Exercise-derived microvesicles: a review of literature

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Short Title: Microvesicles and Exercise

Abstract

Initially suggested as simple cell debris, cell-derived microvesicles (MVs) have now gained acceptance as recognized players in cellular communication and physiology. Shed by most, and perhaps all, human cells, these tiny lipid-membrane vesicles carry bioactive agents, such as proteins, lipids, and microRNA from their cell source, and are produced under orchestrated events in response to a myriad of stimuli. Physical exercise introduces systemic physiological challenges capable of acutely disrupting cell homeostasis and stimulating the release of MVs into the circulation. The novel and promising field of exercise-derived MVs is expanding quickly, and the following work provides a review of the influence of exercise on circulating MVs, considering both acute and chronic aspects of exercise and training. Potential effects of the MV response to exercise are highlighted and future directions suggested as exercise and sports sciences extend the realm of extracellular vesicles.

Key points

- Cells naturally release microvesicles (MVs) known to regulate a variety of physiological processes including haemostasis and vascular adaptations.
- Acute exercise can influence the production and clearance of certain circulating MVs, with remarkable effect in stimulating a transient increase in the concentration of MVs derived from platelets.
- In selected populations, chronic exercise improves vascular function while reducing the blood concentration of endothelial MVs linked to vascular damage at rest.
- Training variables, such as exercise intensity, can be modified to stimulate an acute increase in the concentration of exercise-derived MVs with pro-angiogenic potential.

1. Introduction

When stimulated, different cell types release plasma membrane-derived vesicles into the extracellular compartment, which can enter the circulation and interact with remote tissues. Among these, microvesicles (MVs), also known as microparticles, transport lipids, proteins, and transcripts from their parental cells. Despite the long debate about whether platelets should be classified as cells [1], thrombocytes actively release MVs and comprise a large (if not the largest) fraction of circulating MVs [2–4].

Previously viewed as biomarkers of the parental cell state, we now understand that MVs are not only passive by-products of cell membrane cytoskeleton reorganisation, but active agents released under specific environmental stresses and capable of triggering functional and structural alterations in recipient cells [5–7]. The profile [8–10] and content [9,11] of circulating MVs differ between healthy and clinical conditions, with the concentration of certain MV populations related to impaired vascular health [10,12].

Exercise training is one of the best interventions to maintain cardiovascular function, and recent evidence demonstrates that acute exercise alters circulating MVs concentrations [13–17], especially platelet-derived MVs (PMVs). Although exercise-mediated vascular adaptations like improved endothelial function are mediated in large part by haemodynamic forces (i.e. increased anterograde shear stress) [18–20], systemic circulating factors have also been suggested as involved [21], and circulating MVs have arisen as putative mediators of local and systemic adaptations to exercise. The precise role played by these tiny vesicles is still being unravelled, and the study of extracellular vesicles has received intensive attention due to its potential in physiology and medicine. As such, this review focuses on the promising field of cell-derived MVs considering their application in exercise physiology and medicine and aims to provide future directions for this novel research area.

1.1. Literature search criteria

Electronic databases were searched (Pubmed/Medline, Scopus and Google Scholar) between 2017 and 2018, with no restriction regarding publication date. English language scientific articles were selected based on content concerning (but not restricted to) the exercise and MV literature. Although the present manuscript is not a systematic review, the literature search criteria included the terms “exercise”, “training”, “microvesicles”, “microparticles”, “cell-derived microparticles”. The reference list of selected manuscripts, as well as from PhD theses and MSc dissertations on the topic, were also used searching sources. For the review of specific literature relating to the influence of exercise upon circulating MVs, articles were selected only if they included at least one acute or chronic exercise intervention group and MV outcome.

2. Background – From extracellular vesicles to cell-derived microvesicles

Cells have been known to release biologically active small vesicles with many functions but not all vesicles are created the same, so the term extracellular vesicles has been introduced as an all-encompassing descriptor of vesicles released by cells into the extracellular compartment. Such vesicles can be sub-classified as exosomes, MVs, and apoptotic bodies, depending on their mechanism of formation, size, and the presence of specific markers. Importantly, nomenclature in this field varies with MVs and microparticles referred to as similar constructs, while others defining MVs as an extracellular vesicle category which encompasses both microparticles and exosomes. Although we appreciate that future efforts are necessary for nomenclature standardization, for clarity in this review MVs and microparticles will be used interchangeably, whereas exosomes are considered distinct.

By standard definition, MVs are anucleate vesicular populations derived from plasma membranes, ranging from approximately 0.1 to 1 μm in diameter, and with no synthetic capabilities. MVs differ from exosomes and apoptotic bodies not only in size, but also in how they are formed, their content, and since MVs originate from the plasma membrane, they also carry cell membrane-specific antigens. In contrast, exosomes are produced through a constitutive intracellular process and released by exocytosis upon fusion of multivesicular bodies with the plasma membrane, whereas apoptotic bodies are released as blebs from apoptotic cells and can also carry cell-membrane specific antigens. Although some overlap exists and size definitions vary slightly, exosomes are generally described as ranging from ~40 to 100 nm in diameter, and apoptotic bodies are normally characterised as vesicles larger than 1.5 μm that often carrying nuclear content [22,23].

Differentiating extracellular vesicle populations is important as each differs in function and may exhibit broad physiological and pathophysiological effects. This review focuses on MVs, but the reader is referred to previous publications for specific reviews on exosomes and exercise [24,25].

2.1. Introduction to circulating microvesicles

In 1967 Peter Wolf published a detailed manuscript describing that clot-formation occurred even in platelet free-plasma as long as platelet-derived elements were present, which he named “platelet dust” [26]. These pro-coagulant fragments were later found to have originated from the plasma membrane and several years later, this was confirmed by electron microscopy after platelet stimulation with thrombin [27], providing the first evidence of what we now call MVs.

Over decades, the field advanced with MVs characterized and, to some extent, content was determined, and evidence emerged that platelets were not the sole source of circulating MVs [22]. Presently, diverse MV populations have been identified from cell culture media, and biological fluids including plasma [3,28], urine [29], saliva [28], and synovial fluid [3]. The current view is that upon agonist stimulation most, if not all, cells release MVs carrying specific markers that enable cell origin to be determined like endothelial (EMVs), PMVs, and red blood cell MVs (RBCMVs).

Currently, altered circulating MV concentrations have been associated with subclinical and clinical conditions. For instance, increased circulating concentrations of EMVs occur with obesity [9,12], the metabolic syndrome [30], those with coronary artery disease (CAD) [8], and type 2 diabetes mellitus [10], to list a few. They have even provided prognostic information about cardiovascular mortality in renal failure patients [31]. These conditions all exhibit vascular dysfunction, with increased EMVs concentrations likely reflecting chronic vascular damage, as observed in patients with known poor vascular outcomes such as CAD [8], and in acute stroke [32].

Accordingly, MVs are naturally produced and found in the circulation fluctuating within a physiological range, with chronic alterations in MV concentrations identified as potential biomarkers of pathology. Of particular relevance and based on *in vitro* and *in vivo* evidence, EMVs have received great attention as a surrogate circulating marker of endothelial cell health [33–36].

2.1.1. Microvesicle formation and phenotype

Shedding of MVs from the cell membrane is initiated by complex events that lead to cytoskeleton proteolysis, cell shrinkage, and eventually MV sprouting [37,38]. Our understanding of the overall mechanisms governing MV formation and release derives mainly from platelets, since they were among the first to be identified, and constitute the predominant MV phenotype in human blood.

Briefly, in the basal state the content of phospholipid cell membranes are asymmetric with negatively charged phospholipids, such as phosphatidylserine (PS), mainly a part of the inner leaflet [37]. When

activated or undergoing apoptosis, a randomisation in the phospholipid plasma membrane content occurs, increasing the appearance of PS on the outer membrane, which, coupled with cytoskeleton reorganisation and membrane remodelling, culminates in the shedding of newly formed MVs that may express PS on their surface [38]. Increases in intracellular Ca^{2+} concentration [37,39] with activation of calcium-dependent proteins [39,40] is accepted as a general mechanism leading to these processes, but calcium-independent pathways have also been identified in platelets [41]. Several stimuli that bring about PMV release have been identified *in vitro* and include high shear stress, catecholamines, adenosine diphosphate (ADP), and thrombin [42–44], but some agonists for platelet vesiculation can downregulate the release of MVs from other sources such as endothelial cells [45]. An excellent discussion of biological mechanisms related to MV formation has been published elsewhere [38].

Methods to quantify MVs in body fluids are still limited, but recent developments have facilitated quick progression from time-consuming and semi-quantitative microscopy, to high throughput quantitative MV analyses using enzyme-linked immunosorbent assays (ELISA), traditional flow cytometry, imaging flow cytometry, resistive pulse sensing, and nanoparticle tracking analysis [29,46,47]. Knowing that MVs express PS in the outer leaflet enabled PS-binding agents (e.g. annexin-V staining) as general MV markers; however, PS exposure alone does not necessarily induce vesiculation in platelets [39], and only a fraction of MVs released by unstimulated platelets express sufficient PS to facilitate annexin-V binding [48]. Hence, more recently quantifying specific MV populations has been based on the expression of cell-specific antigens associated with the MV membrane, independent of annexin-V binding [16,34,48]. For example, PMVs can be identified by flow cytometry as glycoprotein IIb (CD41) or glycoprotein Ibα (CD42b) positive events, whereas E-selectin (CD62E) can be used for EMV quantification, and CD45 is employed as a common maker for leukocyte-derived MVs (Table 1).

[TABLE1]

Previous studies indicate that PMVs are the most common MV phenotype in plasma [2–4], although reports of greater abundance of RBCMVs also exists [47]. Such discrepancies in the proportion of RBCMVs may relate to differences in blood sampling and handling procedures, as phlebotomy conditions (e.g. needle Gauge, tourniquet use, and anticoagulant choice) and sample processing protocols (e.g. centrifugation steps, time, and speed) can ultimately affect final MV concentrations [49]. Although a complete list of all antigens expressed by circulating MVs would be useful, it is beyond our scope. However, the most frequently observed MVs antigens in the circulation are also expressed by platelets [12,48], erythrocytes [35,50], monocytes [4,47], neutrophils [47,50], lymphocytes [47] and endothelial cells [11,33,51] suggesting these are the origins of most MVs.

2.1.2. Overall microvesicle function

Circulating MVs likely regulate physiological processes, and the interaction of MVs with target cells occurs through at least three mechanisms. First, MVs may bind to membrane receptors and mediate cell modifications through signalling pathway activation. Next, cell-MV interactions at the plasma membrane may deliver vesicular content upon fusion with the cell membrane [6,22]. Finally, MVs may be internalized by certain cells, altering the function and structure or their recipient targets [52,53]. The latter may also serve as a MV clearance pathway if they are directed to lysosomes. As a result, complex and sometimes contrasting responses arise from studies investigating isolated MV populations.

Platelet-derived MVs are linked to the haemostatic system [22,23] with initial evidence supporting their role in coagulation, even in the absence of intact platelets [26]. Although not all MVs are pro-coagulant [48], a number of MV populations present in a variety of body fluids, including blood and saliva, display thrombogenic functions [3,28,54] similar to PS-rich PMVs [48]. The pro-thrombotic potential of many blood MVs is highly related to their surface PS and tissue factor (TF) content [28,48], and the natural shedding of MVs seems like a necessary physiological process. For instance, Scott syndrome, a rare haemorrhagic disorder, is linked to impaired translocation of PS to the outer leaflet cell membranes and reduced MV shedding [38], which results in defective blood coagulation.

Even though production of MVs is continuous, only a fraction are PS and TF-rich [4,33,48], suggesting roles beyond coagulation. As will be explained, blood MV functions vary widely. For example, specific EMV populations can trigger inflammation [5] and oxidative stress [55] in recipient endothelial cells, and lymphocyte-derived MVs can suppress angiogenesis through vascular endothelial growth factor (VEGF) downregulation [56]. Furthermore circulating MVs isolated from myocardial infarction patients [57] and pre-eclamptic women [58] lead to endothelial dysfunction *in situ*, suggesting that the link between augmented MV concentrations in pathological conditions is beyond correlational.

Conversely, other MVs display opposite functions than those described above. Grasser and Schifferli [59] have shown that granulocyte-derived MVs induce anti-inflammatory properties by downregulating macrophage activity, and using an ischaemic-limb model Leroyer *et al.* [60] demonstrated a 3.5-fold increase in pro-angiogenic MV concentration in mouse muscle homogenates. These ischaemia-related MVs increased progenitor cell differentiation into an endothelial phenotype *in vitro* and *in vivo*, and other studies have shown that endothelial cells undergoing migration release pro-angiogenic EMVs [61]. PMVs may also promote activation of pro-angiogenic pathways, resulting in endothelial cell proliferation, chemotaxis, and protection from apoptosis [7,62].

The identified function of MVs is constantly growing as it depends on a variety of factors, including the MV population, content, and the recipient cell with which they are interacting, which illustrates the complexity of these physiological interactions. More complex than local ischemia, physical exertion imposes unique challenges upon many organ systems often introducing MVs into the human circulation. As such the dynamics of MVs with exercise, and their potential relevance in exercise-induced adaptation is an intriguingly understudied area.

3. Exercise and microvesicles

In the following sections a review of the current state of knowledge on microvesicles and exercise will be presented, but as the reader will see the current understanding about the interaction between MVs and exercise is still incomplete. As such, the subsequent sections aim to provide a starting point and guidance for future research. Certainly, further experiments are warranted to answer specific questions related to exercise and training variables (e.g. exercise volume, intensity, etc.), before more definitive conclusions can be drawn.

3.1. Acute exercise

The phenomenon of microvesicle release with exercise has been appreciated only recently, with most experiments in the field focused on acute aerobic exercise and published in the current decade (Table 2). The dynamics of this response to exercise, however, is MV population specific. For example, circulating RBCMV concentrations did not change in the single study that examined this MV phenotype with exercise, but increases in the concentration of PMVs and MVs with polymorphonuclear neutrophil antigens was observed following a series of maximal cycling protocols [50]. Since MVs are physiologically active, their timed release may be involved in exercise responses.

Most studies have investigated the effect of performing an aerobic cycling session on subsequent blood EMV concentrations. Although some authors observed an increase in certain EMV populations after exercise in healthy individuals [4,16,51,63,64], most experiments report no change [4,13–17,50,65–67], or even a decrease in EMV concentration [63,68], with one study finding an increase in blood EMV in men, but not women [16]. The discrepancies in the EMV response between studies may arise from a number of confounding factors. For example, exercise brings about many acute physiological adjustments that may stimulate or blunt EMV shedding. Cytokines and vascular cyclic strain stimulate the formation of MVs from cultured endothelial cells [33,54,69], whereas high vascular shear stress reduces EMV formation *in vitro* [45]. Since each of these factors increases during exercise, an antagonistic environment may be created where little additional endothelial vesiculation will occur and this may account for inconsistent findings. In addition, plasma volume changes may also confound finding as observed by Wilhelm and colleagues [17] who noted increased EMV concentration with heat stress and strenuous exercise, but this response was abolished when plasma volume shifts were taken into account. This suggests changes in circulating EMVs were mediated by haemoconcentration rather than altered MV dynamics. Most studies, however, do not report blood volume corrected EMV concentrations, making it difficult to establish whether EMV shedding actually occurs.

In contrast to other MV phenotypes, PMVs are the most responsive to an acute exercise bout. Most studies report an increase in these MVs after exercise [4,13–15,50,64,70], with increases up to 2 to 3-fold from baseline, and several agonists known to stimulate PMV formation increase during exertion. Plasma concentrations of ADP originating from exercising limbs in humans increase [71,72], and may lead to platelet activation [72] since it is a PMV production agonist [44]. Similarly, sympathetic activation during exercise can lead to blood noradrenaline spill-over that could stimulate platelet MV formation, similar to *in vitro* effects [43]. Exercise also increases mean and anterograde vascular wall shear rate (surrogate markers of shear stress) in conduit arteries of exercising [19,20], and even non-exercising limbs with prolonged exercise [18,73]. Increased shear stimulates PMV release from *ex vivo* platelets [7,42] and recently a positive correlation between vascular shear rate and plasma PMV concentration was noted in exercising men [15], although a follow-up set of experiments revealed this correlation to not necessarily represents causation [17].

When comparing these results, however, the reader must be aware of intrinsic methodological limitations of the field. Distinct quantification techniques result in different absolute MV concentrations. For instance, in the study by Maruyama et al. [70] PMVs were assessed by ELISA and concentration differed by orders of magnitude from those using traditional flow cytometry, which in turn are not necessarily comparable to studies employing imaging flow cytometry. Nevertheless, the trend of response was the same between techniques (i.e. an increase in PMV with exercise), suggesting that the same physiological phenomenon was being assessed. As shown in Table 2, the vast majority of experiments employed flow cytometry as the main MV quantification technique, but it is recognized that both pre-analytical and analytical procedures influence the MV content in a sample [49]. Although efforts to standardize traditional flow cytometry protocols have been made [74,75] there still lacks methodological agreement between experiments. Furthermore and according to the iceberg concept [29,46], flow cytometers can not identify all MVs due to light scattering limitations of small particles, and as a result swarm detection of small MVs confounds absolute concentration measurements. These factors can complicate comparisons made between studies and should be taken into consideration when interpreting individual results. Future experiments combining quantification techniques such as flow cytometry and nanoparticle-tracking analysis will certainly be useful to robustly quantify absolute MV concentrations after acute exercise bouts.

[TABLE2]

3.1.1. Time-course of circulating microvesicle appearance with acute exercise

The dynamics of blood MV appearance depend on the vesicle source, and until the middle of the current decade our understanding of their time-course of release with exercise was limited to one study exploring EMVs [65], with the remaining experiments exploring post-exercise responses. More recently, plasma PMVs, but not EMV, were shown to increase and stabilise within 30 minutes of continuous cycling, with peak post-exercise values similar to those observed during exercise [15]. Blood PMVs are augmented from a few minutes [4,14,50,70] up to 1 [4,15,70] and 2 h [4,13,50] after exercise, and return towards baseline values thereafter. As such, the current literature indicates that the rise in blood PMVs is a short-lived phenomenon, with these vesicles reaching peak concentrations as early as 30 minutes after the onset of a training session, with values continuing to be elevated for a few minutes to hours into the post-exercise recovery period (Figure 1).

The time-course dynamics of other MV populations are less clear. A single experiment has reported an increase in the concentration of neutrophil-derived MV by the end of a ramp cycling protocol, with a tendency to return to baseline after 2 h of recovery [50], and a delayed rise in circulating monocyte MVs was observed after exercise in well-trained men [4], while it remained unchanged or undetectable in less fit individuals [4,50]. Studies that observed a rise in EMVs expressing markers of endothelial activation were limited to the post-exercise period [4,16,51,63]. From time-course experiments, a late EMV increase may be expected, with peak values between 45-90 min after exercise that returns towards baseline thereafter [4,51], but it is important to recall that several investigations failed to report increases in circulating EMV concentrations (Table 2).

[FIGURE1]

Of relevance is an EMV subpopulation carrying apoptotic antigens (e.g. $CD31^+$ and negative for platelet markers). These MVs have been thought to reflect endothelial apoptosis and vascular damage by several authors [33–35]. One should note that a number of studies in the field of exercise have considered $CD31^+$ EMVs as an apoptotic subpopulation irrespective of annexin-V staining, and this review will follow the same definition when referring to apoptotic EMVs.

The current literature regarding apoptotic EMVs is ambiguous and may depend on the assessment time-point and participant sex/population. For example, Durrer et al. [63] reported reduced concentrations of circulating apoptotic EMVs in the morning following continuous and interval exercise bouts in overweight/obese males, but not females, which was also evident 1 to 3 hours after exercise bouts in healthy men [68]. Conversely, plasma $CD31^+$ EMV remained unchanged in healthy individuals when assessed 5 min after moderate intensity cycling [16]. On the other hand, Schwarz *et al.* [64] observed a 30% increase in plasma $CD31^+$ MVs in men and women within 30 min of completing a marathon, but it is unclear whether the authors' flow cytometry gating strategy excluded platelet markers to ensure appropriate apoptotic EMV quantification. Hence, the limited evidence available suggests that short to moderate duration exercise sessions (i.e. from a few minutes up to 2 h) do not

lead to endothelial injury, as reflected by stable circulating apoptotic EMVs and, if anything, this MV subpopulation may even decrease below baseline values after exercise.

The exact fate of apoptotic EMVs remains unknown but it may include uptake by endothelial cells in the post-exercise period [68]. Internalization of MVs by native endothelial cells have been reported in several tissues [53,76,77] and may serve as a clearance mechanism since haemodynamic forces push circulating MVs towards the vascular endothelium. Previous experiments have identified that endocytosis of MVs by such vascular cells occurs through the anchoring of MV surface PS to endothelial integrins, in a process mediated by endothelial locus-1 glycoproteins [53]. Macrophages have also been reported to remove MVs through phagocytic pathways regulated by the presence of PS, CD31 and Immunoglobulin M on the MV surface [78], and clearance likely involves MV opsonisation by complement components and subsequent uptake by phagocytes [79]. Furthermore, systemically infused MVs localise in the spleen, lungs, and liver, which are all thought to be important organs involved in MV clearance [78]. It is unknown, however, whether these mechanisms apply to the exercise context. Nonetheless, a decrease in apoptotic EMVs compared to resting values has also been reported 1 h into recovery in patients who undergo dobutamine-induced cardiac stress [80], indicating rapid clearance of EMVs which further supports findings from physical stress (exercise) challenges. An extensive review of MV release and clearance has been published by Ayers et al. [78].

3.1.2. Exercise type

Little information exists regarding exercise modalities and MV responses. Most studies have employed continuous or interval cycling and only a single study involved (continuous, incremental) treadmill exercise, where a rise in circulating PMVs occurred [70]. Cycling and running differ in terms of contraction type and muscle mass engaged, which could be factors influencing the MV response. Furthermore, footstrike during running has been proposed to induce mechanical damage of erythrocytes and contribute to exercise-related haemolysis [81,82], which may influence RBCMVs. Although no direct comparison between exercise modalities has not been performed, some conclusions regarding those variables can be drawn from published data. Using an adapted cycling model, Rakobowchuk et al. [14] compared aerobic power matched concentric vs. eccentric exercise and noted similar patterns of increased post-exercise plasma PMV and unaltered EMV concentrations, indicating no influence of contraction type upon MV dynamics. Furthermore, MV release appears to require the activation of only small quantities of skeletal muscle, as circulating PMV increase even with small muscle mass exercise, like with incremental knee extensor exercise [17].

Resistance exercise, on the other hand, does not seem to affect blood EMV levels [67], the only MV population studied to date. One could speculate that PMVs would be released under such conditions since resistance exercise can acutely increase platelet activation as assessed by increased plasma β-thromboglobulin concentrations [83,84] which highly correlates with plasma PMV (r=0.95) [85].

Hence, cycling and running may be expected to induce MV or at least PMV formation, indicating that rhythmic endurance-like exercise can stimulate cell vesiculation, whereas data pertaining to resistance exercise is scarce.

3.1.3. Exercise intensity

The relative intensity of an exercise session often governs acute physiological adjustments and potentially impacts MV responses to physical exertion. If the exercise intensity domains recommended by the American College of Sports Medicine are taken into consideration [86], most studies of MVs and acute exercise have employed moderate to vigorous exercise stimuli, with intensities generally ≥ 50% of peak oxygen uptake. Post-exercise increase in blood PMV concentration

is a consistent response to exercise, even reported with cycling performed below the first ventilatory threshold [14]. A between-study analysis, however, may lead to uncertainty about the influence of exercise intensity on MV dynamics, since augmented appearance of certain blood MVs is reported with near maximal incremental exercise [50,70], but variable responses have been noted after high intensity interval exercise [51,63,66,68], or light to moderate intensity protocols [14,65].

Recent evidence comparing continuous cycling within different intensity domains has helped to clarify this topic. Based on 60 min of moderate or vigorous continuous cycling performed by healthy men, it has become apparent that relative intensity plays an important role in MVs dynamics. Specifically, circulating PMVs increased from baseline during and after vigorous exercise (i.e. ≥ 64% of maximal oxygen uptake), whereas moderate intensity cycling (i.e. ≥ 46% of maximal oxygen uptake) resulted in a very modest non-significant rise [15]. The plasma EMVs concentrations were unaltered, no matter the exercise intensity.

3.1.4. Exercise volume

The influence of exercise volume on acute MV responses lacks systematic evaluation at this point in time. Studies designed to directly isolate exercise volume are needed, and comparison of exercise protocols that induce changes in blood MV concentrations have been limited in terms of exercise duration. Nevertheless, pronounced increases in circulating PMVs occurs even with small volume exercise (e.g. a few minutes of incremental whole-body or isolated-limb exercise) [17,50,70], whereas the longest duration studies (i.e. 4 h cycling performed below the anaerobic threshold or marathon running) showed smaller increases or unchanged concentrations of circulating MVs [64,65]. One could speculate that MVs may have increased and subsequently returned towards baseline concentrations during the latter experiments, but the small number of sequential blood sampling time points limits conclusions.

At this stage, data from Wilhelm et al. [15] helps shed further light on this topic as blood samples were taken throughout 1 h of moderate and vigorous intensity cycling. Plasma PMVs increased from baseline by 30 min of vigorous exercise and remained stable until the end of the 1 h protocol, suggesting little influence of exercise volume upon PMV, since doubling the exercise duration did not further increase plasma MV concentrations. Moreover, if simplistic energy expenditure estimations are made considering a general O_2 caloric equivalent of 5 kcal/l O_2 (i.e. disregarding protocol-specific respiratory exchange rate) it becomes apparent that exercise volume has little influence on circulating MV appearance, since the concentration of plasma PMV was unchanged throughout the moderate cycling session, even though the energy expenditure at the end of 1 h of this protocol (~450 kcal) was greater than at 30 min of vigorous exercise (~330 kcal), when a rise in PMV was already evident.

Together, although still limited, the current body of evidence points toward a greater influence of exercise intensity rather than volume upon MV release.

3.1.5. Physiological significance of exercise-derived microvesicles

The introduction of MVs into the circulation of exercising humans plays a regulatory role in haemostatic control. Sossdorf et al. [4] isolated MVs exposing PS and reported an increase in MV-related prothrombinase activity from post-exercise samples. Fibrin formation was greater in samples from well-trained participants, reinforcing the procoagulant potential of MVs. As one exercises, both the pro and anticoagulant systems may be activated [70,72,87], so the increased MV pro-coagulant potential with acute exercise may play a natural role in fine-tuning the fibrinolytic and thrombotic balance.

Exercise training traditionally improves endothelial function mainly through a vascular shear stress-mediated mechanism [18–20], but circulatory factors are also involved [21]. Since circulating MVs, particularly PMVs, can be acutely increased after an exercise session, speculation that these vesicles are involved in vascular adaptation to training has emerged. Early experimental evidence that exercise increases the interaction between endothelial cells and MVs was provided by Wahl *et al.* [68]. They fluorescently labelled-MVs (PKH26 staining) *in vitro* and loaded these into serum samples obtained from male athletes prior to and after exercise. Human coronary artery endothelial cells were then incubated with the MV-rich sera which increased EMV (but not monocyte MV) uptake by cultured endothelial cells exposed to post-exercise serum, providing some of the first evidence that blood milieu alterations after exercise stimulate the uptake of selected MV populations from the circulation. Moreover, a concomitant decrease in endothelial cell apoptosis was observed as assessed by, caspase-3 activity but unfortunately the lack of a MV-poor serum control condition precludes that the antiapoptotic effect was mediated by MVs specifically.

In subsequent experiments, MVs isolated from the plasma of exercising humans stimulated angiogenesis of cultured endothelial cells when compared to MVs obtained from baseline resting condition [15]. The angiogenic potential of exercise-derived MVs may stem from enhanced endothelial proliferation and migratory capacity induced by these MVs compared to those obtained at baseline. The mechanisms through which exercise-derived MVs induced alterations in endothelial phenotype were not investigated but may relate to delivery of angiogenic growth factors. For example, VEGF is considered a potent regulator of skeletal muscle capillary formation, and MVs shed by platelets *ex vivo* increase endothelial wound-healing and angiogenesis partially through the delivery of VEGF to endothelial cells [62]. Part of the pro-angiogenic effect of VEGF depends on activating the NO pathway [88,89], and MVs from healthy individuals are a source of bloodborne eNOS [9,11]. Moreover, in rodent limb ischaemia models MVs likely facilitate compensatory angiogenesis through the stimulation of progenitor cell differentiation into an endothelial phenotype [60]. Together, these are potential, yet speculative, mechanistic alternatives through which exercise-derived MVs may bring about endothelial adaptation. Although exercise-derived PMVs remain logical candidates for their physiological effects, the specific MV population responsible for the pro-angiogenic and proliferative endothelial stimulation, as well as the exact biochemical pathways through which exercise-derived MVs stimulate endothelial cells still needs to be determined.

As postulated with different extracellular vesicle populations [24], it is possible exercise-derived MVs transport “exerkines” and promote systemic adaptations. An important recent study by Whitham *et al.* [90] suggests that acute exercise might modify the cargo of sampled extracellular vesicles, which contained a fraction of small MVs, as hundreds of proteins transported in extracellular vesicles were altered in the circulation after a cycling bout. Beyond the vascular system, they further demonstrated that systemically infused exercise-derived extracellular vesicles accumulated in the liver of recipient mice, indicating a pathway for communication with non-exercising tissues during exercise, which could include the lungs, kidneys, liver, and brain [53,76,77]. Hence, all these tissues could be prone to phenotypical modifications mediated by the exercise-derived MVs. As such, given the systemic nature of circulating MVs, one can speculate that their timed release with exercise can positively influence several tissues and organs by altering remote cell function and morphology (Figure 2). Future studies ought to further unravel the relevance of these MVs in vascular and systemic adaptations to exercise *in vivo*.

[FIGURE2]

3.2. Physical activity and exercise training

Observational studies reporting altered circulating MVs concentrations in patients with vascular and metabolic dysfunctions, as well as established diseases suggest that MVs respond to chronic physiological challenges. Moreover, the ability of MVs to carry bioactive makers and participate in horizontal gene transfer at remote sites suggests involvement in the pathophysiology. Accordingly, environmental factors and chronic lifestyle modifications, such as exercise training, may influence the basal concentration of circulating MVs.

Early evidence of the influence of physical activity and exercise upon the blood MV profile comes from small, yet well conducted, bedrest and restricted physical activity experiments. Navasiolava et al. [35] limited physical activity of 8 healthy men to a minimum for 7 days, and observed an early elevation in apoptotic EMVs by the third day of inactivity, with reduced microvascular vasodilatory function that was likely prostaglandin-related. In agreement with this previous study, reducing daily physical activity levels of recreationally active men by ~50% (from > 10,000 to < 5,000 steps/day) for 5 consecutive days impaired popliteal artery endothelial function augmented circulating concentrations of apoptotic EMVs [36]. Since inactivity-mediated vascular dysfunction seems to reflect increases in EMVs, a decrease in basal EMVs could be expected with increased physical activity through, for example, exercise training.

Only a few studies have investigated changes in blood MVs after a training period. Babbitt et al. [91] were among the first and reported a near 50% decreased in plasma $CD62^{+}$ EMVs (a MV subset theoretically derived from activated endothelial cells) of middle-aged and older African-Americans after 6 months of light to moderate intensity endurance exercise, which was accompanied by an improvement in blood inflammatory markers and brachial artery flow-mediated dilation. A subgroup analysis also revealed a decrease in apoptotic EMVs [92], suggesting reduced vascular damage and vesiculation at rest after the training period. Unfortunately, the lack of a control group hinders categorical conclusions that the reported effects were exclusive to the training program itself. However, these results importantly indicate that chronic improvements in endothelial function are reflected by a reduction in blood EMV concentrations in a population at elevated risk for cardiovascular events [93–95].

In light of current evidence, we can only hypothesize about the chronic effect of exercise training on resting PMV adaptations. As platelet hyperreactivity to ADP has been associated with long-term cardiovascular complications in some studies [96,97], and considering that: (1) baseline PMV concentration is increased in patients with established or at risk for cardiovascular diseases [8,10,12]; and (2) exercise training can reduce markers of platelet activation at rest and diminish their sensitivity to activation agonists [87]; it appears reasonable that circulating PMV content would drop after a training programme in selected patient populations. However, it is important to stress that the impact of exercise training on resting platelet activation is not unequivocal [87]. Alternatively, the concentration of PMVs might remain unchanged while their content could be modified towards a less thrombogenic and more vasoprotecive phenotype, but again this idea remains merely hypothetical.

4. Further directions in physiology and medicine

Even though alterations in certain circulating MVs concentrations with exercise seems like a normal response in healthy adults, little is known about the influence of acute and chronic exercise upon the cargo of MVs and their dynamics in specific patient populations. For example, Guiraud *et al.* [66] observed no change in circulating PMV and EMV amongst stable CAD patients after an exercise

challenge, whereas subsequent work by Augustine *et al*. [80] revealed that circulating MVs concentrations increased in patients with normal coronary arteries in response to a pharmacologically-mediated cardiac stress, but MVs concentrations were unaltered in patients likely to have further cardiovascular complications. These findings suggest that an increase in selected MV populations with acute stress is an important physiological process that appears blunted in patients at risk. These MV dynamics to stress tests could even be useful in predicting future cardiovascular events in certain populations. Monitoring changes in circulating MVs concentrations prior and after standard tests (such as exercise) in healthy controls and populations with cardiovascular risk factors could provide further insight into the MV dynamics and could progress to the use of exercise-derived MVs as biomarkers in patient stratification.

As discussed, our understanding of the MVs responses to exercise and training is far from complete, and this field will benefit from acute experiments exploring the influence of exercise variables (e.g. volume, intensity, and type of exercise) and their chronic influence to optimise training adaptations. Greater standardization on MV quantification protocols within the field of exercise is also warranted. Moreover, beyond changes in the MV profile, the content of MVs is affected by one's physiological state [9,11], and it is likely that exercise-released MV differ in composition compared to those produced under basal or pathological conditions. It is also tempting to speculate that chronic exercise may impact not just the circulating MV profile, but its cargo as well. Future studies that examine proteomic, lipidomic, transcriptomic, and metabolomic profiles with more appropriate species isolation and quantification techniques will certainly shed light upon the composition of MVs.

The exciting findings that extracellular vesicles can mediate cell-to-cell signalling through horizontal transfer of mRNA and miRNA [98,99] is of particular interest, as acute and chronic exercise can alter the concentration of circulating miRNA [100], and MVs might act as a transport vehicle of transcription-controlling factors and alter the function of remote cells. Interestingly, there is evidence linking PMVs to adhesion and differentiation of early outgrowth endothelial cells *in vitro* and amplification of early outgrowth endothelial cell reendothelization in murine vascular injury models [6]. This suggests the exercise-induced PMV release phenomenon may improve vascular repair and function by enhancing differentiation of circulating endothelial progenitor cells. Integration of *in vitro* and *in vivo* studies exploring these ideas may help unravel the mechanisms of exercise-mediated vascular adaptations.

5. Conclusion

Cell-derived MVs have received increased attention in the scientific community due to their potential to serve as biomarkers of intracellular events and their innate biological activities. These extracellular vesicles had been initially thought of as simple by-products of pathological disorders, and subsequently believed to play a role in maladaptation, but more recent evidence has also shown that MVs are not necessarily harmful, and actually necessary for proper physiological function. Exercise is a powerful factor affecting circulating MV dynamics. Available evidence indicates that the blood MV response to acute and chronic training may resemble the cytokine adjustments to exercise: that is, transient physical exertion may lead to a timed release of MVs, in particular PMVs, which are likely to be involved in acute and subacute exercise adjustments, as is the case with the cytokine response to a single bout of exercise. However, in the long term, and in analogy to pro-inflammatory cytokines, exercise training may decrease resting levels EMVs, which may be involved and reflect reduced vascular injury. Last but not least, a body of evidence indicates that these tiny vesicles play a role in the complex haemostatic control to exercise and are potential novel mediators of endothelial adaptions to training. The upcoming decades will certainly benefit from research investigating the

precise dynamics of MVs in response to specific exercise variables, and will unravel their relevance in human physiology.

Compliance with Ethical Standards

Conflict of Interest

Eurico N. Wilhelm, Laurent Mourot and Mark Rakobowchuk declare that they have no conflict of interest.

Funding

The research was funded by grants from the French Ministry of National Education, of Research and of Technology (EA3920), from Tomsk Polytechnic University Competitiveness Enhancement Program grant, Project № ВИУ-ИСГТ-108/2017 - TPU CEP-HSTI-108/2017 and a Natural Science and Engineering Research Council of Canada Discovery Grant to M.R.

E.N.W. is supported by the Brazilian Education Ministry Foundation CAPES (Postdoctoral Fellowship).

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FIGURE LEGENDS

Figure 1. Projected time-course of circulating microvesicle (MV) appearance during and after an endurance exercise session based on the current literature. A 2 to 3-fold increase in plasma platelet MV (PMV) concentrations during and few hours after exercise have been consistently reported, but data supporting an increase in activation-derived endothelial MV (EMV) concentrations are inconsistent. The concentration of EMVs carrying apoptotic markers may be decreased in the circulation hours to days after exercise.

Figure 2. Exercise-derived microvesicle formation and release in the circulation, and their putative role as ubiquitous mediators of adjustments and adaptations to exercise. Representative figure not to scale. Schematic figure developed using images from the Servier Medical Art image bank.