



Exercise Training Improves Brachial Artery Endothelial Function, but Does Not Alter Inflammatory Biomarkers in Patients with Peripheral Artery Disease: a Systematic Review and Meta-analysis

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Received: 10 August 2023 / Accepted: 6 October 2023 / Published online: 23 October 2023

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Abstract

The study aimed to systematically review the effects of exercise training (EX) on brachial artery flow-mediated dilation (FMD) and inflammatory biomarkers in patients with peripheral artery disease (PAD). Five electronic databases were searched: (i) patients with PAD aged ≥ 18 ; (ii) structured EX ≥ 2 weeks; (iii) measured brachial artery FMD; and (iv) measured blood inflammatory biomarkers. Eighteen studies met the inclusion criteria. EX increased FMD but had no effect on C-reactive protein, interleukin-6, and tumor necrosis factor- α . Subgroups with moderate intensity had a greater increase in FMD than subgroups with vigorous intensity. There was no difference in effect on FMD and three inflammatory biomarkers between subgroups training for ≤ 12 weeks and > 12 weeks of EX, < 50 min and ≥ 50 min of session duration, and < 150 min and ≥ 150 min of weekly volume, respectively. These results suggest that EX-induced improvement in vascular function can be independent of the improvement of systemic inflammation.

Keywords Atherosclerosis · Vascular function · Chronic inflammation · Cardiac rehabilitation

Introduction

Peripheral artery disease (PAD) is a manifestation of systemic atherosclerosis resulting in blood flow restriction by narrowed or blocked peripheral arteries. The vascular endothelium, an inner layer of blood vessels, plays a pivotal role in the development and progression of atherosclerosis [1]. Endothelial dysfunction is defined by an imbalance between vasodilation and vasoconstriction, characterized by reduced bioavailability of vasodilators, particularly nitric oxide (NO), and/or increased endothelium-derived contracting factors [2]. Endothelial (dys)function can be quantified by the measurement of flow-mediated dilation (FMD) of the brachial artery as a non-invasive gold standard [3, 4]. A meta-analysis concluded that every 1% decrease in brachial artery FMD is associated with a 13% increase in the events

of cardiovascular diseases (CVD) [4]. Moreover, brachial artery FMD is independently associated with the presence and extent of atherosclerosis [5]. Thus, the improvement of endothelial function measured by brachial artery FMD is a major therapeutic goal for PAD.

Chronic inflammation plays a critical role in the pathogenesis of atherosclerotic diseases [6]. Various inflammatory mediators such as pro-inflammatory cytokines promote endothelial nitric oxide synthase (eNOS) uncoupling [7] and endothelial activation, morphological and functional modifications triggered by nuclear factor- κ B (NF- κ B) signaling [8], and as a result, these inflammatory mechanisms can directly or indirectly induce vascular dysfunction. Pro-inflammatory cytokines, including certain interleukins (ILs) and tumor necrosis factor (TNF)- α , stimulate the upregulation of adhesion molecules with subsequent monocyte adhesion [8, 9]. Monocytes proceed to differentiate into macrophages that further promote coagulation, platelet adhesion, and aggregation, which consequently induces atherosclerosis [9]. In addition, the pro-inflammatory marker C-reactive protein (CRP) may play a role in the development and progression of PAD [10].

Physical activity increases hemodynamic shear stress, the most powerful physiological activator of eNOS, which

Associate Editor Junjie Xiao oversaw the review of this article.

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promotes the production of a key vasodilator, NO [11]. We previously determined that exercise training (EX) alleviated atherosclerosis and obese-induced vascular dysfunction through increased NO bioavailability and eNOS expression in mice [12, 13]. In patients with PAD, EX increased brachial artery FMD with decreased [14] or unchanged levels of inflammatory biomarkers [15], whereas another study determined that both FMD and inflammatory biomarkers remained unchanged after EX [16]. Similarly, the effects of EX on inflammatory biomarkers seem to be contradictory in patients with PAD. For example, EX decreased levels of CRP or IL-6 [17, 18] in some studies, whereas in other studies CRP, IL-6, and TNF- α were unchanged following EX [15]. Thus, there are contradictory results about the effects of EX on vascular endothelial function and inflammation in patients with PAD.

To our knowledge, no meta-analysis has been conducted to examine the effects of EX on the key prognostic variables for the risk of atherosclerosis and CVD events, vascular endothelial function, and inflammatory biomarkers in patients with PAD. Exercise guidelines for the patients need to be developed based on a quantitative review. Moreover, there are contradictory results about EX-induced changes in endothelial function and inflammation in PAD. Therefore, the purpose of this study was to evaluate the effects of EX on endothelial function assessed by brachial artery FMD and blood inflammatory biomarkers in patients with PAD by conducting a systematic review and meta-analysis. This review carefully examined the specific variables of training regimens (i.e., intensity, duration, and frequency) and demographic characteristics of patients (i.e., age, gender, and body mass index (BMI)) to provide high-level evidence for developing optimal exercise guidelines.

Methods

This current systematic review followed the strategy of the PRISMA statement [19].

Data Sources

Five electronic databases (CINAHL, Cochrane, EMBASE, MEDLINE, and Web of Science) were searched for eligible studies published in English from the earliest date available to April 2023. The following keywords were used for searches: “exercise or training or rehabilitation” AND “endothelial function or flow-mediated dilation or inflammation or inflammatory or biomarker or cytokine” AND “peripheral artery disease or peripheral arterial disease.” Reference lists of included studies and relevant reviews were manually searched to ensure all relevant studies were captured. Two reviewers (JL and AZ) searched all the articles and applied the inclusion and exclusion criteria to the titles and abstracts

searched. Disagreements about inclusion and exclusion were resolved by reaching a consensus through discussion, which involved a senior researcher (YP). When the information was not clear, the full-text papers of the studies were obtained for review. Corresponding authors of potentially eligible studies were contacted if studies reported missing or unclear data.

Study Selection

The inclusion criteria for eligible studies were as follows: (i) patients with PAD of all stages aged ≥ 18 ; (ii) structured exercise intervention ≥ 2 weeks, such as aerobic training (AT), resistance training (RT), or combined AT and RT including supervised intervention, home-based intervention, and mixed intervention in both randomized and non-randomized controlled studies; (iii) measured endothelial function by brachial artery FMD (% of change); and (iv) measured blood inflammatory biomarkers. Measurements had to be administered both at baseline and post-intervention. Non-exercise trials, such as medication treatment, or multi-interventional trials involving additional non-exercise interventions on EX, such as exercise intervention combined with dietary supplements, were excluded from multi-intervention studies to focus on the effects of EX alone. Studies investigating the acute effect of one single exercise session were also excluded. Duplicate studies or sub-studies of included trials were also excluded from the analysis.

Quality Assessment

Two reviewers assessed the quality of the included studies using the PRISMA recommendations [19]. The quality assessment consisted of six items: (i) appropriate generation of random allocation sequence; (ii) concealment of the allocation sequence; (iii) blinding of the assessment and collection outcomes; (iv) proportion of participants lost to follow-up; (v) complete outcome data; and (vi) the intention-to-treat principle [19]. The overall quality of the evidence was also assessed by the Grading of Recommendations Assessment, Development, and Evaluations (GRADE) [20, 21]. GRADE assesses outcomes across five areas: risk of bias, inconsistency, indirectness, imprecision, and publication bias. According to GRADE, interventions were graded as follows: ‘high quality’—we are very confident that the true effect lies close to that of the estimate of the effect; “moderate quality”—we are moderately confident in the effect estimate. The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different; “low quality”—our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect; “very low quality”—we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of the effect [22].

Data Extraction

Data were extracted from all selected studies to record detailed information in terms of subject characteristics, study methods, interventions, outcomes, and adverse events. Means and standard deviation (*SD*) were used, but where standard errors or 95% confidence interval (*CI*) were provided, they were converted to *SD*.

In terms of population characteristics, age, gender, BMI, relative body fat, the number of participants, post-PAD period, and medication were recorded to compare the similarity of participants between trials. The primary outcome was FMD and the secondary outcomes were inflammatory biomarkers. The brachial artery was only selected instead of a femoral or popliteal artery for the measurement of FMD because brachial artery endothelial function is inversely associated with CVD risk and atherosclerosis as a non-invasive gold standard to assess endothelial function [3–5]. Regarding the intervention, total duration, frequency (days per week), intensity, session duration, weekly training volume, type and order of exercise, names of exercise machines or tools, supervisors, and places of intervention were recorded to compare the similarity of training methods between trials. The median values were used for calculation if the studies reported a range of data (e.g., 16 from 15 to 17 repetitions). Detailed interventions about control groups (CON) and any additional interventions were recorded. Measurement technique and region were also extracted.

Data Analysis

Heterogeneity between studies was assessed using the Cochran *Q* statistic [23] and the *I*² test [24]. *I*² ranges from 0 to 100%: a value < 25% indicates a low risk of heterogeneity; 25–75% indicates a moderate risk of heterogeneity, and > 75% indicates a high risk of heterogeneity. In each study, the effect size (*ES*) for the intervention was calculated by the standardized mean difference between pre- and post-intervention using Cohen's *d*. Separate meta-analyses of trials with FMD and inflammatory biomarkers were performed to generate the mean *ES* and 95% *CI*. *ES*s were classified according to Cohen's definition (1988), where 0.2 is considered small, 0.5 moderate, and 0.8 large [25]. We used a fixed-effects model when homogeneity was verified or a random-effects model when heterogeneity was shown by the *Q* statistic [24]. When the *Q* value exceeds the degrees of freedom of the estimate, significant heterogeneity is considered to exist. Publication bias was assessed using Egger's regression test [26]. To evaluate whether an individual cohort had undue influence on the overall meta-analysis result, sensitivity analyses were performed on all outcomes by omitting one of the trials at a time and determining whether statistical conclusions remained the same. The percent changes in

FMD and inflammatory biomarkers between pre- and post-intervention were calculated using the weighted averages. Meta-analyses were performed with SPSS version 20 and STATA version 14.2. Data coding and other calculations were conducted with Microsoft Excel 365.

Subgroup analyses were performed to identify potential factors influencing the effect of EX on outcomes and accounting for the heterogeneity between studies: (i) ≤ 12 weeks versus > 12 weeks of EX; (ii) < 50 min versus ≥ 50 min of session duration; (iii) < 150 min versus ≥ 150 min of weekly volume; and (iv) moderate-versus vigorous-intensity. Random effects meta-analysis regression was conducted to compare the effect estimates (effect size) in different subgroups by considering the meta-analysis results from each subgroup separately. To interpret the results of subgroup analyses, *P* value (*P* < 0.05) between study variations was considered for the statistical difference between subgroups.

Results

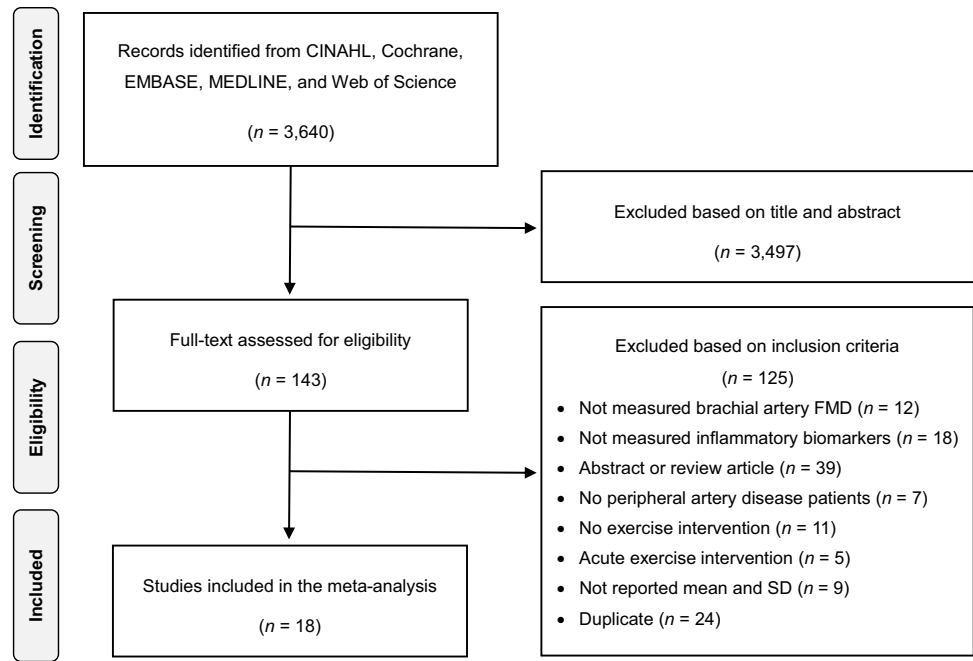
Study Selection and Characteristics

The selection process resulted in 3640 potential studies and is documented in the PRISMA flow diagram (Fig. 1). From the titles and abstracts, 3497 studies were excluded based on the inclusion and exclusion criteria, and then 143 full-text studies were reviewed for eligibility by applying the criteria. Of these, 125 studies were excluded after assessment, and finally, a total of 18 studies [14–18, 27–39] met the criteria. In the selected studies, 9 studies [14–16, 28, 29, 32, 34, 35, 38] had two EX cohorts, and one study had one EX cohort (treadmill) and another EX cohort (treadmill) in a water pool of 24–30 °C [14]. Another heat therapy cohort without EX [27] was excluded from this analysis. Consequently, 29 exercise cohorts in 18 studies were included. Of 18 studies, 13 studies measured brachial artery FMD and 6 studies measured inflammatory biomarkers, such as CRP, IL-6, and/or TNF-α following EX.

Quality Assessment and Potential Bias

In the quality assessment, 83% reported appropriate generation of a random allocation sequence (15 of 18); 44% presented concealment of the allocation sequence (8 of 18); 56% described blinding of the assessment and collection outcomes (10 of 18); 100% explained proportion of participants lost to follow-up (18 of 18); 100% exhibited complete outcome data (18 of 18); and 33% reported that the intention-to-treat principle was used for statistical analyses (6 of 18). Egger's test showed significant publication bias for FMD (*P* = 0.04) and no significant bias for CRP, IL-6, and TNF-α (*P* = 0.13, *P* = 0.78, and *P* = 0.17, respectively) (Fig. 2).

Fig. 1 Study search and selection process. FMD, flow-mediated dilation



Adverse Events

The presence or absence of adverse events was recorded in three [18, 27, 39] of the eighteen studies. Those three studies reported that there were no adverse events.

Participants

Table 1 shows the characteristics of all the studies included. Studies were published from February 2001 [31] to December 2021 [36]. A total of 1301 participants completed their interventions (EX: 980, CON: 321, female%: 33.2%), with sample size ranging from 11 [27] to 185 [33]. The average age of the participants was 66.0 ± 7.9 years (EX: 68.3 ± 8.2 , CON: 66.7 ± 8.4 years). Average BMI was 27.5 ± 5.0 kg/m² (EX: 27.7 ± 5.0 , CON: 26.9 ± 5.0 kg/m²). Most patients with PAD in included studies had some comorbidities including diabetes, hypertension, hyperlipidemia, myocardial infarction, coronary artery disease, or cancer, but it was not reported in four studies [14, 28, 31, 33]. Their medications were maintained throughout interventions but were withheld for at least 24 h before evaluation. The duration of PAD was ≥ 1 month [18] or ≥ 3 months [28, 29], and other studies did not report it.

Interventions

Most studies performed supervised EX in research centers by qualified trainers or researchers, but four cohorts had partial [32] or complete home-based EX sessions [16, 28, 36]. All studies conducted AT using a bicycle or a

treadmill, but seven studies [14, 15, 27, 32, 34, 36, 38] additionally performed resistance training (RT). One EX cohort performed treadmill exercise in a water pool of 24–30 °C [14]. The mean training period was 17 weeks (minimum–maximum: 6 [30]–40 [16] weeks). The training frequency was most often 3 days per week; 2 [15, 27], 4 [16], or 5 [36] days per week were also used. The mean session duration was 50 min (minimum–maximum: 30 [18, 31]–90 [27] min). The mean weekly training volume was 142 min (minimum–maximum: 90 [18, 31]–225 [36] min). AT trials expressed intensity as a percent of maximum heart rate (HRmax), walking ability, the Borg scale, or the metabolic equivalent of task (MET). RT trials established their intensity by a percentage of one-repetition maximum (1-RM) or the Borg scale. Intensities ranged from moderate (64–76% of HRmax, 50–69% of 1-RM, Borg scores 12–14, or MET scores 3.0–5.9) to vigorous (77–95% of HRmax, 70–90% of 1-RM, Borg score 15–17, or MET scores ≥ 6.0). AT trials monitored HR or walking speed of participants during exercise for accurate intensity guidance. RT trials monitored HR or progressively increased their intensity throughout training by increasing loads.

Measurements

All studies measured FMD using a high-resolution ultrasound machine to assess brachial artery endothelial function. There were slight differences in the inflation pressure for measuring FMD between studies: 30 [30] or 50 [16, 17, 32–35, 37] mmHg above systolic blood pressure, 200 mmHg [31], 250 mmHg

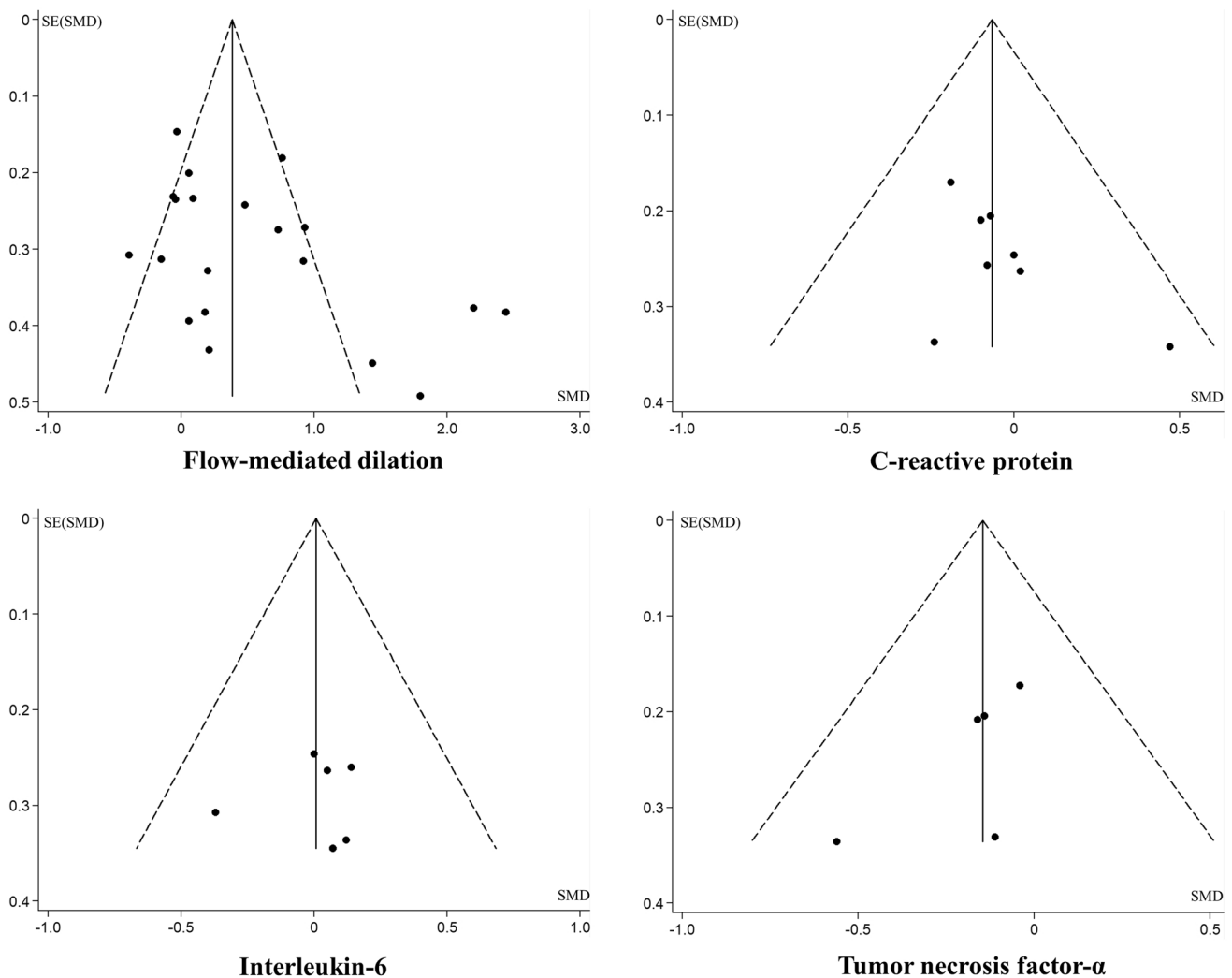


Fig. 2 Funnel plots of publication bias in all 4 outcomes. SE, standard error and SMD, standardized mean difference

[36], or unknown [27–29]. The duration of cuff inflation was 3 min [30], 4 min [33, 34, 36, 37], 5 min [16, 17, 28, 29, 31, 32, 35], or not specified [27]. Inflammatory biomarkers were measured by ELISA [14, 15, 18, 38] or not specified [17, 39].

Effects of Exercise Training

Flow-mediated Dilation

EX in twenty trials of thirteen studies significantly increased brachial artery FMD (mean $ES = 0.54$, 95% $CI = 0.25$ to 0.83 , $P < 0.001$) (Fig. 3). The absolute increase in FMD between the pre-and post-intervention was 19.7%. Univariate meta-regression showed heterogeneity between studies ($Q = 108.8$, $df = 19$, $P < 0.001$, $I^2 = 82.5\%$). In subgroup analyses, subgroups with moderate intensity had a significantly greater increase of FMD than subgroups with vigorous intensity ($P < 0.01$).

There was no significant difference in effect between subgroups training for ≤ 12 weeks and > 12 weeks of EX ($P = 0.41$), < 50 min and ≥ 50 min of session duration ($P = 0.80$), and < 150 min and ≥ 150 min of weekly volume ($P = 0.74$), respectively. Age and baseline BMI were excluded from subgroup analyses as most participants had similar levels of age (65–70 years) and BMI (25–30 kg/m^2). Weekly frequency was also excluded from subgroup analyses as most trials performed EX 3 days per week.

Inflammatory Biomarkers

EX in eight trials of five studies had no significant effect on CRP (mean $ES = -0.07$, 95% $CI = -0.23$ to 0.10 , $P > 0.05$) (Fig. 4). EX in six trials of four studies had no significant effect on IL-6 (mean $ES = 0.01$, 95% $CI = -0.22$ to 0.24 , $P > 0.05$) (Fig. 5). EX in five trials

Table 1 Summary of included studies

Study	Clinical trial number	Age (years)		Number (%female)		BMI (kg/m ²)		Comorbidity	Disease stages	Outcomes	Intervention					
		EX	CON	EX	CON	EX	CON				Duration (weeks)	Frequency (week ⁻¹)	AT volume (min)	RT volume	AT intensity	RT intensity
Akerman et al. (2019) [27]	ACTRN12616001049460	74.4 ± 10.1	-	11 (27)	-	28.5 ± 6.8	-	Hypertension, diabetes, T2D, and MI	Ila–Iib	Brachial FMD ↔	12	2	30	12 stations × 1 set × 2 circuits	n/a	n/a
Allen et al. (2010) [28]	n/a	67.0 ± 2.0	53.0 ± 1.0	18 (n/a)	21 (n/a)	27.8 ± 1.0	28.6 ± 0.9	n/a	n/a	Brachial FMD†	15	3	35	-	n/a	-
Allen et al. (2014) [29]	n/a	69.0 ± 3.0	-	14 (41)	-	25.0 ± 1.3	-	T2D	n/a	Brachial FMD†	15	3	35	-	n/a	-
Andreozzi et al. (2007) [30]	n/a	65.0 ± 13.0	-	22 (0)	-	27.4	-	T2D	n/a	Brachial FMD†	6	3	40	-	60–70% of walking ability	-
Brendle et al. (2001) [31]	n/a	69.0 ± 1.0	-	19 (11)	-	28.1	-	n/a	Ila–Iib	Brachial FMD†	30	3	30	-	50–80% of HR _{max}	-
Correia et al. (2020) [32]	NCT02742220	66.0 ± 12.0	-	50 (39)	-	27.0 ± 5.0	-	T2D, CAD, and hypertension	Ila–Iib	Brachial FMD†	8	3	50	1 station × 10 repetitions × 3 sets	Moderate-vigorous	-
Domanchuk et al. (2013) [33]	NCT01408901	n/a	n/a	93 (n/a)	92 (n/a)	n/a	n/a	n/a	n/a	Brachial FMD†	26	3	45	-	Moderately hard	-
Dopheide et al. (2017) [16]	n/a	68.5 ± 10.4	67.9 ± 9.5	20 (30)	20 (30)	n/a	n/a	Diabetes and hypertension	Ila–Iib	Brachial FMD ↔	40	4	45	-	n/a	-
Januszek et al. (2014) [17]	n/a	65.5 ± 7.7	-	67 (39)	-	25.0 ± 7.6	-	Diabetes and hypertension	Ila–Iib	Brachial FMD†, CRP†, and TNF-α†	12	3	50	-	3.2 km/h	-
McDermott et al. (2009) [34]	NCT00106327	77.1 ± 8.7	68.5 ± 11.9	51 (51)	53 (52)	30.4 ± 6.2	29.9 ± 7.1	Diabetes	n/a	Brachial FMD†	24	3	40	5 stations × 8 repetitions × 3 sets	2.0 mph	n/a
Novaković et al. (2019) [35]	NCT02642276	64.4 ± 9.0	65.6 ± 11.0	10 (28)	8 (27)	30.4 ± 6.2	28.5 ± 2.3	Diabetes	Ila–Iib	Brachial FMD†	12	3	60	-	70% of HR _{max}	-

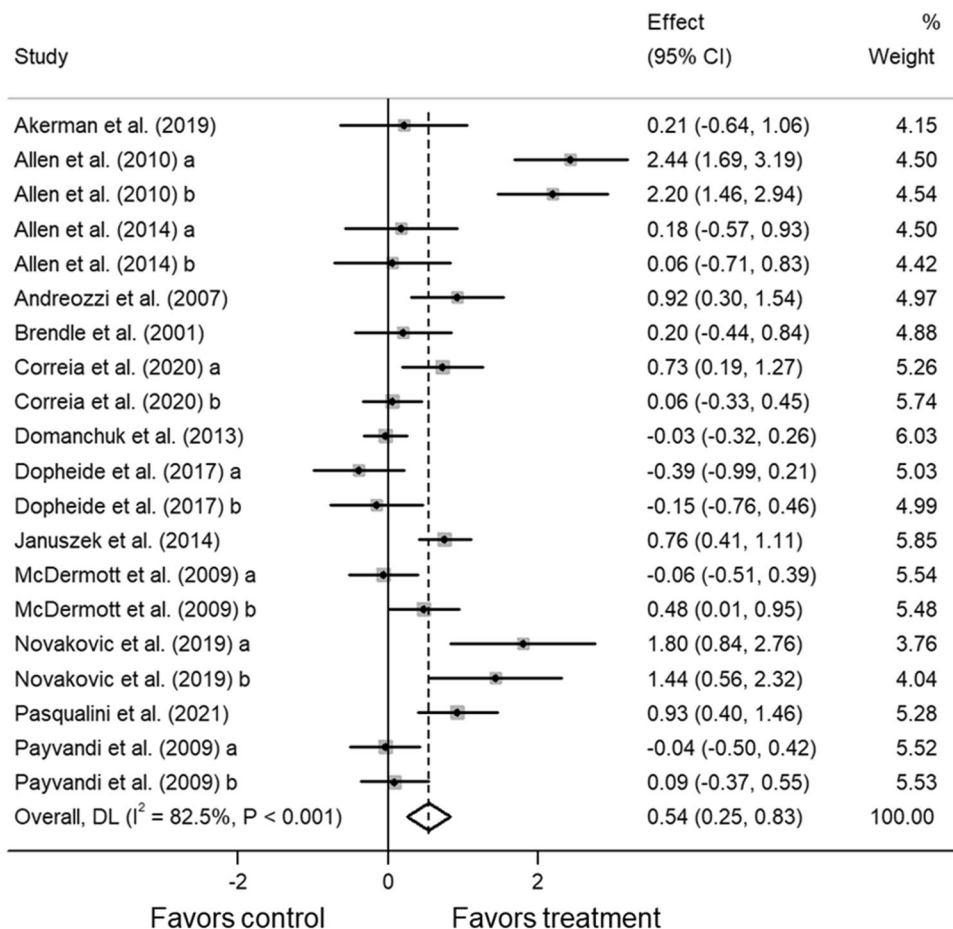
Table 1 (continued)

Study	Clinical trial number	Age (years)		Number (%female)		BMI (kg/m ²)		Comorbidity	Disease stages	Outcomes	Intervention		AT intensity	RT intensity		
		EX	CON	EX	CON	EX	CON				Duration (weeks)	Frequency (week ⁻¹)			AT volume (min)	RT volume
Pasqualini et al. (2021) [36]	NCT04302571	70.5 ± 4.5	72.5 ± 2.3	60 (24)	20 (23)	26.1 ± 2.5	26.6 ± 2.1	Diabetes	I–II	Brachial FMD†	12	5	45	7 stations × 3 sets	80% of maximum walking time	n/a
Payvandi et al. (2009) [37]	NCT00106327	72.2 ± 8.8	66.1 ± 9.3	37 (46)	36 (47)	31.8 ± 7.2	28.7 ± 5.4	MI and hypertension	n/a	Brachial FMD†	n/a	n/a	n/a	-	n/a	-
Alboghadi et al. (2017) [38]	n/a	71.0	67.0	33 (53)	26 (53)	29.9	30.5	MI, cancer, and diabetes	n/a	CRP ↔ and IL-6 ↔	24	3	n/a	3 stations × 8 repetitions × 3 sets	n/a	80% of 1RM
Collins and Twumasi-Ankrah (2013) [39]	n/a	66.6 ± 10.4	66.9 ± 11.2	30 (24)	25 (25)	34.3 ± 9.6	33.9 ± 7.5	Diabetes	n/a	CRP ↔ and IL-6 ↔	24	3	50	-	n/a	-
Delaney and Spark (2016) [15]	n/a	73.4 ± 9.1	-	28 (29)	-	27.0 ± 4.3	-	Diabetes and hypertension	n/a	CRP ↔, IL-6 ↔, and TNF-α ↔	12	2	40	6 stations × 10 repetitions × 3 sets	n/a	n/a
Lin et al. (2021) [18]	NCT03965520	71.1 ± 1.5	70.5 ± 1.9	20 (40)	20 (40)	27.2	26.8	Hypertension and diabetes	n/a	IL-6↓	12	3	30	-	Ventilation threshold	-
Quarto et al. (2017) [14]	n/a	68.0 ± 7.0	-	45 (30)	-	n/a	-	n/a	IIb	CRP↓ and TNF-α↓	12	3	45	3 stations × 10 repetitions × 1 set	3.2 km/h	n/a

Values are *means ± SD*

EX exercise training group, CON control group, BMI body mass index, AT aerobic training, RT resistance training, T2D type 2 diabetes, MI myocardial infarction, n/a not available, FMD flow-mediated dilation, ↔ does not significantly change, ↑ significantly increases, ↓ significantly decreases, HR_{max} heart rate max, CAD coronary artery disease, CRP C-reactive protein, TNF-α tumor necrosis factor-α, IL-6 interleukin-6, and RM repetition maximum

Fig. 3 Forest plot of effect sizes and 95% confidence intervals for 20 cohorts representing brachial artery flow-mediated dilation, based on the random effects meta-analysis results



of three studies had no significant effect on TNF- α levels (mean $ES = -0.15$, 95% $CI = -0.34$ to 0.05 , $P > 0.05$)

(Fig. 6). Univariate meta-regression did not show heterogeneity between studies in CRP ($Q = 3.46$, $df = 7$, $P > 0.05$,

Fig. 4 Forest plot of effect sizes and 95% confidence intervals for 8 cohorts representing blood C-reactive protein, based on the fixed effects meta-analysis results

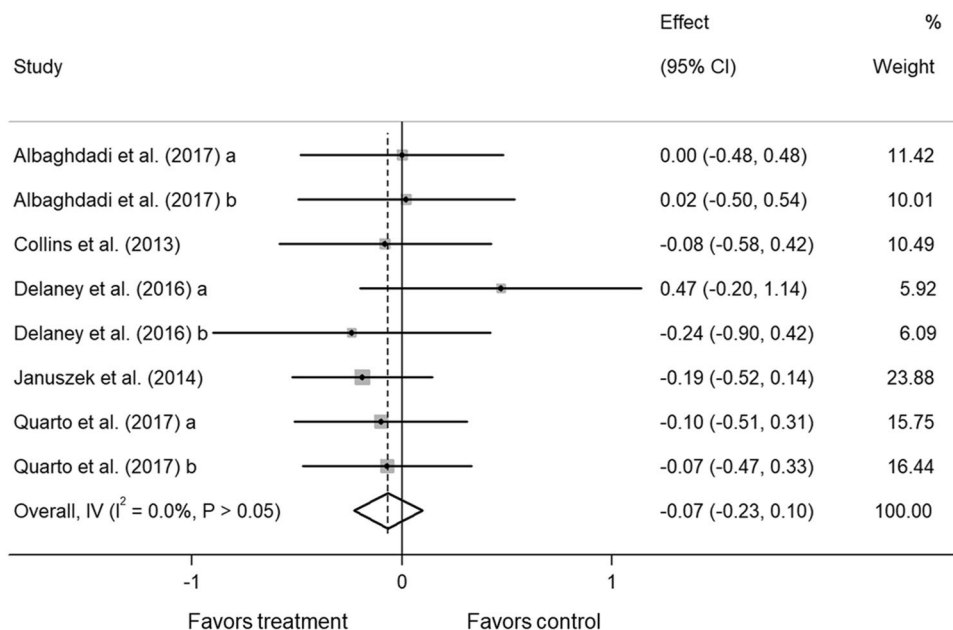
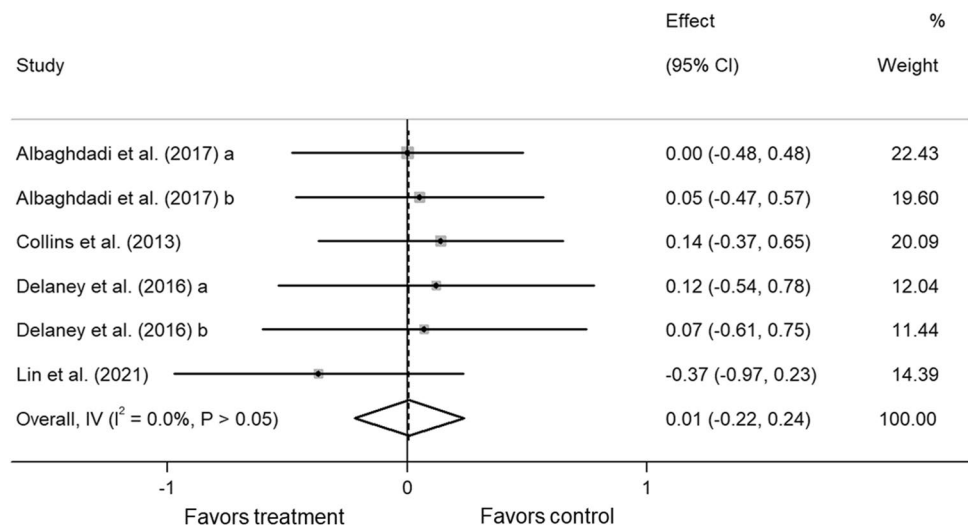


Fig. 5 Forest plot of effect sizes and 95% confidence intervals for 6 cohorts representing blood interleukin-6, based on the fixed effects meta-analysis results



$I^2 = 0.0\%$), IL-6 ($Q = 1.94$, $df = 5$, $P > 0.05$, $I^2 = 0.0\%$), and TNF- α ($Q = 1.92$, $df = 4$, $P > 0.05$, $I^2 = 0.0\%$). In subgroup analyses for CRP, there was no significant difference in effect between subgroups training for ≤ 12 weeks and > 12 weeks of EX ($P = 0.71$), and < 150 min and ≥ 150 min of weekly volume ($P = 0.37$), respectively. For IL-6, there was no significant difference in effect between subgroups training for ≤ 12 weeks and > 12 weeks of EX ($P = 0.55$). For TNF- α , there was no significant difference in effect between subgroups' training for < 150 min and ≥ 150 min of weekly volume ($P = 0.38$). Other training variables, such as intensity and session duration were excluded from subgroup analyses as most trials performed EX with similar levels of intensity (3.2 km/h), session duration (50–60 min), and frequency (3 days per week).

Sensitivity Analysis

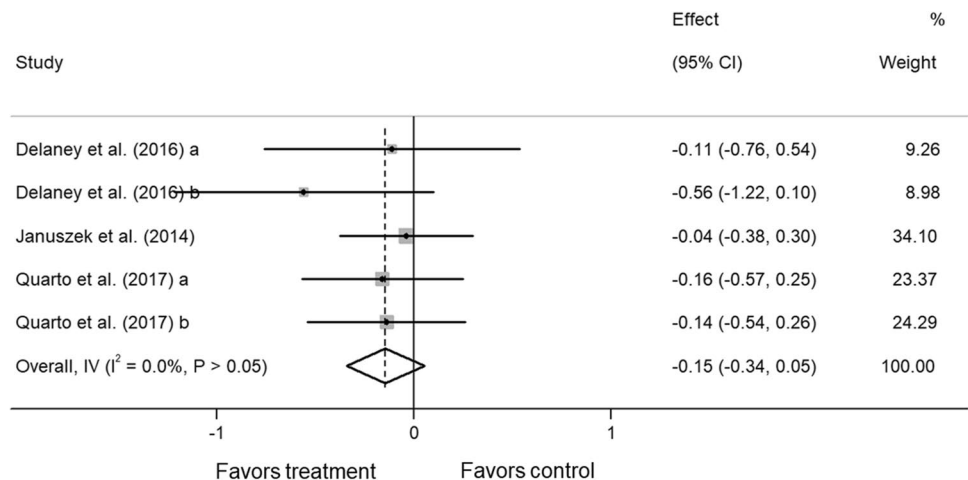
Sensitivity analysis reported that by excluding any of all cohorts from the meta-analysis the estimated effects will still

be within the 95% CI of the mean ES in all four outcomes (Supplementary Fig. 1). These results indicate the results of the meta-analysis will not significantly change after the removal of any one cohort.

Discussion

The primary results of this meta-analysis are that EX significantly increased brachial artery FMD by 20% but had no significant effect on CRP, IL-6, and TNF- α levels in patients with PAD aged 66.0 ± 7.9 years. These results suggest that EX-induced improvement in vascular endothelial function can be independent of the improvement of systemic inflammation (Fig. 7). Brachial artery FMD is negatively associated with the risk of CVD events and the extent of atherosclerosis [4, 5]. Accordingly, EX could substantially contribute to reducing the rate of cardiovascular mortality in patients with PAD. In addition to the main findings, subgroup analyses determined that EX with moderate intensity

Fig. 6 Forest plot of effect sizes and 95% confidence intervals for 5 cohorts representing blood tumor necrosis factor- α , based on the fixed effects meta-analysis results



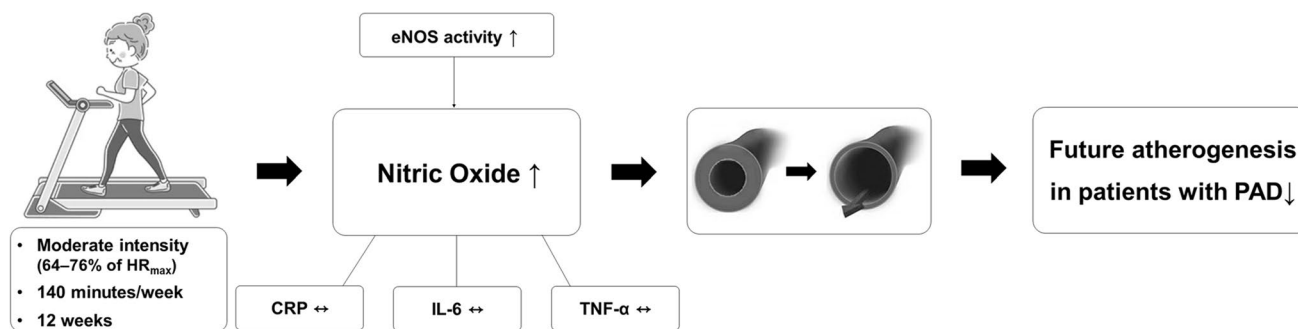


Fig. 7 The exercise training improves brachial artery endothelial function in patients with peripheral artery disease through increased nitric oxide availability without changes in inflammatory biomarkers, which might be associated with improved eNOS activity. Improved endothelial function potentially reduces future atherogenesis. Moder-

ate intensity is based on ACSM's cardiac rehabilitation guidelines. HR_{max}, heart rate max; eNOS, endothelial nitric oxide synthase; CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; and PAD, peripheral artery disease

had a significantly greater effect on FMD than EX with vigorous intensity.

Unchanged Inflammatory Markers Following Exercise Training

Different from our expectation, EX increased FMD but did not affect inflammatory biomarkers. Contrary to our results, a previous meta-analysis [40] found that EX decreased levels of CRP and IL-6 in patients with coronary artery disease. However, another meta-analysis [41] concluded that contradictory inflammatory adaptations to EX can be elicited in patients with CVD according to the type of CVD and training variables. Similarly, included studies in our meta-analysis have differing results of inflammatory biomarkers, which might be attributed to a different training variable. For example, Delaney and Spark [15] determined that IL-1β, IL-2, IL-7, IL-8, IL-10, and IL-12 as well as CRP, IL-6, and TNF-α remained unchanged following 12 weeks of EX. Other two included studies [38, 39] had similar findings that soluble intercellular adhesion molecules, CRP, and IL-6 remained unchanged following 6 months of EX. On the other hand, neutrophils, CRP, IL-6, and/or TNF-α decreased following 12 weeks [17, 18] or 12 months [14] of EX. Our subgroup analyses also indicated no significant difference in effect on the biomarkers between ≤12 weeks and >12 weeks of EX. Accordingly, the training duration does not seem to influence the alteration of systemic inflammation. Another examined training variable, weekly volume (<150 min vs ≥150) did not reveal a significant effect on the inflammatory biomarkers. However, the lowest weekly training volume (90 min) [18] had the greatest favorable ES (−0.37) compared to ESs (−0.19–0.47) of a relatively higher weekly training volume (120–180 min) [14, 15, 17, 38, 39]. Considering PAD mostly occurs in the lower extremities and about

30% of these patients have claudication symptoms [42], a prolonged session duration of EX with a treadmill or bicycle might damage the tissue of the legs and contribute to chronic inflammation [43]. Thus, session duration might be one of the variables to induce different inflammatory adaptations to EX. Although our results indicate no significant improvement in systemic inflammation by EX, it has been also documented that EX exerts anti-inflammatory effects by several molecular mechanisms, e.g., reduced Toll-like receptor expression on monocytes and macrophages [44], which prevents increased expression and secretion of pro-inflammatory cytokines [45]. EX also reduces the expressions of three key proteins, NF-κB p65, cysteinylaspartate specific proteinase 1, and NOD-like receptor protein 3 that directly trigger the activation of proinflammation [46]. However, EX with moderate intensity has more favorable effects on those factors compared to vigorous EX [46]. Taken together, EX is suggested to induce anti-inflammatory adaptations but exaggerated physical stress induced by vigorous intensity or prolonged session duration could decline its benefits. Future studies are warranted to determine inflammatory responses to different exercise durations and intensities.

Increased FMD Following Exercise Training

It is noteworthy that FMD was improved following EX. Exercise increases blood flow, which activates vascular endothelium that produces the potent vasodilator, NO [11–13]. Two included studies [28, 29] observed that EX significantly increased plasma levels of nitrite, a key marker of vascular NO bioavailability [47]. The majority (about 70%) of increased NO bioavailability is attributable to the enhanced activity of eNOS in humans and other mammalian species [48]. We recently determined that EX improved vascular function via increased NO levels and expressions of eNOS in arteries of mice with

apolipoprotein-E-knockout as models of atherosclerosis [13] and obesity [12]. Accordingly, it seems that NO plays a predominant role in modulating a balance between vasodilators and vasoconstrictors, and increased NO bioavailability may be the major beneficial consequence following EX. Taken together, EX can improve PAD-induced endothelial dysfunction by increased NO availability due to enhanced synthesis and activity of eNOS, which might be independent of systemic inflammation levels. However, pro-inflammatory cytokines can contribute to endothelial dysfunction through eNOS uncoupling [7] and NF- κ B signaling [8]. Thus, further studies are needed to investigate the association between vascular endothelial function and cytokines following EX.

A Greater Increase in FMD with Moderate Intensity

Interestingly, subgroups with moderate intensity had a greater increase in FMD than subgroups with vigorous intensity ($P < 0.01$). Considering hemodynamic shear stress is directly proportional to the velocity of blood flow, it may be reasonable that the improvement in vascular endothelial dysfunction is proportional to exercise intensity by increased cardiac output. However, our previous meta-analysis observed similar results that EX with low–moderate intensity increased brachial artery FMD greater than EX with vigorous intensity in patients with type 2 diabetes [49]. These results suggest several notable points. First, increased endothelial NO bioavailability might not solely rely on an increase in shear stress. There are other factors to determine endothelial function such as vascular endothelial growth factor, intracellular calcium concentration, and hormones [50], which might lead to different responses to EX. Second, a stimulus augmenting NO bioavailability could be fully achieved through EX at moderate intensity. Lastly, vigorous exercise can elevate both endothelial inflammation and oxidative stress [43], which eventually reduce NO bioavailability [7, 8, 51]. Thus, EX with moderate intensity might have a greater increase in FMD in this analysis. However, a previous meta-analysis [52] on the population primarily consisting of CVD and healthy conditions determined that EX with higher intensity induced a greater increase in brachial artery FMD compared to EX with lower intensity. Such contradictory results might be due to different populations/pathologies and analytic methods. Future research is warranted to investigate the role of exercise intensity in the improvement of endothelial function.

Recommendation for Exercise Prescription in Patients with PAD

According to subgroup analyses, a significant difference in effect on FMD was not observed between subgroups training

for ≤ 12 weeks and > 12 weeks of EX, < 50 min, and ≥ 50 min of session duration, and < 150 min and ≥ 150 min of weekly volume, respectively. CRP, IL-6 and TNF- α were not influenced by any training variables. These results indicate that EX with a relatively lower weekly training volume for a short duration can be a sufficient exercise regimen to improve FMD in patients with PAD. Age and baseline BMI were excluded from subgroup analyses as most participants had similar levels of age (65–70 years) and BMI (25–30 kg/m²). Aging is a key factor for developing vascular endothelial dysfunction by different mechanisms including exaggerated endothelial oxidative stress and apoptosis which decrease NO bioavailability [51]. Every 10 kg decrease in body weight is related to a 1.1% increase in FMD [53]. Thus, different training strategies could be required for obese or younger patients.

Limitations and Strengths

There are some limitations in this study. Significant publication bias was found in brachial artery FMD ($P = 0.04$). A few studies (e.g., measured FMD of a popliteal artery) were excluded to enhance homogeneity between studies, and only studies published in English were retrieved, potentially increasing the risk of bias. However, approximately 1300 patients in 20 trials were included in this meta-analysis for FMD. Sensitivity analysis also indicated that any one cohort did not predominantly influence our results in all four outcomes. Of 18 included studies, 8 studies did not have CON [14, 15, 17, 27, 29–32], although differences in ESs are insignificant between randomized and nonrandomized trials [54].

This study first investigated the key prognostic variables for the risk of atherosclerosis and CVD events, brachial artery FMD and inflammatory biomarkers following EX in patients with PAD. This analysis observed that brachial artery endothelial function was improved by EX with moderate intensity and a relatively lower weekly training volume for a short-term without the changes in inflammatory biomarkers. Therefore, this study not only reveals the association between EX-induced improvement in vascular dysfunction and inflammation but also contributes to developing a specified EX strategy for patients with PAD.

Conclusions

This meta-analysis determined that EX significantly increased brachial artery FMD but had no effect on CRP, IL-6, and TNF- α levels in patients with PAD. These results suggest that EX-induced improvement in vascular endothelial function can be independent of the improvement of systemic inflammation. EX with moderate intensity elicited a greater effect on FMD compared to EX with vigorous intensity, while no significant difference in effect on

FMD was found between subgroups training for ≤ 12 weeks and > 12 weeks of EX, < 50 min and ≥ 50 min of session duration, and < 150 min and ≥ 150 min of weekly volume, respectively. These results indicate that vascular dysfunction in PAD patients can be improved by EX with moderate intensity and a relatively lower weekly training volume for a short-term. Future studies are warranted to investigate the association between vascular function and inflammatory cytokines following EX.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s12265-023-10451-0>.

Author Contribution JL, EL, MM, and YP conducted a study design. JL, AZ, and YP conducted literature review and data extraction. JL and YP performed statistical analysis. JL, EL, MM, and YP drafted the manuscript.

Data Availability The data used and analyzed in the current study are available upon reasonable request.

Declarations

Ethical Approval No human studies were carried out by the authors for this article.

Conflict of Interest The authors declare no competing interests.

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