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REVIEW ARTICLE

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Effects of exercise on exosome release and cargo in in vivo and ex vivo models: A systematic review

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Abstract

Exercise-released exosomes have been identified as novel players to mediate cell-to-cell communication in promoting systemic beneficial effects. This review aimed to systematically investigate the effects of exercise on exosome release and cargo, as well as provide an overview of their physiological implications. Among the 436 articles obtained in the database search (WOS, Scopus, and PubMed), 19 articles were included based on eligibility criteria. Results indicate that exercise promotes the release of exosomes without modification of its vesicle size. The literature has primarily shown an exercise-driven increase in exosome markers (Alix, CD63, CD81, and Flot-1), along with other exosome-carried proteins, into circulation. However, exosome isolation, characterization, and phenotyping methodology, as well as timing of sample recovery following exercise can influence the analysis and interpretation of findings. Moreover, a large number of exosome-carried microRNAs (miRNAs), including miR-1, miR-133a, miR-133b, miR-206, and miR-486, in response to exercise are involved in the modulation of proliferation and differentiation of skeletal muscle tissue, although antigen-presenting cells, leukocytes, endothelial cells, and platelets are the main sources of exosome release into the circulation. Collectively, with the physiological implications as evidenced by the ex vivo trials, the release of exercise-promoted exosomes and their cargo could provide the potential therapeutic applications via the role of intercellular communication.

KEYWORDS

aerobic training, cfDNA, exercise-released exosomes, extracellular vesicles, microRNAs, physical activity

1 | INTRODUCTION

Exercise is considered as a fundamental intervention to not only help reduce the risk of a multitude of disorders from metabolic diseases to cancer (Hills, 2018; Radak et al., 2019), but also delay the occurrence of aging-related diseases (Estébanez et al., 2019; Whitham & Febbraio, 2016). Although the effects of exercise on different cellular processes through the microRNA (miRNA) and inflammatory modulation, the mitophagy and mitochondrial dynamic processes, or the endoplasmic reticulum stress response have been intensely studied (Cabral-Santos et al., 2019; Domańska-Senderowska et al., 2019; Estébanez et al., 2018; Moreira et al., 2017), the molecular interactions in promoting physiological adaptations to exercise remain unclear. One of the most commonly accepted theories that explains exercise-mediated intercellular interactions is the

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involvement of myokines from skeletal muscle, also known as exercise-promoted cytokines (exerkines) when released into circulation (Pedersen et al., 2007; Whitham & Febbraio, 2016). Although the mechanisms of how these myokines are transported and released need to be further explored, extracellular vesicles (exosomes) have been proposed to facilitate intracellular communication in response to exercise (see Figure 1).

Cellular communication is a fundamental phenomenon in the process of life. The regulation of the whole organism and the maintenance of homeostasis depend on the coordination of all the organs

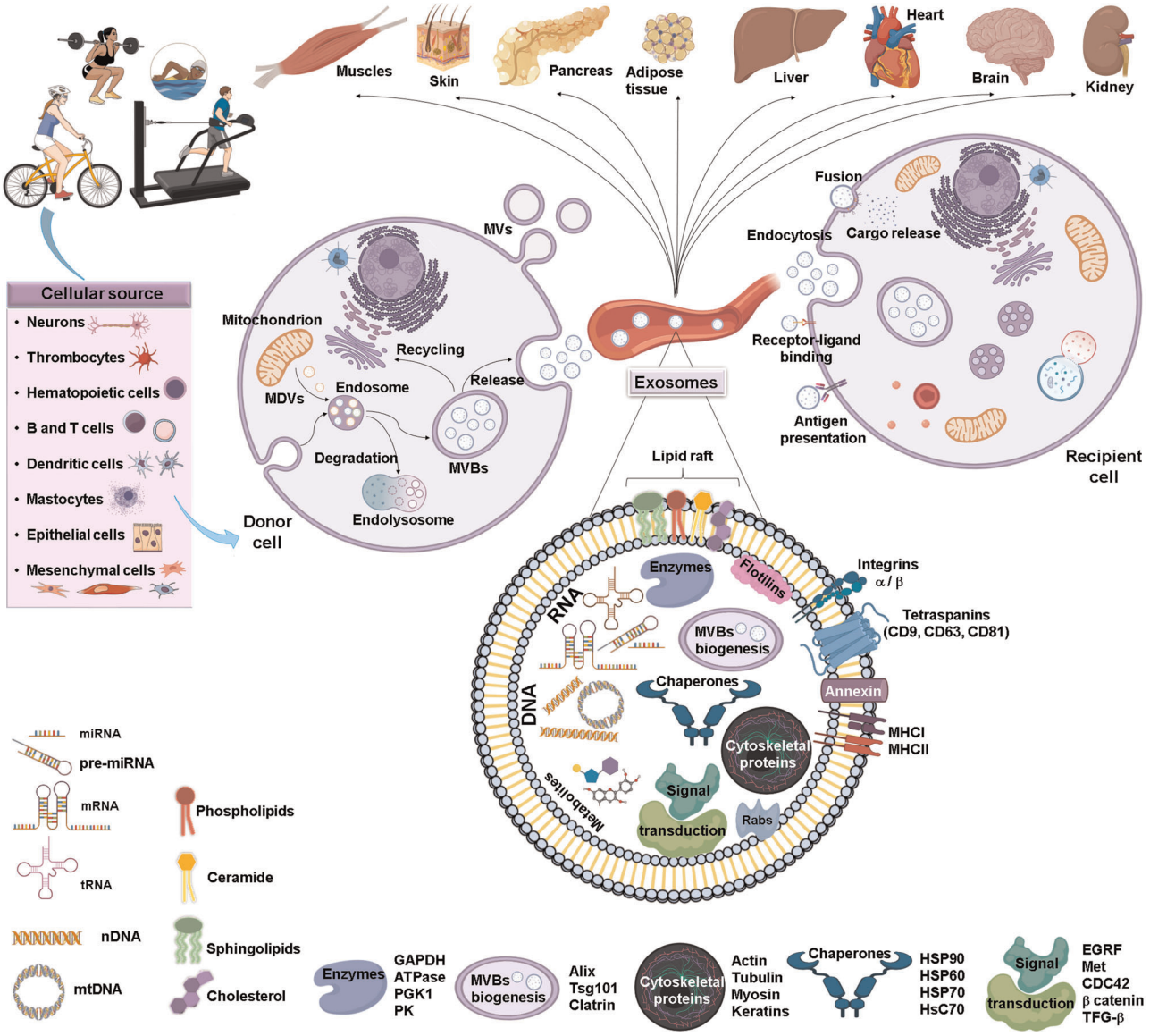

FIGURE 1 Hypothesis of how exercise could promote systemic benefits by exerting peripheral and distal organ crosstalk through exosome release. Physical exercise mediates the release of exosomes, which can derive from a multitude of cell types (neurons, thrombocytes or hematopoietic, dendritic, mast, epithelial, and mesenchymal cells). These exosomes are originated from invaginations of the plasma membrane that later result in endosomes, to which MDVs can be added. Late endosomes, if not degraded by lysosome fusion, results in MVBs that, if not recycled to the trans-Golgi network, then release exosomes to the extracellular medium through their fusion with the plasma membrane. Exosomes are constituted by a lipid bilayer, enriched in domains similar to lipid rafts, and its cargo includes RNA, DNA, and several proteins such as tetraspanins, flotilins, integrins, proteins involved in MVBs biogenesis, membrane trafficking (annexins, Rabs) and signal transduction, cytoskeletal and immunomodulatory proteins (MHCI and MHCII), as well as chaperones, although their molecular composition differs depending on the cell of origin. Donor cells release exosomes into extracellular matrix to potentially act in an autocrine and/or paracrine manner, or into the bloodstream reaching the different organs (muscle, skin, pancreas, adipose tissue, liver, heart, brain, or kidney). Though either intercellular communication, the exosomes contact with and are taken up by recipient cells via cell membrane fusion, binding to surface receptors, or the internalization of endocytosis. Subsequently, exosomes transfer their cargoes into the target cells to modulate their functions, such as survival, proliferation, inflammation, insulin secretion, and glucose metabolism, thereby resulting in physiological improvements. MDVs, mitochondrial derived vesicles; MVs, microvesicles; MVBs, multivesicular bodies. The figure was made with use of Mind the Graph, licensed under a free culture Creative Commons Licence

of the body and cell–cell communication. Thus far, it has been thought that such communication occurs at the paracrine or endocrine level by the secretion of molecules to the extracellular space. However, over the past few decades evidence has demonstrated the release of certain content from cells to systemic environments in the form of vesicles (Samuelson & Vidal-Puig, 2018). Specifically, three types of extracellular vesicles (EVs): exosomes (30–150 nm), microvesicles (150–1000 nm), and apoptotic bodies (800–5000 nm) have been identified to use intercellular communication in mediating cellular processes (Crescitelli et al., 2013; Latifkar et al., 2019). Of particular note, exosomes are small EVs of endocytic origin being constituted by endosomal membranes and grouped in what has been called multivesicular bodies (MVBs). As illustrated in Figure 1, exosomes are released extracellularly when MVBs fuse with the plasma membrane, differently from larger microvesicles (MVs) or apoptotic bodies that are shed from the plasma membrane (Huang-Doran et al., 2017). Then, exosomes interact with a recipient cell, and depending on cell type this communication can be through cell membrane-mediated signaling and/or through clathrin/caveole-mediated endocytosis, phagocytosis, or macropinocytosis internalization (Prattichizzo et al., 2017). Once exosomes bind to the plasma membrane of the recipient cell, their cargo (lipids, proteins, metabolites, messenger RNA, miRNAs [also known as miRs], and genomic and mitochondrial DNA) is released to the recipient cell cytoplasm. The uptake of these exosome cargo seems to be active, when a specialized intracellular transport machinery, participating proteins like tetraspanins, immunoglobulins, and integrins, is involved (Huang-Doran et al., 2017). Research has also demonstrated that exosomes exert a variety of responses in communicating with other cells, as an alternative method to transfer information of both paracrine and endocrine cell-crosstalk in physiological or pathophysiological conditions (Catalano & O'Driscoll, 2020; Prattichizzo et al., 2017). Among the different responses caused by this exosome-induced communication, cell proliferation, apoptosis, immune modulation, cytokine production, and metastasis have been described (Huang-Doran et al., 2017).

Emerging evidence suggests that exercise enhances the biogenesis of MVBs and exosomes (Garner et al., 2020). Subsequently, the release of exosomes following exercise carries exerkines to mediates intercellular communication (Y. Li et al., 2017), thereby resulting in health benefits in various conditions and diseases, including aging (Bertoldi et al., 2018), Type 2 diabetes (G. Li et al., 2019; Safdar et al., 2016), cardiovascular diseases (Bei et al., 2017; Hou et al., 2019; Ma et al., 2018), immunity (Lancaster & Febbraio, 2005; C. X. Wu & Liu, 2018), breast cancer (Dethlefsen et al., 2017), and sarcopenia (Rong et al., 2020). Several studies have proven both paracrine and endocrine effects of muscle-derived exosomes on the maintenance of muscle homeostasis and communication with other tissues, respectively (Qin & Dallas, 2019). In fact, muscle cells, such as myoblasts and myotubes have been reported as a source of exosomes with the expression of Tsg101 and Alix protein markers, along with other proteins involved in signal transduction, miRNAs, and cell-free DNA (cfDNA; Forterre et al., 2014; Guescini et al., 2010). Whereas, other cellular sources of exercise-promoted exosome release, such as antigen-presenting cells, leukocytes, endothelial cells, and platelets, have also been identified (Brahmer et al., 2019). Therefore, this review aimed to systematically investigate all available evidence from in vivo and ex vivo studies in which acute and chronic exercise effects on exosome release and cargo were evaluated in human and animal models, as well as summarize these outcomes with an overview of their physiological implications.

2 | METHODS

This systematic review was performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA; Moher et al., 2009) and prospectively registered in the International Prospective Register of Systematic Reviews (PROSPERO registration number: CRD42020176396).

2.1 | Search strategy and study selection

PubMed, Scopus, and Web of Science (WOS) electronic databases were used to perform the article search from inception to March 19, 2020. The use of Medical Subject Headings (MeSH) terms included "(exosomes OR extracellular vesicles) AND (exercise OR physical activity)," tagging "All fields" in PubMed, "Article title, Abstract, Keywords" in Scopus, and "Topic" in WOS search. Next, the exclusion filters were specified as follows: article types, publication dates, and languages. The screening of eligible articles and data extraction were conducted by two independent reviewers.

Article selection was based on the following inclusion criteria in both in vivo and ex vivo research: (1) studies that assessed the release of exosomes in response to both acute and chronic exercise; (2) studies that examined circulating and tissue exosomes; (3) studies that estimated exosomes number or size; and (4) studies that evaluated any exosomal content (proteins or nucleic acids).

This review only considered EVs as exosomes based on the isolation methods that are used with the aim to study exosomes or small EVs in the literature (ExoQuick™ Kit, magnetic activated cell sorting [MACS] or microbeads, size exclusion chromatography [SEC], or centrifugations at ~100,000*g*, regardless of the use of a filtration step or density gradient). Therefore, articles that analyzed microparticles, MVs or total EVs were excluded in this review. However, to date there are no isolation methods available that specifically isolate exosomes, and especially the ExoQuick Kit was shown not to be exosome-specific (Théry et al., 2018), so we cannot rule out that the evaluated studies have co-isolated other EV subtypes.

The exclusion criteria used for the article output were: (1) meeting abstracts, (2) conference or congress communications, (3) review articles, (4) books or book chapters, (5) project papers, (6) editorials, (7) letters, (8) corrections, (9) retractions, (10) comments, (11) articles in a language other than English, and (12) studies published more than 10 years.

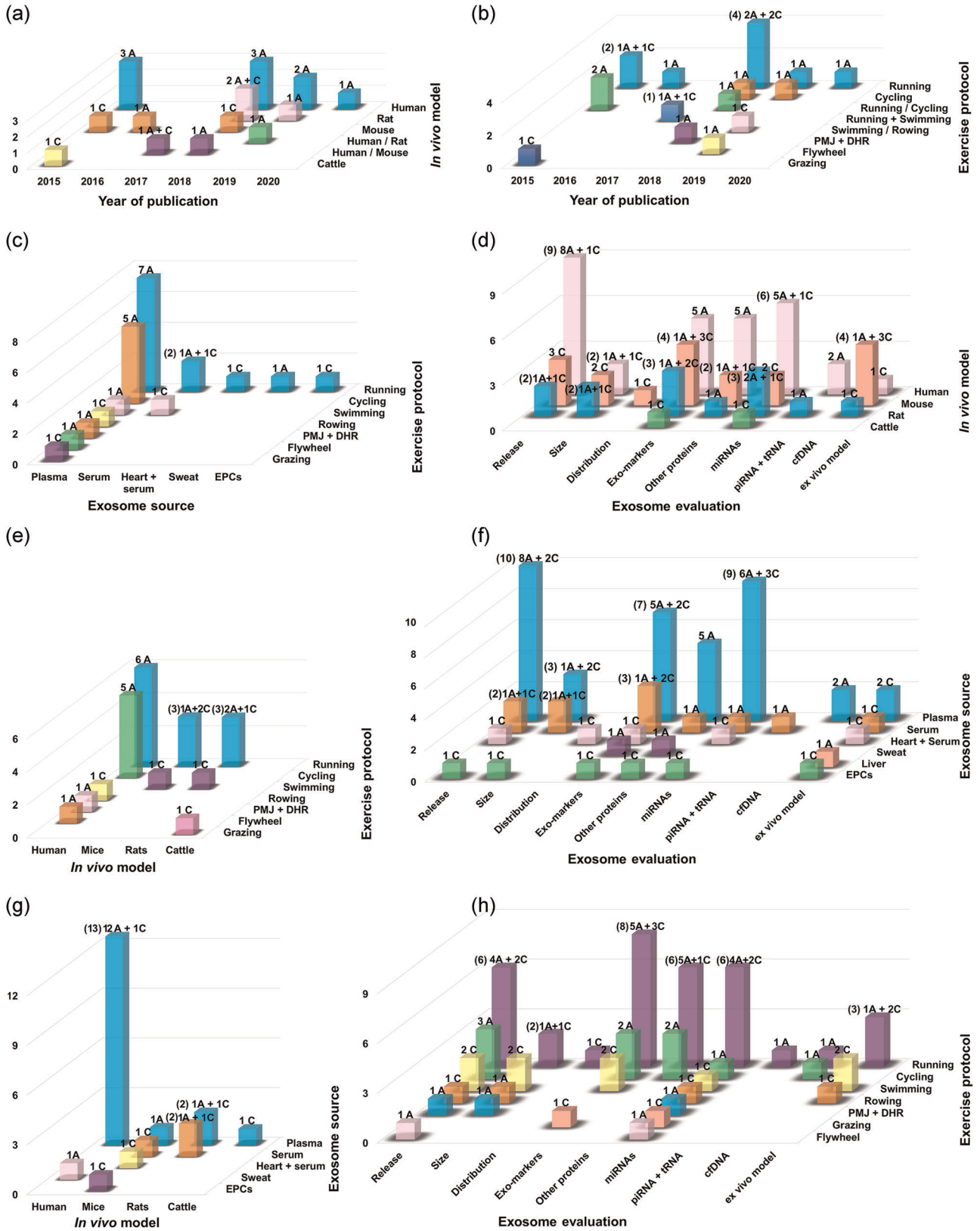

FIGURE 2 (a–h) Main characteristics of the articles included in the systematic review. Number of articles (or studies) published over the last 5 years examining the effects of acute and chronic exercise on the exosomal release and cargo, in both in vivo or ex vivo experiments using human or animal models. A, acute; C, chronic; EPCs, endothelial progenitor cells

TABLE 1 Basic characteristics of articles evaluating exosome release and size and distribution in response to exercise protocols

Research article	In vivo model	Exercise protocol	Exosome source	Isolation method	Characterization method	Results
Annibalini et al. (2019)	Human (n = 8)	Single-bout flywheel	Plasma	UC	NTA	(↑) 2-fold release
Bei et al. (2017)	Human (n = 16)	Bruce stress test (running)	Plasma	Whole plasma	Nano-FCM	(↑) Release
	Mouse (n = 4)	3-Week swimming	Serum	ExoQuick™	NTA	(↑) Release (NS) size
Brahmer et al. (2019)	Human (n = 21)	Incremental cycling test until exhaustion	Plasma	SEC	NTA	(NS) release
Chaturvedi et al. (2015)	Mouse (n = 6)	8-Week running	Heart + serum	UC + filtration	EM + AChE + FC IS	(↑) Release (↑) CD81 and Flot-1 colocalization in cardiac tissues
Frühbeis et al. (2015)	Human (n = 2)	90-min cycling test	Plasma	UC + filtration	NTA	(↑ 90-min post) 2.7-fold release
	Human (n = 2)	360-min running	Plasma	UC + filtration	NTA	(↑ 90-min post) 1.5-fold release
Hou et al. (2019)	Human (n = 32)	1-Year rowing	Plasma	UC	NTA	(NS) release (NS) size
	Rat (n = 8)	4-Week swimming	Plasma	UC	NTA	(↑ Post last training session) release (NS 24-h post) release (NS) size
Lovett et al. (2018)	Human (n = 9)	2-Consecutive bouts of PMJ + DHR	Plasma	SEC	NTA	(NS) release (NS) size
Ma et al. (2018)	Mouse (n = 12–18)	4-Week running	EPCs	MACS	NTA	(↑) EPCs-exosome release (S < L < M) (↑) EPCs-exosome/EPC ratio (NS) EPCs-exosome size
Oliveira et al. (2018)	Rat (n = 18)	40-min running	Serum	ExoQuick™	TRPS	(↑) release (↓) size (H vs. M) (NS) size (H vs. C)
Rigamonti et al. (2020)	Human (n = 23; F/M = 12/11]	30-min moderate running (or voluntary exhaustion)	Plasma	UC + filtration	NTA	(NS) release (↓ Post female vs. male) release Positive correlation with HOMA-IR
Whitham et al. (2018)	Human (n = 11)	60-min cycling	Plasma	UC	NTA	(↑) Release

Abbreviations: C, control; DHR, downhill running; EM, electron microscopy; EPCs, endothelial progenitor cells; F/M, female/male; FCM, flow cytometry; H, high; IS, immunostaining; L, low; MACS, magnetic activated cell sorting; M, moderate; NS, no significant; NTA, nanoparticle tracking assay; PMJ, plyometric jumping; SEC, size exclusion chromatography; S, sedentary; TRPS, tunable resistive pulse sensing; UC, ultracentrifugation.

2.2 | Data extraction

A standardized form was used to extract data from each chosen study, containing the following variables: (1) first author name and year of publication, (2) in vivo model (human, mouse, rat, or cattle), (3) physical activity information or exercise protocol, (4) source of samples (whole blood, plasma, serum, sweat, organs, or cell supernatant), (5) isolation methodology (ExoQuick™, MACS, SEC, and ultracentrifugation), (6) characterization methodology (electron microscopy; flow cytometry; immunostaining; nanoparticle tracking assay [NTA]; tunable resistive pulse sensing), (7) phenotyping methodology (ELISA, EV array, MACSPlex, western blot [WB]), and (8) EVs alterations (exosome number, size, and distribution, and level of exosome markers and other exosome-contained proteins, RNAs [miRNAs, piwi-interacting [piRNAs], and transfer RNAs [tRNAs]], and cfDNA). The performance of a meta-analysis was ruled out since

TABLE 2 Basic characteristics of articles evaluating exosome markers and other exosome-carried proteins in response to exercise protocols

Research article	In vivo model	Exercise protocol	Exosome source	Isolation method	Phenotyping method	Results
Barone et al. (2016)	Mouse (*n* = 24)	60-min running	Plasma	UC + filtration	WB	(↑ 0-min – 15-min vs. control) Alix, HSP60, HSP70 (↑ 15-min vs. 0-min) Alix, HSP60, HSP70
Bei et al. (2017)	Mouse (*n* = 3)	3-Week swimming	Serum	ExoQuick™	WB	(↑) CD63
Bertoldi et al. (2018)	Rat (*n* = 12–18)	2-Week moderate running	Serum	miRCURY™	ExoELISA	(↑ 18-h post) CD63, at all ages (3-, 21-, 26-months)
Brahmer et al. (2019)	Human (*n* = 21)	Incremental cycling test until exhaustion	Plasma	Whole plasma	EV array	(↑) Alix, CD14, CD142, MCH-I, ICAM-1, LAMP-1, tPA (BDT) CD9, CD63, CD81, Flot-1, HSP70, CD41b
				SEC	WB	(↑) CD9, CD63, CD81, CD41b (NS) Tsg101, Syntenin, ApoA1 (ND) alphasarcoglycan, liver markers, Calnesin
					MACSPlex	(NS) CD81 (↑) CD4, CD9, CD14, CD29, CD41b, CD42a, CD44, CD62P, CD63, CD69, CD105, CD146, MCH-II
				Bead-isolation	WB	(↑) CD9, CD81, CD63, Syntenin (ND) Tsg101, alphasarcoglycan, liver markers, ApoA1 $CD9^+$EVs – (NS) CD9, CD63, CD41b $CD63^+$EVs – (↑) CD9, CD81 $CD63^+$EVs – (NS) CD63, CD41b $CD81^+$EVs – (↑) CD9, CD63, CD41b
					MACSPlex	$CD63^+$EVs – (↑) CD4, CD8, CD24, CD40, CD49e, CD62P, CD105, CD146, MCHI, MCHII $CD9^+$EVs – (NS) CD14, CD29, CD31, CD41b, CD42a, CD63, CD81 $CD63^+$EVs – (↑) CD4, CD8, CD9, CD14, CD24, CD29, CD31, CD40, CD41b, CD42a, CD44, CD45, CD49e, CD62P, CD69, CD81, CD105, CD146, MCHI, MCHII $CD81^+$EVs – (↑) CD4, CD8, CD9, CD14, CD24, CD29, CD31, CD40, CD41b, CD42a, CD44, CD45, CD49e, CD62P, CD63, CD105, CD146, MCHI, MCHII $CD81^+$EVs – (NS) CD69
Chaturvedi et al. (2015)	Mouse (*n* = 6)	8-Week running	Heart + Serum	UC + filtration	WB	(↑) CD81, Flot-1
Frühbeis et al. (2015)	Human (*n* = 6)	90-min cycling test	Plasma	UC + filtration	WB	(↑ post) Tsg101, HSP70, Int.aIIb (↓ 90-min) Tsg101, HSP70, Int.aIIb
	Human (*n* = 4)	360-min running	Plasma	UC + filtration	WB	(↑ post) Flot-1 (↓ 90-min to 360-min) Flot-1 (NS) HSP70, Int.aIIb

(Continues)

TABLE 2 (Continued)

Research article	In vivo model	Exercise protocol	Exosome source	Isolation method	Phenotyping method	Results
Helmig et al. (2015)	Human (n = 5)	Incremental running test until exhaustion	Plasma	UC + filtration	WB	(↑ Post – 3-, 5-, 10-, 30-, 90-min) Flot-1, HSP70
Hou et al. (2019)	Rat (n = 6)	4-Week swimming	Plasma	UC	WB	(NS) Tsg101, CD81
Ma et al. (2018)	Mouse (n = 12–18)	4-Week running	EPCs	MACS	WB	(+) CD63, Tsg101 + CD34, VEGFR2 (EPC markers) (S, L, M)
Muroya et al. (2015)	Cattle (n = 3)	7-Month grazing	Plasma	UC	WB	(+) CD9
Oliveira et al. (2018)	Rat (n = 18)	40-min running	Serum	ExoQuick™	WB	(+) CD63 (↑) CD63 (C < L < M < H) (NS) ApoA-IV
C. X. Wu and Liu (2018)	Human (n = 13; F/M]	20-min running	Sweat	UC + filtration + density gradient	WB	(+) Lactoferrin, Alix, CD63, HSP70 (–) a-Tubulin (+) 896 proteins (≠ urine, saliva, plasma) (+) Antimicrobial peptides, immunological factors

Abbreviations: (–), absence; (+), presence; BDT, below the detection threshold; C, control; ELISA, enzyme-linked immunosorbent assay; EPCs, endothelial progenitor cells; EVs, extracellular vesicles; F/M, female/male; H, high; L, low; MACS, magnetic activated cell sorting; M, moderate; ND, no detected; NS, no significant; S, sedentary; UC, ultracentrifugation; WB, western blot.

some articles evaluated different samples, which prevents quantitative estimates of the effects of exercise in experimental animal models. Data extracted from the chosen articles were summarized in graphics (Figure 2a–h) and Tables 1–4.

3 | RESULTS

3.1 | Study selection

The study selection was performed as described in Figure 3. A total of 436 articles were obtained in the database search, of which 153 articles were from WOS, 97 from Scopus, and 186 from PubMed. After removing duplicates, 304 articles underwent title and abstract screening, in which 107 articles were excluded as follows: 90 reviews, two as a book chapter, one as a project paper, one as a correction, 10 as a meeting abstract, two as comments, and one as a retraction. We also excluded 138 articles, based on the study inclusion criteria: 49 articles were unrelated to either exercise and/or exosomes, 69 articles were the study of exosomes but not exercise, and 20 articles were exercise study without the examination of EVs. With the elimination of these articles above, 59 articles were selected to be full-text screened. Subsequently, based on the study selection criteria, of which 35 were unrelated to microparticles and other EVs, and five articles included exercise and exosomes but not exercise-derived exosomes. Finally, a total of 19 studies met eligibility criteria and, therefore, were included in this systematic review.

3.2 | Study characteristics

The main characteristics of the selected articles are represented in Figure 2a–h and summarized in Tables 1–4. All research articles included in this systematic review were published in the last 5 years (2015–2020) in both human and animal models, with five ex vivo studies and just one comparing the effects of age.

3.3 | Effects of exercise on exosome release and cargo in both circulation and ex vivo models

The release of exosomes and their cargo, as well as the impact of exercise-released exosomes in cell culture and animal models are summarized in Tables 1–4 and Figure 4. Specifically, the release, size and distribution of exosomes following different exercise protocols were evaluated in 11 articles (Annibalini et al., 2019; Bei et al., 2017; Brahmer et al., 2019; Chaturvedi et al., 2015; Frühbeis et al., 2015; Hou et al., 2019; Lovett et al., 2018; Muroya et al., 2015; Oliveira et al., 2018; Rigamonti et al., 2020; Whitham et al., 2018). Moreover, 12 articles investigated exosomal markers and other exosome-carried proteins in response to exercise (Barone et al., 2016; Bei et al., 2017; Bertoldi et al., 2018; Brahmer et al., 2019;

TABLE 3 Basic characteristics of articles evaluating exosome-carried RNAs (miRNAs, piRNAs, and tRNAs) and cfDNA in response to exercise protocols

Research article	In vivo model	Exercise protocol	Exosome source	Isolation method	Results
Annibalini et al. (2019)	Human ($n = 8$)	Single-bout flywheel	Plasma	UC	(NS) miR-16, miR-126, miR-133b (↑) miR-206, miR-146a
Chaturvedi et al. (2015)	Mouse ($n = 6$)	8-Week running	Heart + Serum	UC + filtration	(+) miR-323-5p, miR-455, miR-466, miR-29b (↑) miR-455, miR-29b
D'Souza et al. (2018)	Human ($n = 10$)	10 × 60'' intervals of cycling at peak power output	Plasma	UC + filtration	(↑ Post) miR-1-3p, miR-16-5p, miR-23a-3p, miR-23b-3p, miR-208a-3p, miR-105-5p, miR186-5p, miR-222-3p, miR-451a, miR-486-5p, miR-378a-5p, miR-126-3p (↓ 4-h) miR-1-3p (NS) miR-21-5p, miR-24-2-5p, miR-27a-5p, miR-33a-3p, miR-107, miR-134-3p, miR-378b, miR-494-3p, miR-499a-5p
Guescini et al. (2015)	Human ($n = 22$)	2-Bouts of running until exhaustion	Plasma	UC + filtration + density gradient	(Positive correlation with VO_{2max}) miR-1, miR-133b, miR-206, miR-499, miR-181a)
	Human ($n = 22$)	40-min vigorous-intensity running	Plasma	UC + filtration + density gradient	Muscle-specific miR-206 enriched at 1.11 g/ml density fraction (↑) mi-206/miR-16 ratio (SCA-immuno-captured exosomes vs. total or uncaptured exosomes) (↑) miR-181a-5p (NS) miR-1, miR-133a, miR-133b, miR-206, miR-499, miR-146a and miR-24
Helmig et al. (2015)	Human ($n = 5$)	Incremental running test until exhaustion	Plasma	UC + filtration	(↑ Post) cfDNA (NS 90-min) cfDNA (↑) nDNA (supernatants vs. exosomes) (↑) mtDNA (MVs)
		Incremental cycling test until exhaustion	Plasma	UC + filtration	(↑ Post) cfDNA (NS 90-min) cfDNA (↑) nDNA (supernatants vs. exosomes) (↑) mtDNA (MVs)
Hou et al. (2019)	Human ($n = 32$)	1-Year rowing	Plasma	UC	(NS) miR-342-5p
	Rat ($n = 6$)	4-Week swimming	Plasma	UC	(↑) miR-3571, miR-1-3p, miR-342-5p, miR-122-5p, miR-196b-5p, miR-486, miR-208a-3p, miR-3591, miR-184, miR-760-3p, miR-99a-5p (↓) miR-191a-5p (NS) miR-494-3p, miR-206-3p

(Continues)

TABLE 3 (Continued)

Research article	In vivo model	Exercise protocol	Exosome source	Isolation method	Results
Lovett et al. (2018)	Human (n = 9)	2-Consecutive bouts of PMJ + DHR	Plasma	SEC	(+) myomiRs: miR-1, miR-133a, miR-133b, miR-206, miR-208b, miR-486, miR-499a miR-1 → the most abundantly expressed (NS) miR-1, miR-133a, miR-133b, miR-206, miR-486 (↓ 24 h) miR-31
Ma et al. (2018)	Mouse (n = 12-18)	4-Week running	EPCs	MACS	(↑) EPCs miR-126 (↑) EPCs-exosome miR-126 (↑) EPCs-exosome/EPCs ratio miR-126
Muroya et al. (2015)	Cattle (n = 3)	7-Month grazing	Plasma	UC	(↑ 1-month) miR-19b, miR-148a, miR-150, miR-221, miR-361, miR-486 (↑ 2-month) miR-19b, miR-150, miR-223, miR320a, miR-361 (↑ 4-month) miR-223 (↑ 7-month) miR-223 (NS 7-month) miR-19b, miR-148a, miR-150, miR-221, miR320a, miR-361, mir-486, miR-451, miR-29b, miR-30a, miR-30d, miR-103, miR-126-5p, miR-144, miR-155, miR-425-5p, miR-489, miR-1249, miR-2888
	Cattle (n = 3)	7-month housed	Plasma	UC	(↑ 1-month) miR-19b, miR-223 (↑ 2-month) miR-19b, miR-223 (↑ 4-month) miR-150
Oliveira et al. (2018)	Rat (n = 18)	40-min running	Serum	ExoQuick™	(NS) exercise-derived EVs miRNA yields (↑) Rno-miR-330-5p, miR-10b-5p, miR-142-3p, miR-410-3p (↑) miR-128-3p, miR-03-3p, miR-148a-3p, miR-191a-5p, miR-93-5p, miR-25-3p, miR-142-5p, miR-3068-3p (NS) piRNAs (↑) tRNA8336
Yin et al. (2019)	Rat (n = 84)	90-min UH/DH running	Plasma	ExoQuick™	UH → (NS any time point) miR-1, miR-133a, miR-133b, miR-206, miR-208a, miR-499 DH → (↑ post) miR-1, miR-133a, miR-133b, miR-206, miR-208a, miR-499 DH → (↑ 1-h) miR-1, miR-208a DH → (NS 48-h) miR-1, miR-133a, miR-133b, miR-206, miR-208a, miR-499

Abbreviations: (+), presence; cfDNA, cell-free DNA; DH, downhill; MACS, magnetic activated cell sorting; piRNAs, piwi-interacting RNA; SEC, size exclusion chromatography; tRNAs, transfer RNA; UC, ultracentrifugation; UH, uphill.

TABLE 4 Basic characteristics of articles evaluating exercise-released exosomes on cell culture and animal models

Research article	In vivo model	Exercise protocol	Exosome source	Ex vivo model	Results
Bei et al. (2017)	Mouse (n = 3-6)	3-Week swimming	Serum	Mouse serum-derived exercise-released exosomes injected at mice with heart damage	(↓) Infarct size (↑) Heart Bcl2 (↓) Heart Bax
				Mouse serum-derived exercise-released exosomes treating rat H9C2 cardiomyocytes	(↑) Bcl2 (↓) Bax (↑) pERK1/2, pHSP27 (with and without H2O2 treatment) 1.85-increased swim-derived serum exosomes mitigated the ERK1/2 and p38MAP inhibitors-caused ERK1/2 and pHSP27 decrease
				Exercise mimic insulin-like growth factor-1 treating rat H9C2 cardiomyocytes	(↑) Exosome release (↑) Alix, RAB35 (mRNA and protein)
Chaturvedi et al. (2015)	Mouse (n = 6)	8-Week running	Heart	Effects of mouse heart-derived exercise-released exosomes-contained miR-455 mimics and inhibitors in HL-1 cell line	(↓) MMP9 (mimics) (↑) MMP9 (inhibitors)
Hou et al. (2019)	Human (n = 32)	1-Year rowing	Plasma	Human plasma-derived exercise-released exosomes-pre-incubated cultured H/R cardiomyocytes	(↓) Apoptosis (↓) LDH (↑) H/R cardiomyocytes viability miR-342-5p showed greater capacity to reduce apoptosis and LDH release, as well as to increase viability
	Rat (n = 4–7)	4-Week swimming	Plasma	Rat plasma-derived exercise-released exosomes -intramyocardial injected rats (48 h before MI/R)	(↓) Infarct size (↓) Serum LDH
				Rat plasma-derived exercise-released exosomes-pre-incubated cultured H/R cardiomyocytes	(↓) Apoptosis (↓) LDH (↑) H/R cardiomyocytes viability miR-342-5p showed greater capacity to reduce apoptosis and LDH release, as well as to increase viability
Ma et al. (2018)	Mouse (n = 12–18)	4-Week running	EPCs	Mouse EPCs-derived exercise-released exosomes-pre-incubated HG- and hypoxia-challenged ECs	(↑) miR-126 (↑) ECs angiogenesis (migration and tube formation) (↓) ECs apoptosis (↓) SPRED-1 (↑) VEGF
Whitham et al. (2018)	Mouse (n = 6)	90-min running until exhaustion	Plasma	Mouse plasma-derived DiR-labeled exercise-released exosomes-intravenously injected mice	Tropism to the liver

Abbreviations: ECs, endothelial cells; EPCs, endothelial progenitor cells; HG, high glucose; H/R, hypoxia/reperfusion; LDH, lactate dehydrogenase.

Chaturvedi et al., 2015; Frühbeis et al., 2015; Helmig et al., 2015; Hou et al., 2019; Ma et al., 2018; Muroya et al., 2015; Oliveira et al., 2018; C. X. Wu & Liu, 2018). In addition, 11 articles assessed the effects of exercise on exosome-carried RNAs (miRNAs, piwi-interacting RNAs (piRNAs), and transfer RNAs [tRNAs]) and cfDNA (Annibalini et al., 2019; Chaturvedi et al., 2015; D'Souza et al., 2018; Guescini et al., 2015; Helmig et al., 2015; Hou et al., 2019; Lovett et al., 2018; Ma et al., 2018; Muroya et al., 2015; Oliveira et al., 2018; Yin et al., 2019). Finally, five studies were included to evaluate the influence of exercise-released exosomes ex vivo in both cell culture and animal models (Bei et al., 2017; Chaturvedi et al., 2015; Hou et al., 2019; Ma et al., 2018; Whitham et al., 2018).

4 | DISCUSSION

It is well established that exosomes are secreted in different size- and composition-based subpopulations identified by their surface antigens under physiological or pathological conditions (Kang et al., 2017; Y. Li et al., 2017). The release of exercise-promoted exosomes and their cargo modification have been proposed as mediators of systemic adaptations (Safdar & Tarnopolsky, 2018). This systematic review is to summarize scientific evidence regarding the effects of acute and chronic exercise on exosome biology as well as provide an overview of its physiological implications.

4.1 | Effects of exercise on exosome release, size, and distribution

The results of current studies demonstrate an increase in exosome release into the circulation, regardless of exercise protocol (Annibalini et al., 2019; Bei et al., 2017; Chaturvedi et al., 2015; Ma et al., 2018; Oliveira et al., 2018). Among all human research, this review did not observe conclusive results, with only five of nine studies demonstrating an increase in exosome release following exercise (Annibalini et al., 2019; Bei et al., 2017; Frühbeis et al., 2015; Whitham et al., 2018), while the size of exosomes remained unmodified following different exercise modes or modalities (Bei et al., 2017; Brahmer et al., 2019; Chaturvedi et al., 2015; Frühbeis et al., 2015; Hou et al., 2019; Lovett et al., 2018; Ma et al., 2018; Oliveira et al., 2018; Rigamonti et al., 2020).

Although these inconsistent results have been shown in the release of exercise-mediated exosomes, the confounding variables, such as the source of the exosomes and exosome isolation and detection methods may potentially influence the findings. Particularly, Brahmer et al. (2019) examined the potential effect of different techniques in the quantity of vesicles and found no significant change in the total number of particles in human plasma-following an incremental cycling test, with the use of NTA. In contrast, the EV-marker analysis demonstrated an increase in the level of vesicles following acute cycling exercise. This discrepancy could be explained by lipoprotein that may be detected by NTA, thereby resulting in an inaccurate total particle count. In this regard, an increase of non-EV particles in plasma could be due to increased fat intake before exercise or blood sampling (Brahmer et al., 2019). After a thorough literature search, NTA was the only main technique utilized to analyze the total number of particles (Annibalini et al., 2019; Bei et al., 2017; Brahmer et al., 2019; Frühbeis et al., 2015; Hou et al., 2019; Lovett et al., 2018; Ma et al., 2018; Rigamonti et al., 2020; Whitham et al., 2018), which makes difficult when interpreting the results of exosome release. Regarding the distribution of exosomes, research has demonstrated the tropism of acute exercise-liberated EVs to the mouse liver (Whitham et al., 2018) and a greater CD81 and Flot-1 colocalization in mouse cardiac tissues following an 8-week of treadmill running (Chaturvedi et al., 2015).

In addition, the polyethylene glycol (PEG)-based Total Exosome Isolation Reagent Kit has been shown to be more efficient for the isolation of exosomes when compared with the method of ultracentrifugation (Nath Neerukonda et al., 2019). Particularly, the PEG-based kit exhibited eight times higher in relative exosome-carried protein levels (Van Deun et al., 2014). Similarly, Grunt et al. (2020) have also found that the PEG-based kit extracted a higher level of exosomes and carried proteins in both plasma and serum than other technique, the mannuronate-guluronate polymer (MGP)-based method. With the PEG-based kit, a greater level of exosome-carried protein was found to be higher in plasma versus serum (Grunt et al., 2020). However, in the culture supernatants MGP-based method has been shown to be an efficient and effective exosome isolation technique to extract a higher level of proteins compared to the PEG-based kit (Grunt et al., 2020).

When the results from human research were analyzed, all studies used plasma samples as the source of the exosomes and NTA as the characterization method (Annibalini et al., 2019; Brahmer et al., 2019; Frühbeis et al., 2015; Hou et al., 2019; Lovett et al., 2018; Rigamonti et al., 2020; Whitham et al., 2018), with the exception of one study that used flow cytometry (Bei et al., 2017). In terms of the isolation methods, six of nine studies used ultracentrifugation (Annibalini et al., 2019; Frühbeis et al., 2015; Hou et al., 2019; Rigamonti et al., 2020; Whitham et al., 2018) and other two studies utilized SEC (Brahmer et al., 2019; Lovett et al., 2018); however, only one study evaluated the release of exosome using flow cytometry without prior purification (Bei et al., 2017). When these studies were analyzed, the ones using SEC did not show any changes in the release of exosomes (Brahmer et al., 2019; Lovett et al., 2018), while four studies used ultracentrifugation (Annibalini et al., 2019; Frühbeis et al., 2015; Whitham et al., 2018) and one without previous isolation (Bei et al., 2017) showed a significant increase. Finally, when compared with similar acute exercise protocols, the analysis of exosome release showed inconclusive results. Specifically, the number of exosomes was shown to increase following an acute running protocol by the studies of Bei et al. (2017) and Frühbeis et al. (2015); however, Rigamonti et al. (2020) did not observe any changes. This discrepancy could be due to the difference in the sample size of $n = 2$ (Frühbeis et al., 2015) versus $n = 23$ (Rigamonti et al., 2020). It is important to note that Rigamonti et al. (2020) also included both genders and obese subjects in the study, which could make it difficult when interpreting the results. On the other hand, while no change was found in exosome release after acute cycling exercise with the SEC isolation (Brahmer et al., 2019), other two studies using the method of ultracentrifugation showed an increased level of exosomes (Frühbeis et al., 2015; Whitham et al., 2018). Regarding other two exercise protocols, a significant increase in exosome release was shown after completion of a single bout of flywheel exercise (Annibalini et al., 2019), whereas no change was found with 1-year rowing training (Hou et al., 2019). Although the absence of modification in the exosome release could potentially be attributable to the adaptations to different exercise training modalities, limited literature exists on chronic exercise

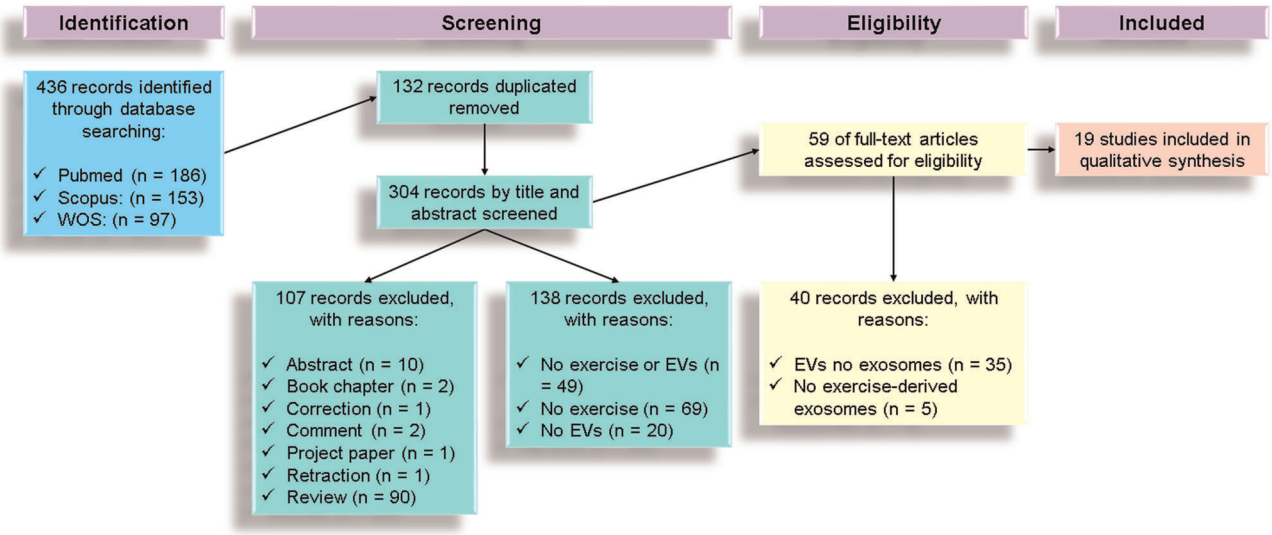

FIGURE 3 Flowchart of the article selection process in the systematic review according to PRISMA guidelines. Modified from Moher et al. (2009). EVs, extracellular vesicles; WOS, Web of Science

protocols in humans, which hamper the comparison of the findings from the rowing study. Thus, additional research is needed with various training modalities and durations to further understand the potential therapeutic role of exosomes.

4.2 | Effects of exercise on exosome cargo (proteins and miRNAs)

Only three articles evaluated the changes of protein content from exercise-mediated exosomes (Brahmer et al., 2019; Frühbeis et al., 2015; Helmig et al., 2015), which makes impossible to conclude the findings of reported proteins in humans. Overall, there is a consensus that exercise increases exosome markers (Alix, CD63, CD81, and Flot-1) and exosome-carried HSPs levels, regardless of the use of isolation, characterization, and phenotyping methodology, and the exercise protocols (Barone et al., 2016; Bei et al., 2017; Bertoldi et al., 2018; Chaturvedi et al., 2015; Frühbeis et al., 2015; Helmig et al., 2015; Hou et al., 2019; Oliveira et al., 2018; Whitham et al., 2018). However, Brahmer et al. (2019) have reported different results in exosome phenotyping, based on the technique used in the study. Specifically, the EV array in the whole plasma showed an increase of some proteins (Alix, CD14, CD142, MCH-I, ICAM-1, LAMP-1, and tPA), whereas the majority of other exosome markers remained under the detection threshold (CD9, CD63, CD81, Flot-1, HSP70, and CD41b), suggesting a necessary process of purification before characterization. On the other hand, following the SEC isolation, the values obtained by WB phenotyping of exosomes were higher than those obtained by MACSplex phenotyping, which may be due to the competition of the antibodies with the same epitopes (tretraspanins) or the absence of plasma components that can increase the signal in the case of WB. Yet, the immunobead isolation seems to be the most accepted and efficient purification method (Brahmer et al., 2019), although there is currently no consensus on the methods to evaluate exosome release in the literature (Hessvik & Llorente, 2018).

The timing of sample recovery is also a key factor that could explain the differences in exosome release and/or cargo, especially in response to prolonged exercise protocols. For instance, Frühbeis et al. (2015) showed an increase in the exosome release and exosome markers (Tsg101, HSP70, Int.aIIb, and Flot-1) immediately following both incremental cycling and treadmill exercise, and the levels of these proteins returned to the baseline at 90 min into recovery. Interestingly, Helmig et al. (2015) also conducted the exhaustion treadmill running test, but the levels of Flot-1 and HSP70 remained elevated, even 90 min following exercise. Furthermore, an observation of elevated exosome membrane protein, CD63 was shown 18 h following the last section of a 2-week aerobic training in aged rats (Bertoldi et al., 2018). Importantly, research has also observed the expression of various miRNAs in exosomes following exercise. For example, D'Souza et al. (2018) showed that the concentration of miR-1 in circulating exosomes was elevated in response to acute cycling exercise, and returned to the baseline level at 4 h into recovery. A recent study by Yin et al. (2019) has also demonstrated an elevation of exosome-carried miR-1, along with the expression of miR-133a, miR-133b, miR-206, miR-208a, and miR-499 immediately following exercise, with a return to the baseline levels at 48 h into recovery. In a study of cattle grazing, the levels of miR-19b, miR-148a, miR-150, miR-221, miR-361, and miR-486 were upregulated during the first month of the study and returned to the baseline toward the completion of an either 4-month or 7-month study period (Muroya et al., 2015), while the control group (housed cattle) showed an increase in miR-19b and miR-223 during the first 2 months but the levels were decreased toward the end of the study. These results do not only support the physiological adaptations to physical activity, but also highlight the importance of the

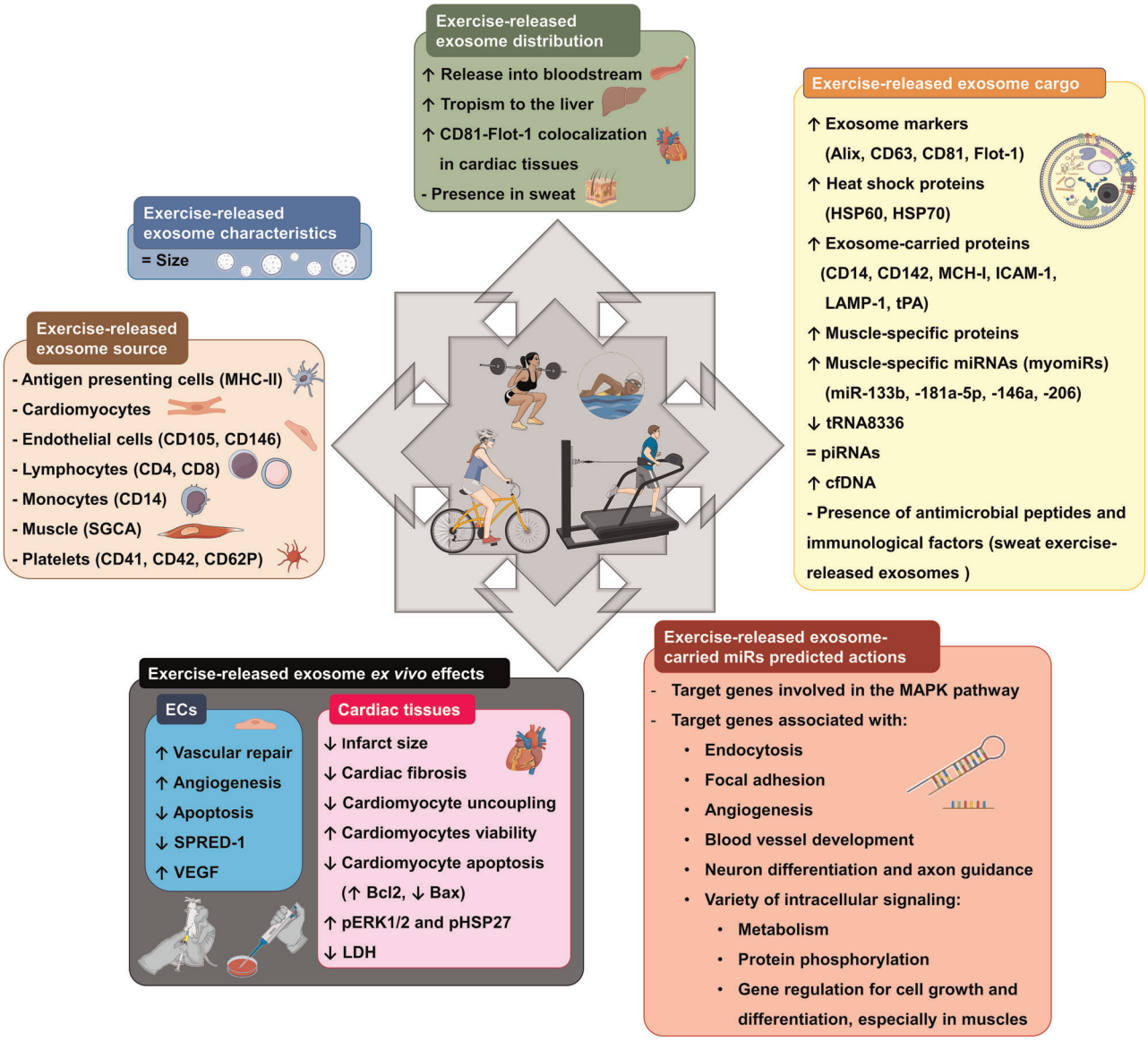

FIGURE 4 Main effects of exercise on exosome characteristics, distribution, source of release, and cargo, as well as exercise-released exosome impact in ex vivo (animal or cell culture) models and exercise-released exosome-carried microRNAs predicted actions. The figure was made with use of Mind the Graph, licensed under a free culture Creative Commons Licence

timing of sample recovery for future research. In addition, Hou et al. (2019) found that the level of exosomes 24 h following a 4-week swimming training in rats remained unchanged, whereas a significant increase was observed immediately after exercise. However, an elevation in the levels of miRNAs (miR-1, miR-486, miR-208a, miR-3571, miR-122, miR-196b, miR-3591, miR-184, and miR-760) was shown 24 h into recovery (Hou et al., 2019). Thus, kinetic studies of exosome release are needed, accompanied with the adjustment of timing of sample collection.

In terms of exosome sources and isolation technique, all human studies in this review used plasma samples and ultracentrifugation (with the exception of one study using SEC) to evaluated the changes in the exosome content of miRNAs in response to exercise, with different exercise modalities and sample sizes as previously described. However, the analyses of target miRNAs were different among these studies. Specifically, two of these studies, coincided evaluating four miRNAs, showing no variations in the levels of miR-1, miR-133a, miR-133b, and miR-206 following acute high-intensity exercise (running and plyometric jumping; Guescini et al., 2010; Lovett et al., 2018). Regardless of the sample sizes in both studies ($n = 9$ and $n = 18$), the different technique, SEC (Lovett et al., 2018) versus ultracentrifugation (Guescini et al., 2010) were utilized to isolate exosomes. However, the proteins co-isolated with the exosomes has been shown to influence downstream RNA profiling (Van Deun et al., 2014), and subsequent quantification of miR-16, commonly used as internal control (Grunt et al., 2020).

4.3 | Physiological implications of exercise-promoted exosomes

The potential role of exosomes has been recently investigated not only as less invasive biomarkers in a wide range of diseases, including various cancer (breast [Hannafon et al., 2016], cervix [Zheng et al., 2019], endometrium [Srivastava et al., 2018], gastric [Tang et al., 2020], lung [Cazzoli et al., 2013], pancreatic [Melo et al., 2015], prostate [Barceló et al., 2019], ovaries [Maeda et al., 2020]), Alzheimer's disease (Hamlett et al., 2017), Parkinson's disease (Kitamura et al., 2018), brain insulin resistance (Mullins et al., 2017), diabetes (Krishnan et al., 2019), cardiomyopathies (T. Wu, Chen, et al., 2018), nephropathies (Guo et al., 2020) and chronic kidney disease (Khurana et al., 2017), and multiple sclerosis (Ebrahimkhani et al., 2017), but also as therapeutic agents for both drug delivery vehicles (tumor therapy [Altanerova et al., 2019; Qi et al., 2016; Y. Tian et al., 2014], Parkinson's disease [Haney et al., 2015], cerebral ischemia therapy [T. Tian et al., 2018]) and a genetic or protein material transfer mediator (Alzheimer's disease [Yang et al., 2020], breast cancer [Sansone et al., 2017], ischemic myocardium [Gollmann-Tepeköylü et al., 2020]) or graft-versus-host disease (Kordelas et al., 2014). In this regard, another potential translational perspective could be the transfusion or transferring of exosomes or enriched exosomes. Thus, research has previously shown that in aged rats, treadmill exercise capacity increased by 25% after the injection with cardiosphere-derived cells and exosomes secreted by these cardiac progenitor cells obtained from young donors (Grigorian-Shamagian et al., 2017). Since physical activity has been considered as a gold-standard therapy to mitigate molecular pathologies associated with obesity and Type 2 diabetes mellitus, exosomes isolated from trained individuals or athletes or exosomes bioengineered to incorporate exerkines could be therapeutically useful for the treatment of age-related diseases and/or metabolic disorders (Safdar & Tarnopolsky, 2018). For example, Chaturvedi et al. (2015) demonstrated that an 8-week aerobic training enhanced the exosome-mediated improvement in cardiac fibrosis and myocyte uncoupling in diabetic mice. Similarly, Ma et al. (2018) developed a high glucose and hypoxia dual injury model to mimic the in vivo diabetic and/or ischemic condition and found the benefits of exercise-mediated exosomes in improving endothelial cell capacity of vascular repair and angiogenesis in diabetic mice. Moreover, the exosome-promoted cardioprotective effects with exercise were observed in both animal and cell culture models to decrease the cardiomyocyte apoptosis and infarct size in mice and rats following heart damage or hypoxia/reoxygenation (Bei et al., 2017; Hou et al., 2019). In addition, besides the unique exosomal protein profile that have been studied in urine (Musante et al., 2014; Pisitkun et al., 2004), saliva (Palanisamy et al., 2010; Xiao et al., 2016), or plasma (Muller et al., 2014), various antimicrobial peptides and immunological factors were found in exercise-mediated exosomes from sweat, suggesting the importance of exosomes in coordinating skin immunity (C. X. Wu & Liu, 2018). This exosome-mediated human immunity with exercise is also supported by Brahmer et al. (2019), demonstrating the release of exosomes from leukocytes following acute incremental cycling test (Brahmer et al., 2019). Furthermore, it has been shown that the immune stimulatory or suppressive functions of exosomes depend on their cellular origin (Kordelas et al., 2014). D'Souza et al. (2018) also identified modifications in the expression of different miRNAs in muscle and exosomes, but not in plasma, which highlights the uniqueness in the sources of exosome-carried miRNAs, irrespective of exercise.

In addition, evidence suggests that exosome-carried miRNAs (e.g., miR-1, miR-133, and miR-206) modulate the proliferation and differentiation of skeletal myocytes via alteration of gene expression (Wang & Wang, 2016). It has been established that muscle miRNAs (myomiRs), such as miR-1, miR-133a, miR-133b, miR-206, and miR-486 play key roles in the regulation of muscle development and differentiation, as well as maintenance, remodeling, repair and regeneration (Kirby et al., 2015). Of particular note, several of these exosome-carried miRNAs (miR-206 and miR-146a) associated with muscle development have been demonstrated to be upregulated in humans in response to acute bout of flywheel resistance exercise, indicating the importance of exosomes as critical mediators to muscle adaptation with exercise (Annibalini et al., 2019). These findings are supported by Guescini et al. (2015), showing an elevated level of alpha-sarcoglycan protein associated with prevention of muscular dystrophy, along with the expression of exosomal miRNAs (miR-206, miR-133b, and miR-181a-5p) in response to acute aerobic exercise. While the bioinformatics pathway analysis revealed that exercise-modified exomiRs were predicted to target genes involved in the MAPK pathway to promote muscle cell growth and differentiation (Muroya et al., 2015; Oliveira et al., 2018), the exercise-induced muscle damage protocol (jumping/running) did not observe any changes in the myomiRs profile of circulating exosomes (Lovett et al., 2018). However, the release of miRNAs (i.e., miR-1, miR-133a, and miR-499) in skeletal muscle of rats were observed following downhill running (eccentric exercise), a typical exercise-induced muscle damage protocol compared to uphill running (concentric exercise), possibly due to the involvement of muscle fiber type and their plasticity (Yin et al., 2019). In fact, the miRNAs, miR-206, miR-208b, and miR-499 are enriched in slow twitch muscle fibers, while miR-1 and miR-133a appear to have uniform expression throughout all muscle types (McCarthy & Esser, 2007; van Rooij et al., 2009). Furthermore, research has recently shown positive correlations between increased muscle MVBs biogenesis and miRNA processing gene expression, but not with the muscle gene expression of markers of exosome release pathways, in response to combined aerobic and resistance exercise (Garner et al., 2020). Taken together, these findings indicate that the upregulation of selected muscle-specific miRNAs from exosomes may be associated with the extent of muscle damage to promote the processes of muscle repair and regeneration.

The literature has also investigated the effects of acute exercise on circulating cfDNA as a potential biomarker for obesity-associated inflammatory responses (Ferrandi et al., 2018), an indicator of training load for intermittent sports (Haller et al., 2018), and a biomarker of muscle damage-related performance decrement

(Andreatta et al., 2018). In relation to the release of exercise-mediated cfDNA from EVs, there is only one study available in the literature. Specifically, an increased cfDNA level in human plasma exosome fraction was found immediately following an incremental treadmill exercise with a higher nuclear DNA in supernatants than in EVs and higher amounts of mitochondrial DNA in the MVs from pellets, implying that the exercise-promoted cfDNA release occurs independent of exosomes (Helmig et al., 2015). An understanding of the potential effects of exercise intervention may support the therapeutic role of exosomes in obese populations.

4.4 | Limitations

The limitations of this systematic review are mainly due to the different exosome isolation, characterization and phenotyping techniques conducted in the current literature. Several studies reported contradicting results on exosome release and size, as well as their cargo, in response to exercise, highlighting the need for homogeneity in the experimental performance. In addition, there is no consensus in establishing time course of sampling release, especially following prolonged exercise or long-tern training protocols. Finally, when evaluating the physiological implications, the main limitation is the low number of ex vivo experiments.

5 | CONCLUSIONS AND PERSPECTIVES

The present systematic review summarizes the effects of different types of physical exercise on the release of exosomes into the circulation and modification of both protein and nucleic acid content, especially miRNAs. These changes in the number of exosomes and their cargo could potentially provide systemic beneficial effects of exercise via exosome-mediated intercellular communication. However, the current limitations in exosome isolation, characterization and phenotyping techniques, as well as sources in response to exercise remain to be investigated. Nonexosome proteins co-precipitation is the main shortcoming of different exosome isolation approaches, thereby resulting in the discrepancies of reported results that could constrain the clinical use. Yet, this systematic review is especially noteworthy in exploring the evidence on the potential therapeutic role of exercise-released exosomes in the pathological conditions as well as the mechanisms by which the effects are implemented. Further investigation is warranted to examine the effects of exercise-mediated exosome release and its cargo in various conditions and diseases, such as aging, obesity, or Type 2 diabetes for the potential therapeutic applications.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

All authors have contributed to planning, designing, conducting, writing and/or revising the manuscript and approved it in its final version.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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