an increased uptake of MPs by cells. Indeed, triathletes/cyclists' serum collected 180 min after high intensities exercises promote the uptake of CD31⁺/CD42b⁻ apoptotic E-MPs in endothelial cells in vitro [117]. Another recent study involving male athletes showed that an incremental cycling protocol until exhaustion determined a significant increase of CD105⁺, CD146⁺ E-MPs, and CD62P⁺ Plt-MPs at the peak of the exercise session [108].

8.2. The Effect of Physical Exercise on MPs with Angiogenic Potential in Healthy Subjects

The physical exercise performed in a regular manner elicits beneficial effects that include improved cardiorespiratory fitness, body composition, decreased risk of noncommunicable diseases and of all-cause mortality [76,120–124].

Data on the effects of aerobic exercise on E-MPs and Plt-MPs are controversial. After 1 h of treadmill run at 70% of VO_{2max}, a decrease in TF⁺ Plt-MPs and neutrophil-derived MPs in healthy men [110] has been observed; moreover, a single session of 30 min of moderate intensity aerobic exercise (60–64% of their VO_{2peak}) reduces the number of CD62E⁺ activated E-MPs and CD31⁺/CD42b⁻ apoptotic E-MPs [115]. On the other hand, in prehypertensive male and female, aerobic exercise performed at 65% of predicted maximal heart rate for a duration of 6 months, with a frequency of three times per week, decreases the blood concentration of CD62E⁺ E-MPs and CD31⁺/CD42a⁻ E-MPs [111]. Some authors also evidenced a possible sex/gender effect on the MPs release: indeed, an acute bout of aerobic exercise at 60–70% of the VO_{2peak} produced a significant increase in CD62E⁺ E-MPs in men, and an increase in CD34⁺ MPs in women [51]. However, results of study involving both male and female are inconclusive. Indeed, another study showed CD62E⁺ E-MPs decrease both during and after moderate intensity continuous exercise (65% of VO_{2max}) in women but not in men [116].

One of the factors that must be taken into account analyzing the effects of physical exercise on the MPs is the timing of the analysis. In response to low to moderate intensity (33% of VO_{2max}) concentric or eccentric cycling, men show higher concentration of CD41⁺ Plt-MPs 5 min following exercise, but these levels returned to baseline levels after 40 min of recovery, while CD62E⁺ E-MPs do not show modifications [56]. Another experimental study performed in young males showed that CD41⁺ Plt-MPs and CD62E⁺ E-MPs were unaffected by 1 h of cycling at ≈46% of VO_{2max}, while when the intensity were set ≈67% of VO_{2max} an increase of CD41⁺ Plt-MPs was observed [17]. Interestingly, the MPs released in response to such type of exercise enhanced endothelial proliferation, migration, and tubule-like formation in vitro, compared with MPs present in resting conditions [17].

Another modality of training that is of increasing interest in research is high intensity interval training (HIIT). HIIT is a useful exercise modality to enhance cardiorespiratory endurance, and such

type of training can be performed also at strenuous intensities [125,126]. Supramaximal sprint cycling protocol consisting of 10 bouts of 15 s at 120% of peak power output separated by 45 s of active recovery lead to a significant increase of circulating CD105⁺ and CD106⁺ E-MPs 90 min after the exercise session, whereas at 180 min post-exercise MPs returned towards resting values [112]. On the other hand, no changes in endothelial MPs concentration was observed after a high intensity interval exercise (95% of VO_{2max}) [116].

Physical inactivity and sedentary behaviors have detrimental effects on vasculature state, contributing to the development of cardiovascular and metabolic diseases [127–129]. After 5 days of reduced physical activity (<5000 steps/day), significant increase of CD31⁺/CD42b⁻ apoptotic E-MPs was registered, whereas CD62E⁺ activated E-MPs remained unchanged, suggesting that inactivity fosters vascular dysfunction increasing endothelial apoptosis in recreationally active men [53].

8.3. The Effect of Physical Exercise on MPs with Angiogenic Potential in Pathologic Subjects

Physical exercise is a fundamental stimulus for improving metabolic and cardiovascular health. Among the various typology, HIIT has the same, or superior capacity to improve health status in pathological subjects (e.g., presenting diabetes, cardiovascular diseases, or cancer) [130,131]. In overweight/obese men, high-intensity continuous exercise (20 min of cycling at just above the subjective ventilatory threshold) and high-intensity interval exercise (10 bouts of 1 min at ≈90% peak aerobic power) reduce E-MPs concentration 18 h following exercise; different results were observed in overweight/obese women in which high-intensity continuous exercise increased activated CD62E⁺ E-MPs, leaving unaffected CD31⁺/CD42b⁻ apoptotic E-MPs, regardless the intensity [15]. However, the chronic exposure of overweight/obese subjects to exercise (2 weeks of HIIT or moderate intensity continuous training) lowered the concentration of activated CD62E⁺ E-MPs [54]. The effect of exercise on MPs release may also depend on the intensity of the physical activity. Indeed, in a recent study involving both over and normal weight subjects, a bout of moderate intensity (60% of their VO_{2max}) aerobic exercise, reduced the total EVs in the MPs range (130–700 nm); however, the release of MPs in normal weight subjects was higher than in obese and in females than in males. [113].

Polycystic ovary syndrome (PCOS) is an endocrine disorder in which endothelial dysfunction and altered angiogenesis are common. Eight weeks of aerobic training consisting of three sessions per week of 1 h of running on a treadmill at 60% VO_{2max} led to a significant decrease in CD105⁺ MPs in women with PCOS compared with a control group of healthy women, while did not have an effect on the amount of CD106⁺ E-MPs [55].

Coronary artery diseases determine a diminished myocardial perfusion that may result in angina, myocardial infarction, and heart failure [132]. In stable coronary heart disease patients, neither moderate-intensity continuous exercise (28.7 min at 70% of peak power output) nor a single bout of high-intensity interval exercise (10 min session with work interval of 15 s at 100% of peak power output interspersed with 15 s of passive recovery) have an effect on the concentration of circulating E-MPs and Plt-MPs [109].

Overall, the numerous studies show contrasting results regarding the qualitative and quantitative changes of MPs in response to acute physical exercise and to exercise training as well. Such divergent conclusions could be due to the heterogeneity of the studies, as they involved different samples in terms of age, sex, training status, possible pharmacological therapies [133], and pathologies [93] (Figure 3). More standardized and harmonized procedures are needed to compare the studies and obtain definitive data on the release of MPs with angiogenic potential in response to physical exercise.

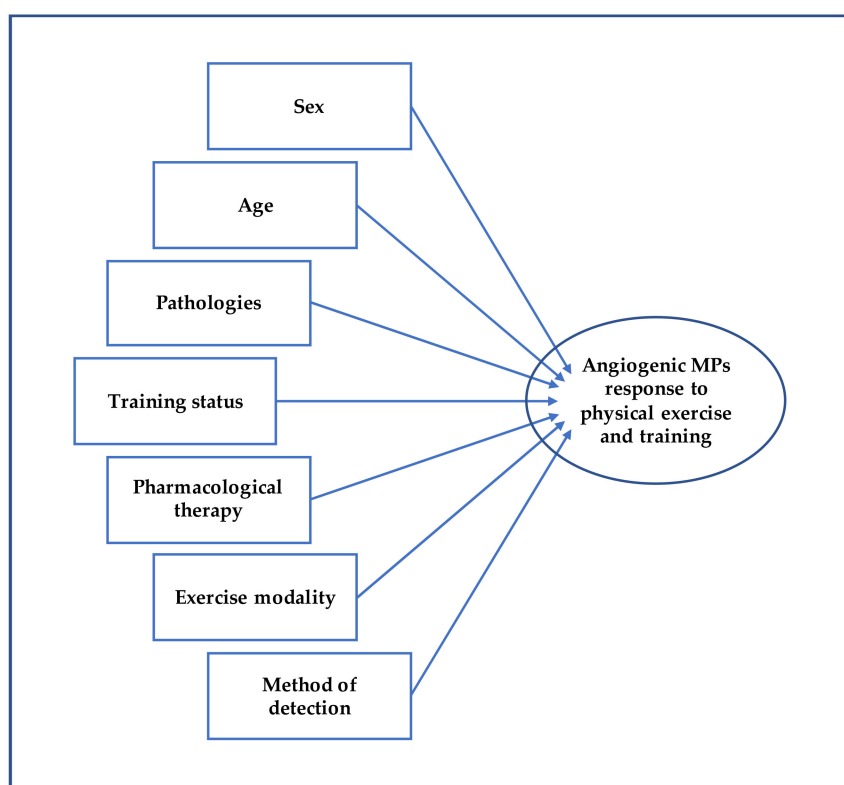


Figure 3. This figure shows the potential influencing factors affecting MPs following physical exercise and training.

9. Conclusions

Various modalities of cardiovascular endurance-based exercise can modify blood concentration of E-MPs and Plt-MPs, known to affect angiogenic processes. It is well known that physical exercise, among different adaptations, can lead to a functional angiogenesis resulting in a wider vascular bed that increases the blood flow to exercising muscles. Results regarding the effect of exercise on MPs are divergent, but it seems that acute exercise results in an increase of MPs while chronic exposure to exercise results in a decrease in most cases. In addition, MPs sensitivity to exercise can be affected by the training status, sex, age, as well as mode of exercise (e.g., run on a treadmill, road run, cycle ergometer), pharmacological therapies, diseases, and method of detection. While only one study directly showed the pro-angiogenic effects of MPs produced following exercise [17], numerous studies highlight the potential of E-MPs and Plt-MPs in communicating with other cells and stimulating angiogenesis [69,105–107]. Thus, future evidence regarding the effect of both acute exercise and training on MPs affecting angiogenesis will be useful to add important insights on the mechanisms by which exercise elicits vascular adaptations and to design specific training programs for various populations.

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