REVIEW

Effects of Exercise on Extracellular Vesicles in Patients with Metabolic Dysfunction: a Systematic Review

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Abstract

The aim of this study was to investigate the effect of exercise on extracellular vesicles (EVs) in patients with metabolic dysfunction. The literatures were searched until Apr 28, 2022, and 16 studies that met inclusion criteria were included in this review. The results showed that the concentrations of platelet-derived extracellular vesicles (PEVs) and endothelial cell-derived extracellular vesicles (EEVs) decreased after long-term exercise, especially for $CD62E^+$ EEVs and $CD105^+$ EEVs. Simultaneously, exercise improved the concentration of clinical evaluation indicators of metabolic diseases, and the changes in these indicators were positively correlated with the changes of EEVs and PEVs. The concentration of skeletal muscle-derived extracellular vesicles (SkEVs) increased after a single bout of exercise. The aforementioned results indicated that long-term exercise might improve endothelial function and hypercoagulability in patients with metabolic dysfunction. The changes in concentrations of EVs could assist in assessing effect of exercise on patients with metabolic dysfunction.

Abbreviations

AT	Adipose tissue
BMI	Body mass index
EVs	Extracellular vesicles
EEVs	Endothelial cell-derived extracellular vesicles
FCM	Flow cytometry
FPG	Fasting plasma glucose
HbAlc	Glycosylated hemoglobin
HIIT	High-intensity interval training
HOMA-IR	Homeostatic model assessment of insulin resistance
IR	Insulin resistance
IL-6	Interleukin-6
LCFA	Long-chain fatty acid
LEVs	Leukocyte-derived extracellular vesicles
MCT	Moderate-intensity training
MEVs	Monocyte-derived extracellular vesicles
MICT	Moderate-intensity continuous training
MINORS	The Methodological Index for Non-Randomized Studies
MIT	Moderate-intensity training
m/lEVs	Medium/large extracellular vesicles
NO	Nitric oxide
NTA	Nanoparticle tracking analysis
PCOS	Polycystic ovary syndrome
PEVs	Platelet-derived extracellular vesicles
RCT	Randomized control trial
ROB	The Cochrane tool of risk of bias
sEVs	Small extracellular vesicles
SkEVs	Skeletal muscle-derived extracellular vesicles
T2DM	Type 2 diabetes mellitus
TNF-α	Tumor necrosis factor-α

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Introduction

Metabolic dysfunction, such as hyperglycemia, dyslipidemias, hypertension, obesity, and insulin resistance (IR), increases the risks for cardiovascular and cerebrovascular diseases [1]. Metabolic dysfunction is characterized by inflammation, oxidative stress, or haemodynamic fluctuations that stimulate the release of extracellular vesicles (EVs) [2]. A meta-analysis that included 48 independent studies reported significantly elevated concentrations of EVs derived from endothelial cells, platelets, and monocytes in patients with type 2 diabetes mellitus (T2DM) [3]. A cohort study revealed that EV concentrations in diabetic patients were significantly higher than those in normal participants, while IR promoted the release of EVs [4]. EVs are a group of heterogeneous membrane vesicles that can be divided into small EVs(sEVs) (< 100 nm or < 200 nm), and medium/large EVs (m/lEVs) (> 200 nm) [5]. EVs have become increasingly popular as potential biomarkers for the diagnosis and prognosis of metabolic dysfunction. They represent a novel mechanism of intercellular communication [4, 6], due to their ability to transfer proteins, lipids, and nucleic acids and affect target organ function [7, 8]. EVs can act as vectors between adipocytes and other cells in the adipose tissue (AT). In obese insulin-resistant animals and humans, adipocyte-derived EVs were found to attract monocytes and lead to adipose tissue inflammation [9, 10]. Metabolic dysfunction affects the production of EVs that, in turn, regulate the metabolic state of the body. Adipose tissue-derived EVs (AT-EVs) from obese patients could promote monocytes differentiating to pro-inflammatory macrophages (M1 macrophages). The M1 macrophages induced the release of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) accelerated IR, which led to metabolic dysfunction [11].

There is strong evidence that exercise can reduce blood glucose in patients with obesity and T2DM by stimulating skeletal muscle glucose uptake and directly affecting insulin sensitivity [12–14]. The concentration of plasma EVs is increased in obese patients [15, 16], and exercise can decrease the EVs concentration [17]. Exercise also affects EVs release via several possible mechanisms. Aerobic exercise increases blood flow to meet the extra oxygen demands of the working muscles, further inducing elevation in shear stress, which is associated with EVs formation [18]. Kawanishi observed that chronic exercise could regulate the expression of EVs, prevent macrophage infiltration in adipocytes, and downregulate toll-like receptor 4 expression to adjust the transformation of macrophages from M1 pro-inflammatory phenotype to M2 anti-inflammatory phenotype, thereby inhibiting inflammation and improving insulin sensitivity [19]. Exercise can increase the release of EVs derived from endothelial progenitor cells and promote the expression of miR-126, thereby enhancing the protective effect of endothelial cells by reducing apoptosis [20]. However, the changes in EVs caused by different forms and exercise intensity need to be analyzed in more detail.

The effect of exercise on different types of extracellular vesicles has gained attention. Our previous study on healthy participants has found platelet-derived extracellular vesicles (PEVs) expressed a transient increase after a single bout of exercise, while the proportion and duration of PEV increment reduced in long-term exercise. In addition, most studies reported a decline in leukocyte-derived extracellular vesicles (LEVs) in trained participants after a single bout and long-term exercise [21].

Exercise is one of the non-drug therapies for improving metabolic dysfunction. Nevertheless, the changes in extracellular vesicles concentrations and subtypes in patients with metabolic dysfunction after exercise have not been reviewed. Therefore, based on the existing literatures, we aimed to summarize the concentration of EVs and their different subtypes in patients with metabolic dysfunction and the changes after exercise in order to evaluate the effect of exercise on patients.

Methods

Criteria for Considering Studies for This Review

Types of patients: patients with metabolic dysfunction were included. Metabolic dysfunction refers to a collection of risk factors associated with many diseases, such as hyperglycemia, dyslipidemias, hypertension, obesity, insulin resistance, glucose intolerance and diabetes mellitus [1]. Fasting plasma glucose (FPG) $\geq$ 7.0 mmol/L or glycosylated hemoglobin (HbAlc) $\geq$ 6.5% can be used as the basis for the diagnosis of diabetes mellitus, which includes type I or type II diabetes mellitus. Impaired fasting glycaemia (FPG < 7.0 mmol/L) and impaired glucose tolerance (FPG: 5.6–6.9 mmol/L) which are called pre-diabetes were also included [22]. Obesity is defined as a chronic disease caused by excess adipose tissue, with a body mass index (BMI) > 25 kg/m^2 as diagnostic and evaluation criteria [23]. Hypertension is diagnosed when a person has a systolic blood pressure is $\geq$ 140 mmHg and/or diastolic blood pressure $\geq$ 90 mmHg in the office or clinic following repeated examination [24].

Types of interventions: exercises or physical activities were included. It mainly comprised moderate-intensity continuous training (MICT) or high-intensity interval training (HIIT) based on aerobic exercise, resistance training, and endurance exercise. Swimming, diving, and climbing were excluded.

Types of outcomes: Detection of different types of EVs, including sEVs and m/lEVs, used flow cytometry (FCM), nanoparticle tracking analysis (NTA), western blotting, and ELISA [5].

Types of studies: prospective studies such as randomized controlled trials (RCTs), before–after self-control studies, cohort studies, case–control studies, and descriptive studies were included [25].

Types of work: full articles, meeting abstracts or oral reports were included.

Search Strategy

Databases of PubMed, Web of Science, Embase, and the Cochrane Library were comprehensively searched until Apr 28, 2022. "Exercise," "Cell-Derived Microparticles," "Exosomes," "Extracellular Vesicles," "Hyperglycemia," "Dyslipidemias," "Hypertension," "Obesity," "Insulin Resistance," "Diabetes Mellitus," "Glucose Intolerance," "Hyperlipidemias," and "Adipose tissue" were used as the retrieval words. Search strategies included Medical Subject Headings (MeSH) and keywords adapted to databases.

Study Selection and Data Extraction

Retrieval results were imported into EndNote X8, and duplicates were excluded. Two independent reviewers filtered all the titles and abstracts according to the eligibility criteria, and evaluated full texts for inclusion. Divergences were resolved by discussion. If no agreement was reached, the corresponding authors were consulted. Studies that met all criteria were included. After study selection, two authors independently extracted the relevant data from the included studies using an extraction sheet. The data sheet included the authors' name, published time, sample size, types and changes of EVs, and exercise protocols and outcomes.

Methodological Quality Assessment

The methodological quality assessment was independently conducted by two reviewers, and the Cochrane collaboration's tool was used for assessing the risk of bias (ROB) of RCTs. It included six items of selection bias, performance bias, detection bias, attrition bias, reporting bias and other bias. "High risk," "unclear risk," and "low risk" were used to describe the concentration of each project. The Methodological Index for Non-Randomized Studies (MINORS) [25] was applied to evaluate the quality of non-randomized studies. This validated index contains 12 items; the first 8 items are specifically designed for noncomparative studies, and the remaining 4 items are applied to comparative studies. Items were scored as 0 (not reported), 1 (reported but inadequate), and 2 (reported and adequate). The maximal ideal score for noncomparative studies and comparative studies was 16 and 24, respectively.

Summary Analysis

Due to the complexity of exercise modes, various types of EVs, diverse study designs, and different extraction and detection methods, qualitative synthesis was adopted.

Results

Identification of Studies

Among a total of 722 assessed studies, 400 duplicates and 167 inconsistent studies were removed. Next, 155 studies were roughly screened, among which 117 were inconsistent with the designed research. Among 38 studies whose full text was screened, 22 imperfect-design studies (unclearly described interventions or incomplete data presentation) were excluded. Finally, 16 studies met the selection criteria and were enrolled in this systematic review. Figure 1 presents the flow diagram of the study collection [17, 26–40].

Characteristics of Studies and Patients

As shown in Table 1, a total of 16 studies were included in this review. Diseases included after retrieval contained obesity, T2DM and concomitant diseases, and hypertension. The referred studies contained 5 RCTs, 3 case–control studies, and 8 descriptive studies. The sample size ranged from 10 to 86; 4 studies only included men and 2 only included women, while both men and women were enrolled in the remaining 10 studies. Among the included articles, exercise protocols in 12 studies were aerobic exercise and combined aerobic, while the remaining included a resistance training program. Twelve studies used differential centrifugation to extract EVs. The time points of drawing blood were pre-exercise and post-exercise and 3 h to 3 days after exercise. Ethylenediaminetetraacetic acid and citrate were used as anticoagulants in 5 and 7 studies, respectively. Twelve studies used FCM to quantify extracellular vesicles and three utilized NTA. Extracellular vesicles covering endothelial-derived extracellular vesicles (EEVs), LEVs, PEVs, and skeletal muscle-derived extracellular vesicles (SkEVs) were included in the eligible literature. The details are shown in Table 1.

Quality of Evidence

The Cochrane assessment tool was applied for assessing RCTs. Four RCTs showed a low risk of attrition and reporting bias but performance bias was high risk. Two studies showed a low risk of selection bias. The MINORS index, a

Fig. 1 Flow diagram of the study search and selection process
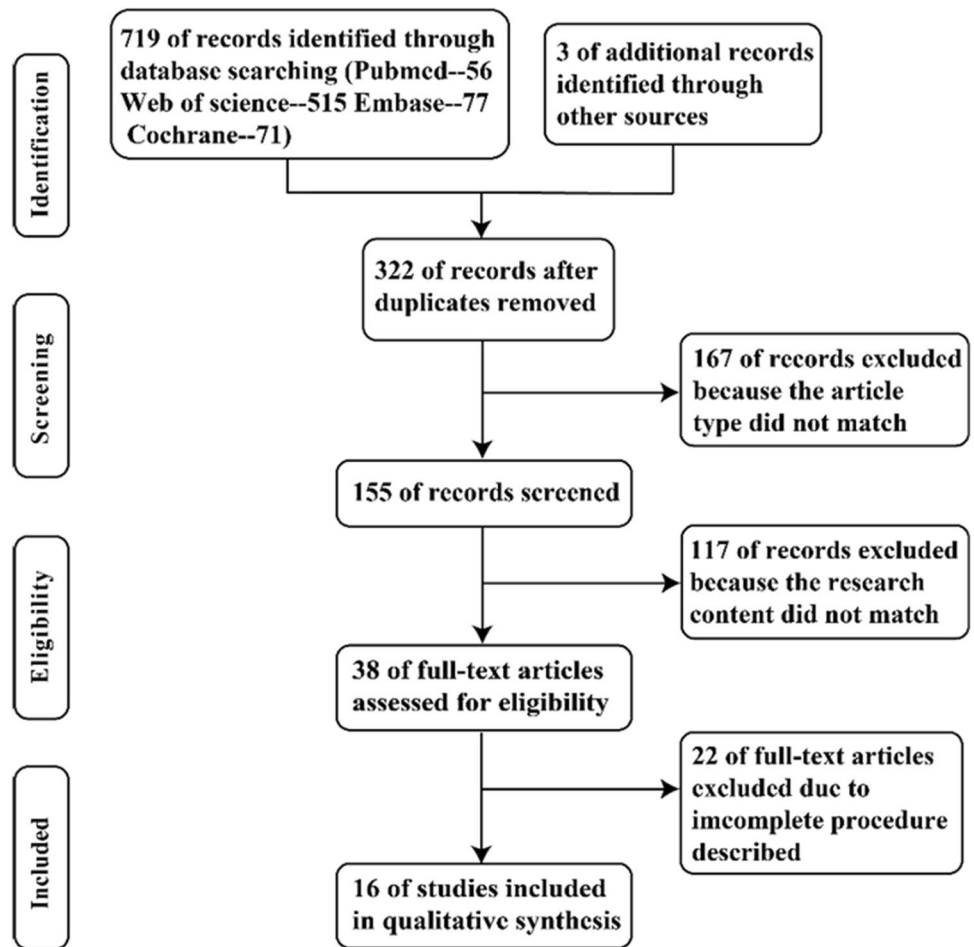
tool for evaluating non-randomized studies, was used to assess the methodological quality. In this review, MINORS scores ranged from 9 to 17, demonstrating a relatively medium or high concentration of methodological quality among studies. The results are shown in detail in Tables S1 and S2.

The Effects of Exercise on the Concentration of EVs in Patients with Metabolic Dysfunction

It is well-known that exercise can lower body weight, regulate glucose and lipid metabolism disorders, and reduce the risk of cardiometabolic diseases. Most included studies reported that the concentration of EEVs and PEVs was decreased after exercise while SkEVs were increased and were associated with clinical evaluation indicators of metabolic diseases. The details are shown in Table 2 and Fig. 2.

Obesity

Nine studies examined the changes of EVs concentrations in obese patients after exercise [17, 26, 27, 29, 32–35, 39], where 4 studies addressed a single bout of exercise [27, 32, 35, 39], and 5 dealt with long-term exercise [17, 26, 29, 33, 34].

One study showed the concentration of EVs in obese patients was higher than in those with normal weight at baseline after long-term exercise. After performing an 8-week HIIT, the counts of EVs increased some 30%, and a significant reduction of BMI was obtained in both groups. Positive associations were found between EVs and TNF-α and IL-6 while negative association were found between EVs and HDLc [29]. While, the significantly decreased of total EVs was found at the end of the moderate-intensity training (MIT) and after 3 and 24 h. EV concentrations were not associated with BMI but were positively correlated with homeostatic model assessment of insulin resistance (HOMA-IR) ($P < 0.05$) [35].

Out of 9 studies, 6 focused on the EEVs, and the concentration of EEVs declined after a single bout of exercise and three long-term exercises [26, 27, 33–35, 39]. Only one showed an increment of EEVs after a single bout of exercise. The concentration of CD105$^+$ EEVs and CD31$^+$/CD41$^-$ EEVs decreased after a moderate-intensity exercise session [39]. An interval exercise decreased AV$^-$ CD105 EEVs compared to continuous training [34]. The baseline number of EEVs was significantly higher in the usual care than the intervention group, and a significant reduction in the number of circulating EEVs was shown in the

Table 1 Details and protocols of exercise

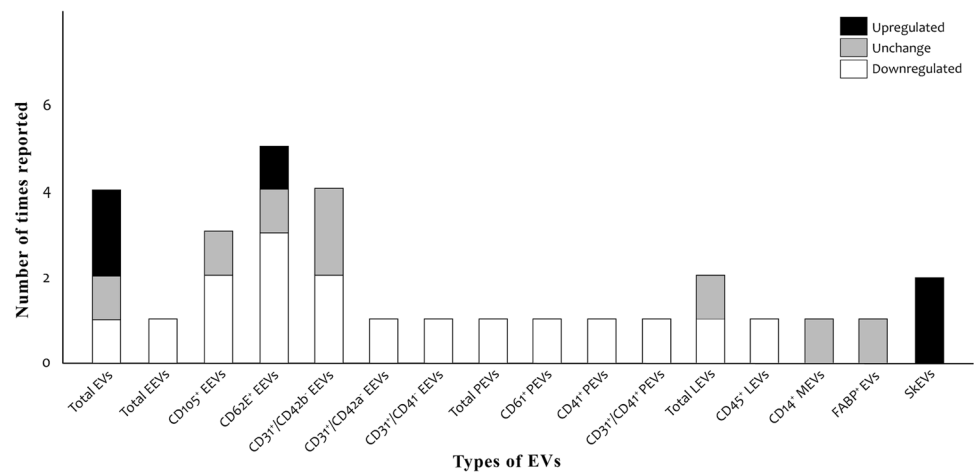| Author/year	Sample size	Types of diseases	Types and changes of EVs		Extraction method of EVs	Exercise protocol	Time of drawing blood	Testing methods
Heiston et al., 2022	10	Obesity	IEVs	CD105$^+$EEVs, CD31$^+$/CD41$^-$ EEVs, CD41$^+$ PEVs, CD31$^+$/CD41$^+$ PEVs, CD45$^+$ LEVs, TX$^+$ EVs↓	5000 g, 15 min→17000 g, 10 min	A moderate-intensity exercise session	After an overnight fast	FCM
Warnier et al., 2021	32	Prediabetic	sEVs	→	3000 g, 10 min→2000 g, 15 min→2000 g, 60 min→20000 g, 60 min	Normoxia or hypoxia acute exercise	Pre- and 30-min, post-exercise	NTA
Waclawovsky et al., 2021	33	Hypertension	IEVs	CD45$^-$/CD42$^-$/CD31$^+$ EEVs→	-	Aerobic exercise, resistance exercise or combined exercise	Pre- and 10-min, 70-min post-exercise	FCM
Apostolopoulou et al., 2021	43	T2DM/IR/IS	sEVs	↑	1500 g, 10 min→10000 g, 10 min→100000 g, 70 min	12-week HIIT for 3 days/week	Pre- and 72-h post-exercise	NTA
Yan et al., 2021	18	Hypertension	IEVs	CD31$^+$/CD42b$^-$ EEVs↓	1200 g, 30 min	acute moderate-intensity exercise	Pre- and post-exercise	FCM
Rigamonti et al., 2020	23	Obesity	sEVs and IEVs	SCGA$^+$ EVs↑ CD62E$^+$ EEVs, CD105$^+$ EEVs →, CD14$^+$ MEVs, FABP$^+$ EVs, sEVs →, CD61$^+$ PEVs↓	1000 g, 15 min→11000 g, 75 min	MIT on a treadmill (60% VO$_{2max}$)	Pre- and 0-h, 3-h, and 24-h post-exercise	NTA&FCM
Eichner et al., 2020	18	Obesity	IEVs	AV$^+$CD31$^+$/CD41$^+$ PEVs, AV$^+$/$^-$CD45$^+$/ CD41$^-$ LEVs, AV$^+$/$^-$CD45$^+$ LEVs, AV$^+$/$^-$CD31$^+$/ CD41$^-$ EEVs →, AV$^+$/$^-$CD105 EEVs↓	5000 g, 15 min→17000 g, 10 min	INT (alternating 3 min intervals at 90% and 50% HR$_{peak}$, respectively) or CONT (70% HR$_{peak}$) training for 12 supervised sessions over 13 d	Pre- and post-exercise	FCM
Nielsen et al., 2019	27	Obesity/T2DM	IEVs	CD36$^+$SkEVs, FATP4$^+$SkEVs↑	2100 g, 10 min	Ergometer exercise test (70% VO$_{2max}$) for 60 min	Pre- and 0-h and 3-h post-exercise	FCM
Rafiei et al., 2019	24	Obesity	IEVs	CD31$^+$ EEVs →CD62E$^+$ EEVs↓	1500 g, 15 min→1500 g, 15 min	2 weeks of HIIT and MICT	Pre- and 3-day post-exercise	FCM

Table 1 (continued)

Author/year	Sample size	Types of diseases	Types and changes of EVs		Extraction method of EVs	Exercise protocol	Time of drawing blood	Testing methods
Lechner et al., 2019	29	Metabolic syndrome	IEVs	LEVs↓ EEVs↓	-	MCT, HIIT with low and high volume of 16 weeks	-	-
Bratseth et al., 2019	74	CAD with T2DM	IEVs	CD146+ EEVs, CD62E+ EEVs, CD146+/CD62E+ EEVs, CD309+/CD34+ PEVs, CD14+/CD11b+ MEVs, CD14+/CD142+ MEVs, CD62P+ PEVs→	2500 g, 15 min→20000 g, 30 min	12-month combined aerobic and resistance training program	Pre- and 12-month post-exercise	FCM
Dimassi et al., 2018	15	Obesity	IEVs	↑	1500 g, 15 min→13000 g, 2 min→20000 g, 90 min	8-week (three times a week) HIIT	Pre- and 48-h post-exercise	FCM
Bruyndonckx et al., 2015	61	Obesity	IEVs	CD31+/CD42b− EEVs↓	1525 g, 20 min→1525 g, 20 min	10-month residential treatment program	Pre- and 5- and 10-month post-exercise	FCM
Durrer et al., 2015	13	Obesity	IEVs	CD31+/CD42b− EEVs ↑ (female) ↓ (male) CD62E+ EEVs ↓ (male)	1500 g, 15 min→1500 g, 15 min	No exercise control, HICE and HIIE in a randomized counter-balanced crossover design	18-h post-exercise	-
Kim et al., 2015	21	Prehypertension	IEVs	CD31+/CD42a− EEVs, CD62E+ EEVs↓	1500 g, 20 min→1500 g, 20 min	Aerobic exercise 3 days/week for 6 months	-	FCM
Murakami et al., 2007	86	Obesity	IEVs	PEVs↓	2000 g, 10 min→200 g, 10 min	Aerobic exercise for 12 weeks	-	FCM

EEVs, endothelial-derived extracellular vesicles; *FABP*, fatty acid binding protein; *FATP4*, fatty acid transport protein 4; *FCM*, flow cytometry; *HIIT*, high-intensity interval training; *IR*, insulin-resistant; *IS*, insulin-sensitive; *LEVs*, leukocyte-derived extracellular vesicles; *MEVs*, monocyte-derived extracellular vesicles; *MICT*, moderate-intensity continuous training; *MIT*, moderate-intensity training; *NTA*, nanoparticle tracking analysis; *PEVs*, platelet-derived extracellular vesicles; *SkEVs*, skeletal muscle-derived extracellular vesicles; *T2DM*, type 2 diabetes mellitus; *TX⁺ EVs*, tetraspanin-derived EVs↑, upregulated;→, unchange;↓, downregulated

Table 2 The correlations between different types of EVs and clinical evaluation indicators of metabolic diseases

Author/year	Types of EVs		Correlations with EVs
Apostolopoulou et al., 2021	sEVs	-	Quantitative proteomic analysis of SEV revealed that HIIT-mediated proteins related to glycolysis in T2DM, to antioxidative metabolism in IR (insulin resistant) and to lipid metabolism in IS (insulin sensitive)
Rigamonti et al., 2020	sEVs and lEVs	Total EVs, sEVs, and CD61$^+$PEVs	Positively with homeostatic model assessment of insulin resistance (HOMA-IR) ($P < 0.05$)
Eichner et al., 2020	lEVs	AV$^+$/CD105 EEVs AV$^-$/CD31$^+$ PEVs	Positively with dietary sugar intake ($r = 0.60$, $P = 0.01$); negatively with VO$_{2peak}$ ($r = -0.60$, $P = 0.01$) Positively with early phase glucose tolerance (trend: $r = 0.40$, $P = 0.10$)
Dimassi et al., 2018	lEVs	-	Positively with TNFα ($r = 0.487$; $P < 0.01$), and IL-6 ($r = 0.534$; $P < 0.005$); Negatively with HDLc ($r = 0.45$; $P < 0.05$)
Kim et al., 2015	lEVs	CD31$^+$/CD42a$^-$ EEVs, CD62E$^+$ EEVs	Negatively with endothelial cell mitochondrial content ($r = -0.768$, $P = 0.008$, and $r = -0.684$, $P = 0.021$)
Murakami et al., 2007	lEVs	PEVs	Positively with BMI, waist circumference, fat tissue mass, and subcutaneous fat area

Fig. 2 The results of EVs changes after exercise (one cuboid represents one study)

latter compared to a rise of EEVs in the former following a 10-month supervised training program. Moreover, the residential intervention also decreased BMI and body fat percentage [26]. In addition, it was found that gender differences might influence the concentration of EVs in exercise. Baseline concentrations of CD62$^+$ and CD31$^+$ EEV populations were lower in the healthy controls than in overweight and obese women. Two weeks of progressive HIIT or MICT lowered the concentration of CD62E$^+$ EEVs, accounting for 7% and 5.7%, respectively. It also led to a significant reduction in body mass and BMI. Still, neither HIIT nor MICT affected on CD31$^+$ EEVs [33]. In males, both high-intensity continuous training and HIIT reduced EEVs compared to control, while the former group had increased CD62E$^+$ EEVs whereas CD31$^+$/CD42b$^-$ EEVs were unaltered by either exercise type in females. HIIT led to a significant increase in fasting insulin and insulin resistance in males but an obvious reduction in females when compared to control [27]. However, there was

no significant difference in CD62E$^+$ EEVs, CD105$^+$ EEVs before and after exercise [35].

Besides, the concentration of PEVs and SkEVs also showed a significant fluctuation after exercise. A single bout of exercise decreased CD41$^+$ PEVs and CD31$^+$/CD41$^+$ PEVs [39]. A remarkable reduction in CD61$^+$PEVs after exercise and a positive correlation with HOMA-IR was reported [35]. Compared with the non-obese group, the patients in the non-diabetic obese group had a higher plasma concentration of PEVs in the baseline data that significantly decreased after a 12-week calorie-restricted diet and diet with aerobic exercise. Meanwhile, the number of PEVs was positively correlated with BMI waist circumference, fat tissue mass and subcutaneous fat area [17].

The two studies found that the concentration of SkEVs increased after a single bout of exercise [32, 35]. The concentration of SkEVs ascended immediately after exercise [35] and the total SkEV post-exercise was 27% higher in obese men with T2DM than obese men without T2DM. Additionally,

the concentration of SkEVs that carrying FATP4 and CD36 was significantly increased immediately after exercise in both groups [32]. One study showed that the concentration of CD45$^+$ LEVs and tetraspanin-derived EVs declined after a moderate exercise [39], while the concentration of AV$^{+/-}$ CD45$^+$ LEVs, AV$^{+/-}$ CD45$^+$/CD41$^-$ LEVs, CD14$^+$ monocyte-derived extracellular vesicles (MEVs), and fatty acid binding protein (FABP$^+$) EVs were examined in the included studies, and there was no significant difference in before and after exercise [34, 35].

T2DM and Concomitant Diseases

Four studies observed the effect of exercise on EVs in patients with T2DM and concomitant diseases [30, 31, 36, 40].

After 12 weeks of HIIT, the number of EVs increased by $52 \pm 19\%$ and $58 \pm 21\%$ in T2DM and insulin-resistant patients, respectively. Quantitative proteomic analysis of EV revealed that HIIT-mediated proteins were linked with glycolysis in T2D, antioxidative metabolism in IR, and lipid metabolism in IS [36]. Another study reported that the EEVs counts reduced in patients with metabolic syndrome after high-volume MICT and HIIT. Moreover, the overall concentration of LEVs decreased, and the relative representation of T-cell extracellular vesicles increased among all LEVs following the latter interventions [31].

Nevertheless, the concentration of EEVs, LEVs, PEVs, and MEVs did not significantly vary during the intervention period in patients with coronary artery disease with T2DM [30]. And the total concentration of EVs was not affected by an acute exercise in patients with prediabetic [40].

Hypertension

The concentration of CD31$^+$/CD42a$^-$ EEVs and CD62E$^+$ EEVs in individuals with prehypertension decreased after aerobic exercise training for 6 months; the same trend was also observed with triglycerides. Moreover, both two subtypes of EEVs were negatively correlated with endothelial cell mitochondrial content [28]. The concentration of CD31$^+$/ CD42b$^-$ EEVs decreased after acute exercise in patients with stage 1 hypertension and the blood pressure reduced too[38]. Yet, another study found that after a single bout of aerobic exercise, resistance exercise, or combined exercise, the concentration of CD45$^-$/CD42b$^-$/CD31$^+$ EEVs remained unchanged [37].

Discussion

Metabolic dysfunction characterized by endothelial dysfunction, pro-inflammatory cytokines release, hypercoagulable state, and unstable vascular homeostasis has been found to be closely related to cardiovascular and cerebrovascular diseases. In addition, continuous inflammation, thrombin production [41], and impaired mitochondrial network can result in metabolic dysfunction [42, 43], which is associated with changes in plasma concentrations of extracellular vesicles as well as alterations in their EVs cargo [1].

EVs released by cell activation or apoptosis, carrying proteins, lipids, and microRNAs, have been gaining increasing interest in the field of cardiovascular disease and oncology [44]. The concentrations of EVs were reported to be significantly elevated in patients with coronary heart disease and had an increasing trend with the degree of: myocardial infarction > unstable angina > stable angina, thus suggesting that EVs might be a potential biomarker to identify coronary heart disease [45]. A previous study reported that GPC-1 protein was highly expressed in sEVs derived from breast cancer cell lines, which was used for preoperative and postoperative monitoring of breast cancer patients [46]. EVs have abundant targets for detection and may be an important means for non-invasive liquid biopsy. After high-throughput proteomics analysis of fluid body samples from tumor patients, it was found that the newly discovered markers can be used as markers of EVs from tumor cells in addition to the traditional markers [47]. EVs might have great potential for diagnosing and treating diseases in the future.

Undoubtedly, the role of EVs in metabolic diseases cannot be underestimated. The elevated concentration of EVs in obese patients may be partly due to the response of EVs to a high fat that induced vascular dysfunction and increased oxidative stress [48] and leukocyte infiltration [49]. Hyperglycemia promotes greater coagulant activity, reactive oxidative species, and blunts endothelial constriction [50]. High glucose conditions increased the activity of NADPH oxidase in EEVs thereby accelerating vascular inflammation [51]. The use of exercise, the preferred nondrug therapy applied to improve the metabolic state for EVs regulation, has attracted widespread attention. This systematic review summarized the effect of exercise on EVs in patients with metabolic dysfunction to further the understanding and provide additional references for future studies.

The Changes of Subtypes and Numbers of EVs After Exercise

Total EVs

Our results showed that the baseline concentration of EVs was higher in patients with metabolic dysfunction compared to healthy participants [17, 29, 32, 33]. Patients with metabolic dysfunction exhibited a chronic low-grade inflammation, hypercoagulability, and endothelial dysfunction [52] that

could induce more EVs secretion [29]. Improving metabolic abnormalities through exercise is recommended as a basic non-drug therapy. However, the three studies investigating changes in total EVs displayed contradictory changes in extracellular vesicles concentrations, which might be related to the small sample size of included studies. The effect of exercise on total EVs has not been confirmed, and its credibility needs to be further confirmed. In addition, different intensities and frequencies of exercise and combining data might influence the results [29, 35]. Long-term aerobic exercise can improve chronic cardiovascular inflammation and insulin sensitivity, reduce the risk factors of cardiovascular disease such as hyperglycemia, hyperlipidemia, and obesity, and promote metabolic health [53–55]. Although regular moderate-intensity exercise is beneficial for regulating risk factors in chronic disease, most patients remain inactive [56]. HIIT was found to be superior to moderate-intensity continuous training in improving glucolipid metabolism in lifestyle-induced cardiometabolic diseases [57]. After 1-year intervention in patients with T2DM, in both MCT and HIIT groups, carotid intima-media thickness was reduced, while only the HIIT group had improved peripheral arterial stiffness index and dilation coefficient [58]. The research demonstrated that HIIT could effectively improve the cardiorespiratory health of coronary artery disease patients [59]. At the same time, it also significantly improved the VO_2 peak by 10% compared with 4% in the MICT group. HIIT was feasible and safe, and adherence to MICT was similar at 12-month follow-up. This suggested that HIIT could be incorporated into cardiac rehabilitation programs as an adjunct to or as an alternative to MCT [60]. Eight to 12-week HIIT increased the counts of EVs in normal-weight, obesity, T2DM, and IR groups; it also improved insulin sensitivity in the latter two groups [29, 36]. The sedentary obese patients had a higher prevalence of cardiovascular risk factors such as hypertension and inflammation with a higher circulating EV concentrations. The cardiovascular risk appears to increase when exercise is strenuously executed at high intensity and for long times, mainly due to hypercoagulability [61]. The results of this review revealed that HIIT had a more obvious effect on regulating the clinical evaluation indicators of metabolic diseases and EV variations.

EEVs

All of the included literatures reported a decrement in EEVs after long-term exercise, especially for CD62E^{+} EEVs and CD105^{+} EEVs [26–28, 31, 33, 34], while only one research displayed the increased concentration of CD62E^{+} EEVs after a single bout of exercise. EEVs are being used as novel circulating biomarkers of endothelial cell damage and dysfunction, and cardiovascular risk [62]. CD105, a constitutively expressed protein on the surface of endothelial cells, is present in almost all EEVs [33]. CD62E^{+} EEVs are a sign

of early phase endothelial dysfunction [63]. An important role of exercise is to improve vascular endothelial function, which might be related to regulating the release of EVs [64]. Firstly, exercise can increase nitric oxide (NO)-dependent vasodilation and decrease endothelial cells activation and apoptosis in the arterial wall [65, 66]. Moreover, an impaired endothelial mitochondria network has been related to dysfunctional endothelium [42, 43]. Also, exercise elevated long-term laminar shear stress to alleviate endothelial dysfunction by motivating mitochondrial biogenesis in the vascular [28]. Finally, the adaptation of vascular to exercise is partly mediated by shear stress acting on endothelial cells [67]. Exercise-induced increment of endothelial blood flow might reduce the concentration of EEVs as the high shear stress conditions limited EEV secretion in vitro studies [68].

PEVs

The concentration of PEVs was decreased in obese patients undergoing a moderate or high-intensity exercise [17, 35]. Metabolic dysfunction is featured with hypercoagulability and chronic low-grade inflammation. Platelet was overactivated in diabetic patients and its aggregation and adhesion were enhanced. Activated platelets activate phospholipase A2 to promote arachidonic acid production and accelerate platelet release [69]. At the same time, the metabolism of arachidonic acid in platelets was changed due to the synthetic reactive oxygen species by hyperglycemia, resulting in the production of platelet coagulants [69, 70]. PEVs are released upon platelet activation by agonists such as thrombin in obesity with T2DM [71, 72]. Insulin resistance also increases EV secretion [4]. The glycosylated products of insulin resistance reduce the fluidity of platelet membranes and make platelets more prone to activation [73]. In addition, insulin resistance reduces the synthesis of endothelial-derived nitric oxide and prostaglandin, weakening the sensitivity of platelets, which leaves the platelets in a state of obvious activation [74]. Exercise can reduce postprandial glucose concentrations by improving insulin resistance, which might explain the drop of PEVs [75]. Patients with metabolic dysfunction exhibited a chronic low-grade inflammation, which could increase the information of PEVs [76, 77], while exercise was reported to decrease the pro-inflammatory cytokines such as IL-6 and TNF-α to regulate EVs [78].

SkEVs

Two studies involving SkEVs reported an ascending change after a single bout of exercise [32, 35]. Still, the effect of long-term exercise on SkEVs was not mentioned. There is growing evidence suggesting that skeletal muscle

functions as a secretory organ [79], releasing EVs containing hundreds of peptides [80]. Intramuscular injection of fluorescently labeled EVs could cause fluorescence in the distal and contralateral muscles, thus strengthening the concept of paracrine-like effects of muscle release of EVs [81]. A preliminary observation suggested that exosome marker, such as apoptosis-linked gene 2 interacting protein X, was present in the skeletal muscle and seriously depleted immediately after exercise. This supports the premise that EVs of skeletal muscle are released in response to exercise [82]. Exercise can improve the body's metabolism, the balance of oxidative stress, and chronic inflammation [83, 84]. Furthermore, exercise induced the translocation of glucose transporter-4 from intracellular to the cell membrane surface thereby regulating glucose transport in skeletal muscle [85], which can maintain blood glucose homeostasis. Currently, there are no studies on the changes of EVs concentrations and subtypes in metabolic dysfunction after exercise so further studies are needed for in-depth discussion.

AT-EVs

The concentration of FABP$^+$ EVs was unchanged before and after exercise [35]. A study has recently showed that human ATs also secrete EVs that are able to affect local IR in adipocytes through macrophage activation, thus suggesting that AT-EVs have a paracrine role [86]. The AT-EVs also participate in regulating transforming growth factor beta pathway expression in hepatocytes [87] and appetite or weight at central level [88], or even involving in obesity-related cancer [89]. The amount of EV-TFGBI corrected for EV-caveolin, an adipose tissue-specific protein, was significantly elevated in circulating vesicles compared with non-IR obese patients and lean individuals [90]. At present, there are few studies on the effect of exercise on AT-EVs in patients with metabolic dysfunction, and more in-depth studies are needed to demonstrate their specific mechanisms.

The Change of EV Cargos in Response to Exercise

Various miRNA species and peptide are altered by exercise that can influence the pathophysiology of obesity and T2DM through endocrine-like effects. EVs are endocrine-like intercellular communication vesicles that carry proteins, miRNAs, and other nucleic acids which in turn are taken up by target cells [82]. Among the studies we included, three studies investigated the change of EV cargos in response to exercise. After the training program, miR-150, miR-21, miR-223, and miR-146a expression in circulating EVs was significantly enhanced in obesity. A significant positively

correlation was found between the EV concentration of miR-150 and adiponectin [29], a well-known cytokine that is downregulated in obese subjects [91]. This suggests that exercise positively modulates metabolic risk biomarkers. One study found impaired regulation of transporter-mediated long-chain fatty acid (LCFA) uptake processes may contribute to intracellular triacylglycerol accumulation and cellular insulin resistance [92]. By comparing changes in gene expression and SkEVs carrying LCFA transport protein concentrations, it was found exercise-induced changes in skeletal muscle CD36 mRNA expression were positively correlated with CD36-carrying SkEV concentrations in patients with T2DM [32], indicating the beneficial effects of exercise on diabetes. Simultaneously, HIIT can downregulate EV proteins involved in the MAPK, PLC, and PKA signaling, which might help stimulate the downstream insulin signaling pathway in T2D. It also enhanced the antioxidant system in the EVs released by IR, which ultimately improved peripheral insulin sensitivity [36]. This provided new evidence that EV-mediated exercise regulated the metabolic state of the body.

Correlation Analysis Between EV Subtypes and Clinical Evaluation Indicators of Metabolic Diseases

A reduction of total and subtypes of EVs after exercise was positively associated with clinical evaluation of indicators of metabolic diseases in several studies. One study found that HOMA-IR, a measurement used to assess an individual's concentration of insulin resistance, was positively correlated with the postexercise release of total EVs, and CD61$^+$ PEVs in obese patients [35]. The HOMA-IR index in normal individuals was 1, and with the increase of insulin resistance, the index will be higher than 1. Obese patients are frequently insulin-resistant, which might lead to a more sensitive postexercise response in EVs. Moderate increases in exercise are associated with significant reductions in both fasting glucose and HbAlc [93]. In addition, compared with aerobic and resistance exercise, combined exercise can significantly improve the HbAlc concentrations [94]. Another study showed AV$^+$ CD105 EEVs were positively linked to dietary sugar intake and the same association was displayed between AV$^-$/CD31$^+$ PEVs and early phase glucose tolerance [34]. Hyperglycemia can increase the concentration of EVs to promote greater coagulant activity and endothelial dysfunction [50]. As exercise is known to improve glucose regulation, it is reasonable to infer that exercise might modulate EVs released by improving glucose control [14, 95]. This review also showed that PEVs were positively correlated with BMI, waist circumference, fat tissue mass, and subcutaneous fat area [17]. One possible explanation is the positive association of PEVs with leptin,

which decreases the proportion of BMI in obese patients [96]. Moreover, high concentrations of leptin promote ADP-induced platelet aggregation by leptin receptors expressed on the platelet surface [97–99]. Exercise seems to increase t-PA and decrease PAI-1 activity [100] and moderate-intensity exercise can also inhibit platelets activation [101]. Exercise may reduce the release of PEVs, thereby regulating leptin in order to improve hypercoagulability and promote weight loss in obese patients.

Exercise-Related Factors on EVs and EV-Related Detecting Methods

In addition to considering the changes of EVs in metabolic diseases, the impact of exercise-related factors on vesicles cannot be ignored. Among the included studies, 5 focused on a single bout of exercise [27, 32, 35, 37, 39], while the remaining focused on long-term exercise [17, 26, 28–31, 33, 34, 36]. SkEVs increased after a single bout of exercise, while the EVs derived from other cells remained unchanged or decreased. Exercise induced the increase of SkMVs expressing vital long-chain fatty acid (LCFA) transport proteins and increased LCFA oxidation was promoted by the rapid and continued upregulation of LCFA uptake primarily by CD36 and FATP4 [102, 103]. The SkEVs carrying CD36 and FATP4 could reflect real-time expression levels of parental skeletal muscle cells, thus facilitating translocation from intracellular pools to the sarcolemma [32]. The total of EVs in circulation increased after long-term exercise; however, the concentrations of subgroups of EVs remained unchanged or declined. Several proteins from EVs regulated by HIIT overlapped with proteins released from a skeletal muscle cell, which supported the premise that skeletal muscle was likely the key contributor to exercise-induced EVs release. The central role of skeletal muscle in exercise responses and high secretory activity in myokines might help support this argument [104]. The concentration of other subgroups of EVs, for example, EEVs, PEVs, and LEVs decreased after a moderate-intensity single bout of exercise while long-term exercise might be associated with endothelial function improvement, an inhibition of platelet vesiculogenesis and antioxidation of exercise [84, 105].

According to the American College of Sports Medicine, the intensity of exercise is divided into five levels, i.e., very light, light, moderate, high, and near maximum to maximum, on the basis of individual factors such as age, sex, body weight, and fitness level [56]. In this review, all included studies focused on moderate- or high-intensity exercise. After moderate-intensity exercise, the concentration of PEVs was decreased in patients [17, 35, 39], whereas, after interval exercise, PEVs remained unchanged [30, 34]. Moderate-intensity aerobic exercise promoted the synthesis and release of NO, and increased the concentration of plasma NO. Subsequently, it activated the guanylate cyclase system in platelets and increased the level of cGMP, thereby inhibiting platelet activation [106]. Yet, high-intensity exercise induced a hypercoagulative state that might activate the platelets and be related to the release of EVs [61].

In spite of the universalities, we can summarize from the available results, the methods for detecting EVs, including sEVs and m/lEVs, cannot be ignored. Out of 14 studies, 12 used FCM to detect EVs and three researches applied NTA, which are the commonly used detection methods [5]. FCM, which has good statistical accuracy and strong applicability, is applied for rapid quantitative analysis and cell origin of EVs [107, 108]. The diameter of EVs ranging from 200 to 2000 nm can be directly measured and FCM can detect the specific marker. EVs have a diameter between 40 and 200 nm and need to be tracked and tested by NTA. Nanoparticle tracking analysis determines suspended particles size and concentration by tracking the Brownian motion of submicrometer particles using a dark field microscope [109]. However, due to the complex composition of blood after exercise, the analysis of EVs is hindered, thus challenging the separation and characterization of EVs. The use of a two-step EV isolation protocol (containing size and density) and complete identification of EVs (including biochemical EV-marker analysis, single-EV imaging, and single-particle tracking) are essential [110].

Limitations

This review has several limitations. First, different types of diseases, and exercise patterns, had a complex impact on outcomes. Furthermore, most of the included studies were observational studies with relatively low grades. And the small sample size limits the application to the general participants. Finally, current methods of extraction and detection of EVs continue to improve so the economic burden of EVs as a biomarker of patient response to exercise should be strictly considered. Hence, comprehensively designed clinical studies are essential to provide data support and further evidence.

Conclusions

This systematic review summarized the effect of exercise on different types of EV concentration in patients with metabolic dysfunction. Our review showed that the concentration of EEVs decreased after long-term exercise, especially for CD62E$^+$ EEVs and CD105$^+$ EEVs. The same trend was also observed in PEVs. Nevertheless, completely opposite changes were seen in the concentration of SkEVs after a

single bout of exercise. It was also found that the changes in EEVs were positively linked to dietary sugar intake and PEVs were positively linked with glucose tolerance and BMI after long-term exercise. The pathological mechanism of EVs on the improvement or aggravation of metabolic function should be further studied. In conclusion, the changes in EVs concentrations could assist in assessing the effect of exercise on patients with metabolic dysfunction.

Author Contribution TL, XWH, and SQC have contributed equally to this work and share first authorship. Theme and design of the research: XWH and TL; article retrieval: SQC and BFW; data extraction: YT and WTC; verification of data: ZWL and YL; literature evaluation: WWX, YYJ, and LW; writing of the manuscript: TL, XWH, and SQC; critical revision of the manuscript for intellectual content: LSL and MJZ; obtaining funding: MJZ and XWH.

Declarations

Conflict of Interest The authors declare no competing interests.

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