Exercise Training and Vascular Function in Post-menopausal Individuals: A Systematic Review and Meta-analysis

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Running Title: Menopause, Exercise and Endothelial Function

Key Points

Question: What influence does aerobic exercise have on vascular function, assessed by flow-mediated dilation, for post-menopausal individuals?

Findings: Nine studies (N = 182), evaluating the effect of exercise on vascular function were included. Overall, exercise improved vascular function and this improvement was greater in controlled interventions compared to pre-post interventions. The improvement in vascular function was predicted by resting blood pressure and the increase in cardiorespiratory fitness.

Meaning: Based on these findings, postmenopausal individuals can improve their vascular function. Exercise can be of benefit to those with a higher resting blood pressure, and a low cardiorespiratory fitness.

Abstract

Importance: Cardiovascular disease (CVD) is a leading cause of morbidity and mortality for menopausal individuals. Flow mediated dilation (FMD); a surrogate marker of CVD, improves with aerobic exercise training in healthy and diseased cohorts. However, systematic evaluation and precise estimate of this effect for menopausal individuals is unknown.

Objective: We conducted a systematic review with meta-analysis to evaluate the influence of exercise training on FMD in post-menopausal individuals.

Evidence Review: Studies were identified from systematic search of major electronic databases (PubMed, ScienceDirect and Cochrane Library) from inception to February 2021. Healthy, post-menopausal individuals were included, following an aerobic exercise intervention assessing FMD. A random-effects meta-analysis was used to calculate a pooled effect size (mean difference (MD)) with 95% confidence interval (CI). Heterogeneity was assessed using I^2 statistics. Meta-regression was used to assess the association between changes in FMD and physical characteristics (e.g., blood pressure, age, baseline FMD) and intervention details (metabolic equivalents and change in maximal oxygen uptake [$\Delta \dot{V}O_{2max}$]). For variables that significantly correlated, a multiple meta-regression model was used to assess the accounted variance in between-study ΔFMD%. Study quality was assessed using the National Heart, Lung and Blood Institute assessment tool.

Findings: Nine studies, including 11 interventions [6 controlled interventions and 5 pre-post interventions; N = 182], with age ranges of 52 ± 4 to 64 ± 7 years underwent quantitative pooling of data. Exercise training significantly improved ΔFMD% (MD: 0.99, 95% CI: 0.46 – 1.52, $P < 0.001$). Between-study heterogeneity was large and statistically significant (I^2 = 93.8%, $P < 0.001$). Posthoc analysis based on study design identified significant heterogeneity in the MD in ΔFMD% between controlled and pre-post study interventions ($P < 0.05$). According to multiple meta-regression, diastolic and systolic blood pressure, and $\Delta \dot{V}O_{2max}$ significantly predicted ΔFMD% (Q = 15.74, df = 3, $P < 0.01$, $R^2 = 0.72$).

Conclusions & Relevance: Aerobic exercise training improves FMD for post-menopausal individuals and this observation was greater among controlled versus pre-post interventions. A higher resting blood pressure and the greatest $\Delta \dot{V}O_{2max}$, yielded the largest improvements in FMD.

Key words: endothelial function, post-menopausal, exercise, flow-mediated dilation

Introduction

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality for women [1]. Menopause is an established CVD risk factor due, at least in part, to the decline in the cardioprotective hormone, oestrogen [2]. This multifunctional hormone plays a critical role in mediating cardiovascular health by stimulating vasodilation, modulating inflammatory processes, regulating oxidative stress and maintaining endothelial function [3].

Endothelial function is a surrogate marker of CVD and can be measured non-invasively using the flow mediated dilation (FMD) test at the brachial artery [4]. This is a clinically meaningful outcome since CVD risk can be reduced by 8-13%, per percent point increase in brachial FMD [5–8].

It has been well established that ageing is accompanied by impaired endothelial function as measured by FMD [9–11]. This age-related decline in FMD becomes exaggerated for individuals around the timing of menopause onset, and therefore elevates CVD risk [12,13]. Pharmacological treatments including hormone therapy (HT) have shown promise in attenuating and improving FMD in post-menopausal individuals via reductions in oxidative stress [14,15]. However, the use of HT has also shown to be associated with an increased risk of cancer and CVD [16–19]. Equally, there is evidence to show no association with HT use and cancer risk [20,21]. Unsurprisingly, there remains great controversy regarding this approach for vascular benefits and non-pharmacological interventions are likely preferrable to avoid such risks.

Aerobic exercise improves FMD across a range of healthy and diseased populations, with higher aerobic and resistance exercise training volumes and intensities yielding the greatest benefits [22,23]. A single meta-analysis that included four interventions comprising of post-menopausal individuals with existing cardiovascular and metabolic disease [22] demonstrated exercise-induced improvements in FMD. Aside from the small number of interventions, the translation of this finding to healthy menopausal women is challenging. This is because the responsiveness of the endothelium to exercise training may be dampened by the menopause related loss of oestrogen that facilitates the release of nitric oxide and therefore, vasodilation [24,25]. To date, the impact of exercise on FMD in healthy post-menopausal individuals has not been evaluated with meta-analysis. Therefore, the aim of this study was to perform a systematic review with meta-analysis to investigate the influence of aerobic exercise training on FMD in post-menopausal individuals with appraisal of study quality in this field. We hypothesized that

FMD in post-menopausal individuals would significantly improve following aerobic exercise training.

Methods

This study was conducted in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA 2020) guidelines and the checklist was completed [26].

Protocol and Registration

The systematic review examining the impact of aerobic exercise training on FMD in post-menopausal individuals was registered with PROSPERO, the international prospective register of systematic reviews (Registration no. CRD42021269150).

Eligibility Criteria

This study was guided by the participants, interventions, comparisons, outcomes and study design framework [27].

Participants

The population of interest included individuals described or defined by study authors as post-menopausal individuals, reporting as healthy without known cardiovascular, metabolic (including diabetes), respiratory diseases (or studies that used exclusion criteria pertaining to associated diseases) and were not on HT).

Intervention (Exposure)

Studies that employed an aerobic exercise intervention of any frequency, duration, and intensity. For multi-modality interventions (for example, diet plus exercise), the placebo group/no diet group were extracted as the control group.

Comparison

Eligible comparators included no exercise.

Outcome

Studies were included if they determined vascular function using the FMD test, acquired by ultrasound imaging at the brachial artery. Studies were required to use a forearm occlusion with

no limitation imposed on the method of analysis post cuff deflation. Studies may have used a fixed time point or continuous assessment of artery diameter over a given time period [28].

Study Design

Studies were included if they were either a controlled intervention (randomized trial (RT), randomized controlled trial (RCT), quasi-experimental trials) or, pre – post interventions without a non-exercise control group.

Information Sources

Two authors (ÁB and AB) designed the electronic database searches, which were performed on PubMed (title and abstract), ScienceDirect (title, abstract and keywords) and the Cochrane Library (title, abstract and keywords; see online Supplementary Digital Content 1 for complete search strategies). The authors identified peer-reviewed journal articles published in English-language from inception to 11th February 2021. Reference lists of included articles and relevant review articles were screened for any further articles that were not identified from the systematic search.

Study Selection and Data Extraction

Records were imported into Mendeley for de-duplication and subsequently reviewed for inclusion. The title and abstracts of all retrieved articles were independently screened by ÁB and AB. Abstracts that met the initial screening criteria by at least one reviewer were automatically retrieved as full-text articles. Full-text articles were then independently screened by two reviewers against study inclusion prior to extraction. For studies where at least one reviewer recommended exclusion, further review was conducted by NS for final decision on exclusion.

Two authors (ÁB and AB) extracted the data in Microsoft Excel. Population characteristics (e.g., age, description of post-menopausal status, systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI) and fitness, measured and reported as either peak ($\dot{V}O_{2peak}$) or maximal ($\dot{V}O_{max}$) oxygen uptake before and after the intervention), exposure (e.g., exercise intervention duration, frequency, intensity, and type) and outcomes (e.g., relative FMD and baseline diameter). For studies that reported multiple time points throughout the intervention period, only post-intervention values were used. For studies that investigated the influence of exercise on FMD with a co-intervention (e.g., hormone treatment) without a non-

exercise placebo condition, the data from the placebo group was extracted and treated as a pre-post study design.

Data were extracted as mean ± standard deviation (SD). In the interest of consistency for the reader, where studies reported the standard error of the mean (SEM), a manual conversion was applied using the formula: SD = SEM × $\sqrt{N}$, where N is the number of participants [29]. Data were extracted from the text and within tables and figures. For the latter, study authors were contacted by email to ascertain the mean ± SD, however, if the authors did not respond, the data were manually extracted using the calibrated measuring function within 'ImageJ' (Image Processing and Analysis in Java, Maryland, USA) [30]. When absolute $\dot{V}O_{2max}$ ($L.min^{-1}$) was reported, relative $\dot{V}O_{2max}$ ($mL.kg.min^{-1}$) was calculated as: ($L.min^{-1}$) * 1000 / body mass (kg).

Study Quality Assessment

We used the National Heart, Lung and Blood Institute assessment tools (NHLBI, Bethesta, MD) checklists for each study design to determine the extent to which a study has addressed the possibility of bias in its design, conduct and analysis. Specifically, all studies were screened for potential sources of bias including sampling, flawed application and measurement of exposure, flawed measurement of outcomes, selective/incomplete outcomes, unidentified confounding factors and inappropriate statistical analysis. The difference in ratings were resolved through discussion. Accordingly, each individual study was classified as 'poor', 'fair' or 'good' in accordance with NHLBI guidelines. Two authors (ÁB and AB) independently conducted the quality assessment for all studies before conferring the allocated classification along with consultation with another author for arbitration (NS) (Table 1).

Statistical Analysis

All statistical analyses were conducted using Comprehensive Meta-Analysis (CMA; Biostat, V3, Englewood, NJ, USA). Since the metric of endothelial function was the same across studies (i.e., FMD%), pooled random effects difference in mean ΔFMD% were calculated to determine the influence of aerobic exercise interventions on vascular function. For controlled interventions studies, ΔFMD% post values for intervention and control groups were used. For within participant (pre-post) designs, ΔFMD% was recorded before and after the intervention.

Between-study heterogeneity was calculated as Tau^2, Cochrane's *Q* and I^2 statistic, and classed as either low, moderate, or high at 25%, 50%, and 75%, respectively [31]. Categorical moderator analysis was used to compare within (pre-post) and between (controlled intervention) participant designs by using separate within subgroup Tau^2. Mixed effects (method of moments) meta-regression were used to determine relationships between ΔFMD% and covariates: age, SBP, DBP, BMI, $\Delta\dot{V}O_{2max}$, intervention duration and total metabolic equivalents, when 10 or more comparisons were available [31]. Metabolic equivalents of the task (METs) were calculated by converting the prescribed exercise intensity (%HRmax and %HRR), to age-relative METs according to Garber *et al*., (2011) [32]. This was performed to yield daily and weekly METs as well as the total METs for each intervention. The latter (total METs) was incalculable for one the treadmill intervention group from Jo *et al*., (2019) [33] as exercise frequency was not clearly stated. Where exercise was progressed in terms of intensity, frequency and/or duration, METs were calculated for each prescribed training *block* and then totaled (i.e. 2 weeks at 30% HR plus 22 weeks at 60% HRR [11]), to be used in the meta-regression. Although not mandatory for study inclusion, $\Delta\dot{V}O_{2max}$ was determined for the intervention group only and reflected the effectiveness of the aerobic exercise training. Due to their interrelationship, SBP and DBP were linked to form 'blood pressure' (BP) and then used in the meta-regression using proprietary software embedded within CMA. Covariates including age, SBP, DBP and BMI were pooled at baseline for control and exercise groups in the controlled intervention studies and entered into the meta-regression. This was performed since the outcome variable (i.e., the difference in mean ΔFMD%) was calculated between the intervention groups and control groups. For variables that were significantly associated with ΔFMD%, these were collectively entered into a multiple meta-regression model to determine the accounted variance using analogue R^2. Publication bias was assessed using two-tailed Egger's regression [34] and statistical significance was granted at $p \leq 0.05$.

Results

Search Outcome and study designs

See Figure 1 for the PRISMA flow diagram of studies pertaining to the identification, screening, eligibility and inclusion. We identified 9 relevant studies [11,14,33,35–40] that included, 1 RT [39], 4 RCTs [14,33,36,38], 3 quasi-experiments [35,37,40] and 1 pre-post design [11]. There were 2

studies that had more than 1 exercise intervention [33,39] which enabled for 11 separate interventions (e.g. high intensity interval training (HIIT) and continuous training reported as separate interventions [39]). One RCT presented data investigating the influence of exercise on FMD with placebo hormone treatment without a non-exercise placebo condition [14]. Similarly, another study was described as an RCT but directly allocated some participants into the intervention groups and thus, was considered a pre-post experimental design [40]. Another study consisted of 2 intervention arms with randomized allocation, yet without a non-exercise control group [39]. Together, these 3 study designs were considered controlled interventions but presented data that reflect pre-post analysis. Taken together, 6 comparisons were derived from 5 studies [33,35–38], and are collectively described herein as controlled interventions, comprising of between participant designs. In contrast, 5 comparisons were taken from 4 separate studies [11,14,39,40], and are collectively described as pre-post interventions, comprising of within participant designs.

*****INSERT FIGURE 1 NEAR HERE*****

Study characteristics, protocols, and quality

Information pertaining to the study participant characteristics and FMD outcomes as determined from statistical analyses are detailed in Table 1 and 2. Similarly, descriptions of FMD protocol, exercise interventions and study quality are presented in Table 2.

***** INSERT TABLES 1 AND 2 NEAR HERE *****

Participants

Sample sizes ranged from 6 to 26 (N = 182) with an age range of 52 ± 4 to 64 ± 7 years and a mean age of 59 years. Participants were described as post-menopausal having had amenohorrea for at least 1 year [14,36,37,39,40], at least 2 years [35], or in some instances, no indication as to how

menopausal status was confirmed [11,33,38]. Three studies provided information regarding the duration of time since menopause onset [14,37,40] (Table 1).

Intervention protocol

The exercise interventions ranged from 2 weeks[39] to 24 weeks[11], with a mean duration of 11 weeks. The mode of intervention included conventional aerobic exercise (walking, running, cycling, rowing) in 10 interventions [11,14,33,35–37,39,40]. Non-traditional forms of exercise training included exergaming in 1 intervention [33]. Exercise intensity varied across all interventions and including self-paced [33], %maximum heart rate (HR_{max}) [14,35,38,40], % heart rate reserve (HRR) [11,33,36,37] and %peak power output [39]. Within these, the majority of interventions were of a moderate intensity, while some interventions progressively increased in intensity throughout the study duration [11,35–38]. All interventions were designed for participants to progress towards a pre-defined time. All studies reported $\dot{V}O_{2max}$, with a $\Delta\dot{V}O_{2max}$ ranging from 0.5 to 11.2 mL.kg.min^{-1}, with an average $\Delta\dot{V}O_{2max}$ of 4.6 ± 3.7 mL.kg.min^{-1}.

Flow Mediated Dilation Protocol

All studies reported a 5-minute cuff inflation period of the forearm in the assessment of brachial artery FMD, with inflation pressures described as 180-200 mmHg [33], 50 mmHg above SBP [35,36,38,39], >200 mmHg [11,37] and 250 mmHg [14,40]. Images were recorded after cuff deflation for 40-60 seconds [33], 2 minutes [14,38,40] and 3-minutes [11,36,37,39]. Eight studies [11,14,33,35–37,39,40] referred to published guidelines or cited methodological justifications for image acquisition and/or analysis of the FMD protocol (see Table 2).

Study Quality Assessment

Of the controlled intervention study designs, 1 study was rated 'good'[36], 4 were considered 'fair' [14,35,37,38], while 3 were determined to be 'poor' [33,39,40]. In general, the studies that scored as 'poor quality', was largely due to >15 percentage points differential drop out between

intervention arms [41]. Study quality for the only pre-post study design was considered to be 'good' [11]. See Supplementary Digital Content 2 for full evaluation of study quality.

Flow Mediated Dilation

Figure 2 illustrates the overall effect of aerobic exercise on ΔFMD% including all 11 interventions. The meta-analysis identified that there was a significant improvement in the ΔFMD% following exercise training when combining all study designs (N = 9 studies, N = 182 women; MD: 0.99, 95% CI: 0.46 – 1.52, $P < 0.001$). However, separate to the meta-analysis, the overall between-study heterogeneity was large and statistically significant (I^2 = 93.8%, $P < 0.001$). Egger's regression identified no significant publication bias (intercept: -0.72, 95% CI: -5.79 – 4.34, $P > 0.05$).

Posthoc analysis that compared controlled interventions and pre-post interventions, identified significant heterogeneity in the mean difference in ΔFMD% ($P < 0.05$). Both controlled interventions (MD: 3.62, 95% CI: 1.55 – 5.69, $P < 0.01$), and pre-post interventions (MD: 0.81, 95% CI: 0.26 – 1.36, $P < 0.01$) demonstrated that ΔFMD% significantly increased after exercise training. Nonetheless, between-study heterogeneity was significant and large for controlled interventions (I^2 = 92.02%, $P < 0.001$), yet small and non-significant for pre-post interventions (I^2 = 0.00%, $P > 0.05$).

*****INSERT FIGURE 2 NEAR HERE*****

Meta-Regression (s)

Univariate meta-regression

The ΔFMD% was significantly associated with SBP (β = 0.21, 95% CI: 0.08 – 0.34, $P < 0.01$), DBP (β = 0.33, 95% CI: 0.16 – 0.49, $P < 0.001$) and $\Delta\dot{V}O_{2max}$ (β = 0.52, 95% CI: 0.15 – 0.89, $P < 0.01$). This would suggest that for every 1 mmHg increase in baseline SBP and DBP, there is a subsequent increase in FMD% of 0.21 and 0.33, respectively. Similarly, with every 1 $mL \cdot kg \cdot min^{-1}$ increase in $\dot{V}O_{2max}$ there is an associated increase of 0.52 FMD%. Since DBP and

SBP together represent blood pressure, these covariates were linked and found to be significantly associated with ΔFMD% ($P < 0.001$).

Daily or weekly METs were not significantly associated with ΔFMD% (β = -7.5, 95% CI -21.14 – 6.05, $P > 0.05$; β = 2.13, 95% CI -0.34 – 4.59, $P > 0.05$ respectively). Total intervention METs were also not significantly associated with ΔFMD% (β = 0.14, 95% CI: -3.72 – 3.99, $P > 0.05$). All other covariates including baseline FMD (β = 0.30, 95% CI: -0.85 – 1.45, $P > 0.05$), age (β = -0.10, 95% CI: -0.61 – 0.42, $P > 0.05$) and intervention duration (β = 0.17, 95% CI: -0.17 – 0.50, $P > 0.05$) were not significantly associated with ΔFMD%.

Multiple meta-regression

Covariates significantly associated with ΔFMD% were entered into a multiple meta-regression model to determine the account variance in ΔFMD%. The model including DBP, SBP and $\Delta\dot{V}O_{2max}$ significantly predicted ΔFMD% (Q = 15.74, df = 3, $P < 0.01$, $R^2 = 0.72$).

Discussion

This is the first meta-analysis performed to understand the influence of exercise on FMD in healthy post-menopausal women that also included a study quality appraisal. This study contributes novel insight to existing literature that aerobic exercise appears to improve endothelial function, quantified using FMD, by an average of 2.6% in healthy, non-medicated post-menopausal individuals. According to previous meta-analysis, this may reduce CVD risk by 21 – 47%, since a 1% increase in FMD is associated with an 8 – 13% reduction in CVD risk [5–8]. This finding may therefore be of clinical significance although ought to be interpreted with caution since FMD is merely a surrogate marker of CVD . The observed improvements in FMD were greater in controlled interventions compared with pre-post interventions. Lastly, a higher resting blood pressure, albeit within the normotensive range, and a greater change in cardiorespiratory fitness ($\dot{V}O_{2max}$) are positively associated with the ΔFMD. Exercise may therefore be a feasible, non-pharmacological approach to improving vascular function in healthy post-menopausal individuals and thus, may indirectly reduce CVD risk.

Influence of aerobic exercise on FMD

The findings from this meta-analysis agree with those from previous meta-analysis also reporting exercise-induced improvements in FMD [22,23]. Prior to our meta-analysis, it has been challenging to generalize those findings to healthy menopausal individuals since oestrogen decline can potentially impede the capacity of the endothelium to respond to exercise training [24,42]. Given the heterogeneity between studies included in this meta-analysis, the time since menopause onset may be an important factor to consider. However, only three studies have reported this metric and it is therefore challenging to understand the influence of exercise timing post-menopause on endothelial function [14,37,40]. It is however plausible since post-menopausal individuals improve endothelial function to a lesser extent when compared with age-matched men, in response to light-intensity exercise training [14,40]. To further support this concept, Moreau and colleagues (2013) showed that FMD improves for individuals receiving HT alongside exercise training, compared to an exercise only training group. Together, this suggests that exercise related vascular improvements may be oestrogen-mediated, although requires further study[14]. That said, our analysis shows that exercise can increase FMD without HT, since we only included studies with non-HT treated individuals. Given that the long-term use of HT is associated with increased risk of cancer and CVD, exercise could be an alternative non-pharmacological therapy to improve or at least attenuate the menopause induced decrements in vascular health [16–19,43,44]. The majority of the individuals included in our analysis were <65 years of age; the combination of aging with chronic oestrogen decline may compound vascular responsiveness to exercise, however, this warrants further investigation [24]. Nonetheless, the present meta-analysis advances our knowledge to suggest that exercise is effective for improvements in endothelial function in a cohort of apparently healthy, non-medicated post-menopausal individuals.

Influence of the exercise prescription, aerobic fitness and blood pressure on FMD

Exercise prescription varied extensively and may explain the large heterogeneity observed between studies. According to Early *et a*l., (2017) [23] exercise intensity may be an important factor for vascular outcomes, since they observed that higher weekly volumes (<150 min/week vs. ≥150 min/week), and intensities (*'moderate'* and *'vigorous-near maximal'* vs. *'very light-light'*), were associated with superior improvements in FMD. Additionally, a 10% increase in

relative intensity ($\dot{V}O_{2peak}$) is reportedly associated with a 1% unit improvement in FMD [22], implying that exercise intensity could be a mediating factor.

To understand the influence of the exercise prescription on FMD, we quantified the daily, weekly, and total METs for each intervention [45]. According to meta-regression, neither daily, weekly, nor total METs were related to the FMD response. Ashor *et al.,* (2015)[22] as demonstrated no association between aerobic exercise frequency or duration on FMD. This aligns with our finding whereby total METs; that accounts for exercise duration and frequency, is unrelated to FMD improvements. The same authors did show an association between exercise intensity and FMD, contrarty to our observation that may be explained by the majority of studies in our analysis being of low-to-moderate intensity. That said, 8-weeks of high intensity aerobic exercise did not yield any improvements in popliteal FMD [46]. According to the authors, intensity is not a determinant of FMD response in menopausal individuals. Instead, FMD response to exercise may be more closely dependent on intraindividual factors including oestrogen receptors and endothelial nitric oxide synthase expression, and from our analysis, the potential at improving aerobic capacity.

To allude further, we extracted aerobic capacity as a surrogate for the exercise intervention. Through meta-regression analysis, our observations corroborate those from Ashor *et al.,* (2015) [22], whereby the greatest increases in aerobic capacity were associated with subsequent increased FMD. For example, the largest increase in $\dot{V}O_{2peak}$ (11.0 – 11.2 mL.kg.min^{-1}) was accompanied by the largest increase in FMD (7.5 – 10.5 percent points) [33]. In contrast, studies with a minimal change in $\dot{V}O_{2peak}$ (<1 mL.kg.min^{-1}) reported negligible changes in FMD (-1.2 – 0.8 percent points) [14,47]. This highlights the interrelationship between FMD and aerobic capacity and implies increased relative aerobic capacity may be necessary to improve FMD, at least at the brachial artery. While these data encouragingly suggests that improved cardiorespiratory fitness may have an indirect and positive impact on endothelial function, our findings can only be generalized to upper limbs. Hoier *et al.,* (2021) [46] reported an 18% increase in oxygen uptake following 8-weeks of high intensity aerobic exercise training although demonstrated no improvements in popliteal FMD. Since this could imply limb specific endothelial adaptation to improved cardiorespiratory fitness, this data warrants to be interpreted with caution.

Systolic and diastolic blood pressure is commonly reported to be higher in postmenopausal compared to premenopausal individuals [48,49]. In this analysis, studies with participants that had

a higher resting blood pressure, albeit still normotensive, were associated with the greatest improvements in FMD. Previous studies have shown a direct relationship between blood pressure and cardiovascular mortality [50]. Accordingly, low levels of physical activity and fitness is associated with a 30% to 50% greater risk for high blood pressure, respectively [51]. Exercise improves blood pressure and FMD in adults, alongside concurrent increases in high density lipoprotein (HDL) cholesterol and hormonal alterations, namely reduced norepinephrine [52]. These parallel improvements may be related to improved sympathetic activity control, since brachial FMD and norepinephrine are inversely related and dependent on the production of dilatory molecules, such as nitric oxide [53,54]. Equally, increased FMD may arise via improved lipid profiles, since HDL protects blood vessels from atherogenesis, by preventing the generation of oxidatively modified low-density lipoprotein cholesterol [55–57]. While the precise mechanisms underpinning exercise related enhanced endothelial function are to be fully established, these data suggest that post-menopausal individuals with a higher baseline blood pressure, albeit still normotensive, benefit the most from aerobic exercise.

Our analysis reported greater FMD improvements in controlled interventions compared with pre-post interventions, prompting a detailed evaluation of the study quality appraisal. Despite the larger effect of controlled interventions on FMD compared with pre-post interventions, both encouragingly reported an improvement. However, while the controlled interventions showed a greater improvement, this was accompanied by large heterogeneity between studies. This may be explained by variations in randomization and blinding processes, inadequately powered studies, and levels of drop out that are deemed less than acceptable by the study quality assessment tool [41].

Heterogeneity of the Research

This meta-analysis adopted a strict inclusion criterion by focusing on aerobic exercise in healthy post-menopausal women, not taking medication (including HT), that had endothelial function assessed by brachial artery FMD. Heterogeneity was high for the change in percent FMD with exercise both overall, and when separated by study design (randomized controlled trials versus pre-post interventions. According to our analyses, between study heterogeneity was not explained by the exercise prescription despite contrasting intensities and durations. The heterogeneity may therefore be explained by differential physiological profiles recruited to the studies, or potentially, the inclusion of non-responders within study samples [58], although

this warrants further study. For example, studies where participants had higher pre-intervention systolic blood pressure, and the greatest improvements in aerobic capacity had the greatest improvements in FMD that may be explained by the different protocols for the conduct and analysis of FMD. This critical finding identifies the importance of interpreting the results in the context of adherence to gold standard guidelines for FMDs.

Study Limitations

Firstly, this systematic review and meta-has limitations. Firstly, whilst, it is possible that some articles may have been missed. This may pertain to the use of 3 databases only and the inclusion of articles in the English-language only. Although we conducted a thorough search using multiple databases and reference checking relevant review articles in an attempt to capture all relevant literature. Secondly, the study inclusion focused on the non-invasive assessment of vascular function derived from FMD, however, it is important to acknowledge that other research has shown exercise to improve invasive outcomes of vascular function assessed at the femoral artery [59]. Lastly, we acknowledge that while the sample size is small, it is sufficient for a meta-analysis.

Future recommendations

We propose several directions that future research may wish to consider to effectively advance current evidence in the following areas:

Exercise protocol

(1) It is clear that an optimal exercise dose remains to be established to influence FMD in post-menopausal individuals consistently across studies. Based on our interpretation of the studies performed in this area, the exercise prescription should be of sufficient duration and intensity to observe positive associations between FMD and aerobic capacity. **(2)** Since many of the studies to date have focused on light-to-moderate intensity aerobic exercise, a greater understanding regarding the value of resistance and high intensity exercise needs to be established.

FMD assessment

(3) It is paramount that the most up to date FMD guidelines be followed to ensure consistency, rigor, and comparability between studies in regard to the preparation, image acquisition and analysis to arrive at an FMD%. **(4)** Lastly, given the systemic nature of the vascular system and the heterogeneity in the development of atherosclerosis between vascular beds, upper and lower limbs warrant consideration to help develop our understanding of endothelial dysfunction manifestation in post-menopausal individuals.

Study quality

(5) While the study quality assessment tool is not subject specific, quality control of studies would undoubtedly be improved by providing clarity regarding the number of sonographers acquiring and analyzing images for an exercise training study. **(6)** Improved transparency can be achieved by reporting the sonographer skill level that can be represented by the measurement error (e.g., coefficient of variation, interclass correlation coefficient) of intra- and inter-rater reproducibility. By addressing these issues, prospective studies will improve scientific rigor in this area and perhaps lead to homogenous findings across studies.

(7) This research area would be improved by the addition of adequately powered studies since we could only evaluate 11 studies with a total of only 182 participants. **(8)** Lastly, greater transparency and detail regarding the randomization processes and consistent conforming to established study quality criteria [41].

Conclusion

This systematic review and meta-analysis have demonstrated that FMD is increased with aerobic exercise training in menopausal individuals. This improvement was greater in those involved in controlled interventions versus pre-post interventions. Those with a higher resting blood pressure and studies that observed the greatest improvement aerobic fitness, yielded the largest improvements in endothelial function. Together, exercise appears to improve FMD in healthy post-menopausal that may be dependent aerobic capacity and resting blood pressure, and not on the exercise duration, frequency, or intensity. Future research should aim to be of

high quality and conform to best practice FMD guidelines. In doing so, we can develop existing insight into the usability of FMD as a surrogate marker for CVD in postmenopausal women.

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Figure Legend

Figure 1 PRISMA flow diagram of study identification, screening, eligibility and inclusion. HT, hormone therapy; FMD, flow mediated dilation.

Figure 2 Forest plot illustrating the overall effect of aerobic exercise on ΔFMD%, represented by the difference in mean. *Closed square* study effect size; the size of the symbol and CIs represent study weight and precision, respectively, in the meta-analysis; *closed diamond* overall summary effect, diamond width represents overall summary effect precision; *Con/Pre* represent controls and pre-exercise intervention respectively; *CI* confidence interval; *1 and 2* denote multiple athlete–control comparisons from the same study.

Supplementary Digital Content

Supplementary Digital Content 1.docx

Supplementary Digital Content 2.docx

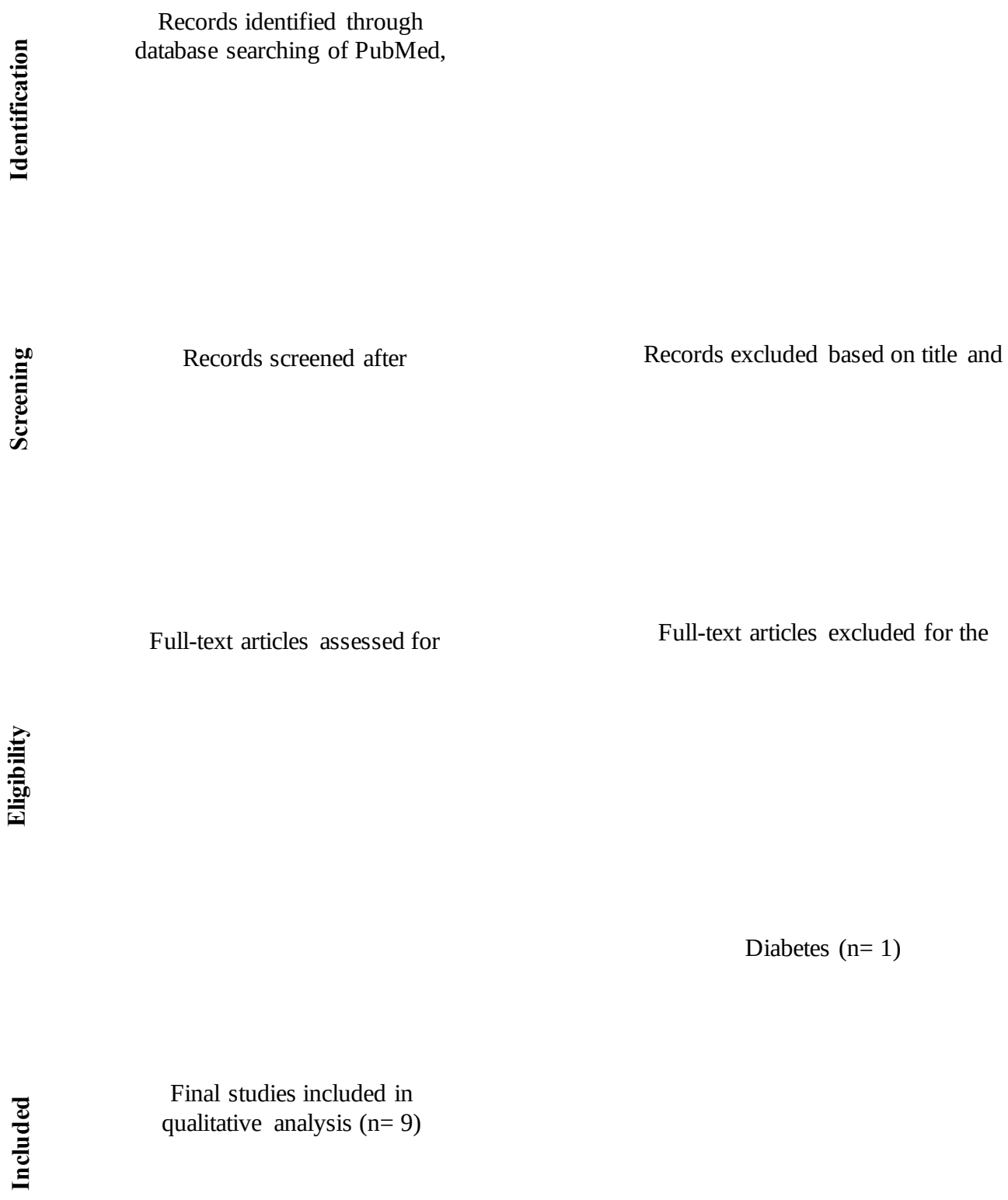

Fig 1. PRISMA flow diagram of study identification, screening, eligibility and inclusion.

Group by Study Type	Study name	Sample size		Statistics for each study				Difference in means and 95% CI
		Con/Pre	Exercise	Difference in means	Lower limit	Upper limit	p-Value	
Pre-Post	Black et al. (2009)	6	6	2.289	-0.200	4.778	> 0.05	
Pre-Post	Klonizakis et al. (2014)[1]	7	7	-1.900	-8.563	4.763	0.576	
Pre-Post	Klonizakis et al. (2014)[2]	11	11	-1.600	-6.384	3.184	0.512	
Pre-Post	Moreau et al. (2013)	10	10	0.820	0.227	1.413	0.007	
Pre-Post	Pierce et al. (2011)	15	15	0.325	-1.758	2.408	0.760	
Pre-Post	Overall effect for Pre-Post designs	49	49	0.807	0.256	1.357	0.004	
RCT	Akazawa et al. (2012)	10	11	1.700	-0.148	3.548	> 0.05	
RCT	Azadpour et al. (2017)	12	12	5.890	5.198	6.582	< 0.001	
RCT	Bailey et al. (2016)	7	14	1.900	0.448	3.352	0.010	
RCT	Jo et al. (2019)[1]	7	13	3.896	1.998	5.794	< 0.001	
RCT	Jo et al. (2019)[2]	6	21	7.204	5.378	9.030	< 0.001	
RCT	Yoshizawa et al. (2010)	10	10	1.000	-0.650	2.650	0.235	
RCT	Overall effect for Controlled interventions designs	52	81	3.620	1.550	5.691	< 0.001	
Overall	Overall effect for Pre-Post and Controlled interventions designs	101	130	0.992	0.460	1.524	0.000	

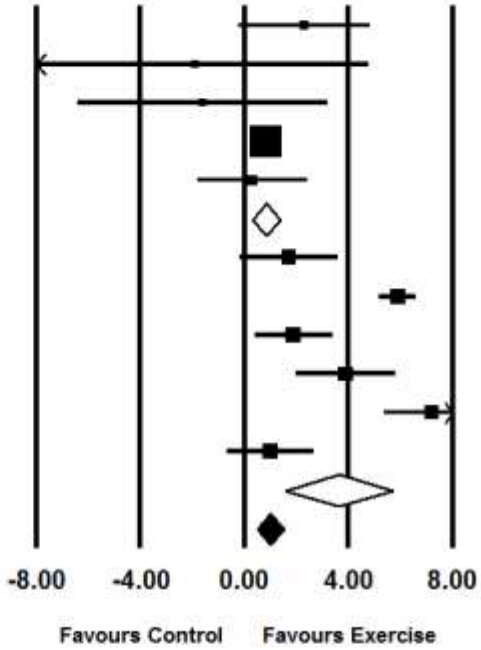

Table 1 Flow mediated dilation and exercise protocols with quality assessment.

Study overview		FMD protocol			Exercise Intervention					
Author	Design	Artery	Procedure	Methodological guidance cited	Type	Study duration	Session duration	Frequency	Intensity	Study quality
Akazawa et al. (2012) [33]	Quasi – experimental	Brachial	5 min cuff inflation at 50 mmHg above SBP Images recorded every 5 seconds for 2 min after cuff deflation	[62] cited in [63]	CON: maintain normal PA INT: partially supervised cycling and walking	8 weeks	30 min increased to 40-60 min	3-4 days/week increased to 4-5 days/week (cited Yoshizawa, 2009).	~60% HR_{max} increased to 70-75% HR_{max}	Fair
Azadpour et al. (2017) [34]	RCT	Brachial	5 min cuff inflation at 50 mmHg above SBP Images recorded for 3 min after cuff deflation	[62]	CON: maintain normal PA no exercise intervention) INT: supervised treadmill walking and jogging	10 weeks	25-40 min First 2 weeks = 25 min Weeks 3 and 4 = 30 min Weeks 5 and 6 = 35min Last 4 weeks = 40min	3 days/week	50-70% HRR First 2 weeks = 50% HRR Weeks 3 and 4 = 55% HRR Weeks 5 and 6 = 60% HRR Last 4 weeks = 70% HRR	Good
Bailey et al. (2016) [35]	Quasi – experimental	Brachial	5 min cuff inflation at >200 mmHg Images recorded for 3 min after cuff deflation	[64]; [65]; [66]	CON: no exercise intervention INT: aerobic – supervised walking, running, cycling, cross-training and rowing	16 weeks	30 min Week 12 = 45 min	3 times/week Week 12 = 4-5 times/week	30% HRR Week 12 = 60% HRR	Fair

			Analysed using custom edge-detection and wall-tracking software							
Black et al. (2009) [11]	Pre – Post	Brachial	5 min cuff inflation at >200 mmHg Images recorded for 3 min after cuff deflation Analysed using edge-detected software	[65]; [66]	INT: aerobic – partially supervised treadmill walking and cycling	24 weeks	30 min	3 sessions/week Week 7 = 5 sessions/week	30% HRR Week 13 = 60% HRR	Good
Jo et al. (2019) [31]	RCT	Brachial	5 min inflation at 180 – 200 mmHg (50 mmHg above SBP) Diameter recorded for 40 – 60 sec after cuff deflation	[62]	CON: maintain normal PA INT: supervised treadmill walking and jogging INT: supervised exergaming with running and jumping	12 weeks	Treadmill: 40 min Exergaming: 40 min	Treadmill: NR Exergaming: Per day	Treadmill: 60-80% HRR Exergaming: Self-paced. Mean HR, 120 ±19 beats.min^{-1}	Poor
Klonizakis et al. (2014) [37]	RT[a]	Brachial	5 min inflation at 50 mmHg above SBP Images recorded for 3 min after cuff deflation Analysed using Brachial analyser for research (Medial Imaging	[64] for resting procedure and [65] for cuff down procedure.	INT: supervised continuous cycling training (CT) INT: supervised high intensity cycling intervals (HIIT)	2 weeks	CT: 40 min HIIT: 10 x 1 min intervals with 1 min active recovery	CT: 3 times/week HIIT: 3 times/week	CT: 65% PPO HIIT: 100% PPO interspersed by 30% PPO	Poor

			Applications; Coralville, Iowa)							
Moreau et al. (2013) [14]	RCT[a]	Brachial	5 min cuff inflation at 250 mmHg Images recorded for 2 min after cuff deflation Analysed using software (Vascular Analysis Tools 5.5.1; Medical Imaging Applications, Iowa City, Iowa)	[62] cited in [12]	INT: unsupervised walking	12 weeks	40-45 min	5-7 days/week	65-80% HR_{max}	Fair
Pierce et al. (2011) [38]	Quasi – experimental[a]	Brachial	5 min cuff inflation at 250 mmHg Images recorded for 2 min after cuff deflation Analysed using software (Vascular Analysis Tools 5.5.1; Medical Imaging Applications, Iowa City, Iowa)	[67], [68] and [62]	INT: unsupervised walking	8 weeks	40-45 min	6-7 days/week	70-75% HR_{max}	Poor
Yoshizawa et al. (2010) [36]	RCT	Brachial	5 min cuff inflation at 50 mmHg above SBP.	NR	INT: partially supervised walking and cycling	8 weeks	25-30 min/day increased to 40-45 min/day	3-4 days/week increased to 4-5 days/week	~60% HR_{max} increased to 70-75% HR_{max}	Fair

	Images recorded for 2 min after cuff deflation.	

CON, control; CT, continuous training; FMD, flow mediated dilation; HIIT, high-intensity interval training; HR, heart rate; HR_{max}, heart rate maximum; HRR, heart rate reserve; INT, intervention; CON, control; NR, not reported; PA, physical activity; PPO, peak power output; RCT, randomised controlled trial; SBP, systolic blood pressure; VO_2, volume of oxygen uptake; $\dot{V}O_{2peak}$, peak oxygen uptake. [a] data presentation/analysis follow pre-post design.

Table 2 Study characteristics and flow mediated dilation outcome.

Study Overview		**Participant characteristics**							**Outcomes**	
Author	**Study Type**	**Post-menopausal description**	**Group/ Intervention (n)**	**Age (Years)**	**SBP (mmHg)**	**DBP (mmHg)**	**BMI ($kg.m^2$)**	**$\dot{V}O_{2max}$ ($mL.kg.min^{-1}$)**	**FMD (%)**	**Findings**
Akazawa et al. (2012) [33]	Quasi-experimental	Amenorrhea for at least 2 years	CON: maintain normal PA (n= 10)	64 ± 6	112 ± 12	69 ± 5	21.5 ± 1.0	Pre: 22.9 ± 1.4 Post: 22.5 ± 1.1	Pre: 4.0 ± 1.7 Post: 3.8 ± 1.7	
			INT: cycling and walking (n= 11)	59 ± 5	112 ± 10	71 ± 8	22.7 ± 1.0	Pre: 25.3 ± 1.2 Post: 27.3 ± 1.2	Pre: 3.9 ± 2.4 Post: 5.5 ± 2.5	↔
Azadpour et al. (2017) [34]	RCT	>1 year without menstruation	CON: maintain normal PA (n= 12)	57 ± 4	130 ± 4	82 ± 1	31.3 ± 1.4	Pre: 23.1 ± 6.2 Post: 22.6 ± 5.4	Pre: 5.8 ± 0.5 Post: 5.3 ± 0.5	
			INT: treadmill walking and jogging (n= 12)	58 ± 4	128 ± 5	82 ± 2	32.2 ± 1.8	Pre: 22.6 ± 5.7 Post: 27.8 ± 5.1	Pre: 6.0 ± 0.7 Post: 11.2 ± 1.1	↑
Bailey et al. (2016) [35]	Quasi-experimental	1 – 4 years since last menstrual cycle. >4 hot flushes over a 24-hour period	CON: no exercise intervention (n= 7)	52 ± 6	127 ± 10	77 ± 11	28.0 ± 7.2	Pre: 23.2 ± 2.4 Post: 22.6 ± 3.1	Pre: 5.6 ± 1.9 Post: 5.5 ± 1.8	
			INT: walking, running, cycling, cross-training and rowing (n= 14)	52 ± 4	128 ± 5	78 ± 8	29.0 ± 5.8	Pre: 22.5 ± 3.3 Post: 27.3 ± 4.1	Pre: 5.0 ± 1.2 Post: 7.4 ± 1.5	↑
Black et al. (2009) [11]	Pre – Post	NR	INT: treadmill walking and cycling (n= 6)	60 ± 5	124 ± 17	68 ± 10	30.0 ± 4.9	Pre: 23.0 ± 4.9 Post: 30.0 ± 2.5	Pre: 4.4 ± 1.3 Post: 6.6 ± 2.8	↔

Jo et al. (2019) [31]	RCT	NR	CON: maintain normal PA (n= 13)	63 ± 14	135 ± 18	76 ± 12	27.3 ± 4.6	Pre: 21.0 ± 0.8	Pre: 7.9 ± 0.5	
								Post: 23.3 ± 3.5	Post: 10.5 ± 2.1	
				57 ± 8	135 ± 16	80 ± 10	27.0 ± 3.0	Pre: 23.0 ± 0.8	Pre: 6.8 ± 0.4	
			INT: walking and jogging (n= 13)					Post: 34.2 ± 3.5	Post: 14.4 ± 2.0	↑
				62 ± 10	131 ± 18	77 ± 10	27.7 ± 3.0	Pre:22.4 ± 0.8	Pre: 7.0 ± 0.5	
			INT: Exergaming (n= 21)					Post:33.4 ± 3.6	Post: 17.7 ± 2.0	↑
Klonizakis et al. (2014) [37]	RT[a]	Assessed by questionnaire	INT: continuous cycling training (CT) (n= 7)	64 ± 4	114 ± 13	68 ± 7	NR	Pre: 25.0 ± 7.4	Pre: 8.9 ± 7.9	
								Post: 26.7 ± 5.4	Post: 7.0 ± 4.3	↔
			INT: high-intensity interval training (HIIT) (n= 11)	64 ± 7	127 ± 17	70 ± 4	NR	Pre: 20.4 ± 3.4	Pre: 8.1 ± 7.2	
								Post: 22.6 ± 3.1	Post: 6.5 ± 3.7	↔
Moreau et al. (2013) [14]	RCT[a]	Amenorrhea ≥ 1 year. FSH ≥ 30 IU/L. 8.8 ± 8.0 years since menopause	INT: walking (n= 10)	56 ± 7	116 ± 14	66 ± 6	24.5 ± 5.7	Pre: 23.1 [a]	Pre: 5.4 ± 0.7	
								Post: 23.9 [a]	Post: 6.2 ± 0.6	↑
Pierce et al. (2011) [38]	Quasi-experimental[a]	Post-menopausal for at least 1 year. 9.4 ± 6.6 years since menopause	INT: walking (n= 15)	63 ± 4	114 ± 15	68 ± 8	24.6 ± 2.7	Pre: 26.2 ± 4.3	Pre: 5.0 ± 3.0	
								Post: 28.0 ± 5.0	Post: 5.4 ± 2.8	↔
Yoshizawa et al. (2010) [36]	RCT	NR	CON: (n= 10)	58 ± 3	110 ± 19	66 ± 13	22.2 ± 2.5	Pre: 28.3 ± 4.7	Pre: 4.9 ± 1.2	
								Post: 26.9 ± 5.1	Post: 4.7 ± 1.5	
			INT: walking and cycling (n= 10)	57 ± 3	116 ± 13	68 ± 6	23.7 ± 2.9	Pre: 27.7 ± 4.1	Pre: 4.6 ± 1.5	
								Post: 30.2 ± 5.7	Post: 5.7 ± 2.2	↔

Data are presented as mean ± standard deviation (SD) unless stated otherwise. BMI, body mass index; DBP, diastolic blood pressure; FMD, flow mediated dilation; SBP, systolic blood pressure; $\dot{V}O_{2max}$, maximal oxygen uptake; FSH, follicle-stimulating hormone; NR, not reported; RT, randomised trial; RCT, randomised controlled trial; CON, control; INT, intervention; PA, physical activity. [a] data presentation/analysis follow pre-post design.