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# **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  $\Delta FMD\%$ . 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  $52 \pm 4$  to  $64 \pm 7$  years underwent quantitative pooling of data. Exercise training significantly improved  $\Delta FMD\%$  (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  $\Delta FMD\%$  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  $\Delta FMD\%$  ( $Q = 15.74$ ,  $df = 3$ ,  $P < 0.01$ ,  $R^2 = 0.72$ ).

**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

## 37 **Introduction**

38

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

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

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

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

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

## 71 **Methods**

72

73 This study was conducted in accordance with the Preferred Reporting Items for Systematic  
74 Review and Meta-Analysis (PRISMA 2020) guidelines and the checklist was completed <sup>26</sup>.

## 75 **Protocol and Registration**

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

## 79 **Eligibility Criteria**

80 This study was guided by the participants, interventions, comparisons, outcomes and study  
81 design framework <sup>27</sup>.

## 82 **Participants**

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

## 87 **Intervention (Exposure)**

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

## 91 **Comparison**

92 Eligible comparators included no exercise.

## 93 **Outcome**

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

96 no limitation imposed on the method of analysis post cuff deflation. Studies may have used a  
97 fixed time point or continuous assessment of artery diameter over a given time period<sup>28</sup>.

## 98 **Study Design**

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

## 102 **Information Sources**

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

## 110 **Study Selection and Data Extraction**

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

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

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

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

136

### 137 **Study Quality Assessment**

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

### 148 **Statistical Analysis**

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



155 Between-study heterogeneity was calculated as Tau<sup>2</sup>, Cochrane's *Q* and *I*<sup>2</sup> statistic, and classed  
156 as either low, moderate, or high at 25%, 50%, and 75%, respectively<sup>31</sup>. Categorical moderator  
157 analysis was used to compare within (pre-post) and between (controlled intervention)  
158 participant designs by using separate within subgroup Tau<sup>2</sup>. Mixed effects (method of  
159 moments) meta-regression were used to determine relationships between ΔFMD% and  
160 covariates: age, SBP, DBP, BMI, Δ $\dot{V}O_{2max}$ , intervention duration and total metabolic  
161 equivalents, when 10 or more comparisons were available<sup>31</sup>. Metabolic equivalents of the task  
162 (METs) were calculated by converting the prescribed exercise intensity (%HRmax and  
163 %HRR), to age-relative METs according to Garber *et al.*, (2011)<sup>32</sup>. This was performed to  
164 yield daily and weekly METs as well as the total METs for each intervention. The latter (total  
165 METs) was incalculable for one the treadmill intervention group from Jo *et al.*, (2019)<sup>33</sup> as  
166 exercise frequency was not clearly stated. Where exercise was progressed in terms of intensity,  
167 frequency and/or duration, METs were calculated for each prescribed training *block* and then  
168 totaled (i.e. 2 weeks at 30% HR plus 22 weeks at 60% HRR<sup>11</sup>), to be used in the meta-  
169 regression. Although not mandatory for study inclusion, Δ $\dot{V}O_{2max}$  was determined for the  
170 intervention group only and reflected the effectiveness of the aerobic exercise training. Due to  
171 their interrelationship, SBP and DBP were linked to form 'blood pressure' (BP) and then used  
172 in the meta-regression using proprietary software embedded within CMA. Covariates including  
173 age, SBP, DBP and BMI were pooled at baseline for control and exercise groups in the  
174 controlled intervention studies and entered into the meta-regression. This was performed since  
175 the outcome variable (i.e., the difference in mean ΔFMD%) was calculated between the  
176 intervention groups and control groups. For variables that were significantly associated with  
177 ΔFMD%, these were collectively entered into a multiple meta-regression model to determine  
178 the accounted variance using analogue R<sup>2</sup>. Publication bias was assessed using two-tailed  
179 Egger's regression<sup>34</sup> and statistical significance was granted at  $p \leq 0.05$ .

## 180 **Results**

### 181 **Search Outcome and study designs**

182 See Figure 1 for the PRISMA flow diagram of studies pertaining to the identification,  
183 screening, eligibility and inclusion. We identified 9 relevant studies<sup>11,14,33,35-40</sup> that included,  
184 1 RT<sup>39</sup>, 4 RCTs<sup>14,33,36,38</sup>, 3 quasi-experiments<sup>35,37,40</sup> and 1 pre-post design<sup>11</sup>. There were 2

185 studies that had more than 1 exercise intervention <sup>33,39</sup> which enabled for 11 separate  
186 interventions (e.g. high intensity interval training (HIIT) and continuous training reported as  
187 separate interventions <sup>39</sup>). One RCT presented data investigating the influence of exercise on  
188 FMD with placebo hormone treatment without a non-exercise placebo condition <sup>14</sup>. Similarly,  
189 another study was described as an RCT but directly allocated some participants into the  
190 intervention groups and thus, was considered a pre-post experimental design <sup>40</sup>. Another study  
191 consisted of 2 intervention arms with randomized allocation, yet without a non-exercise control  
192 group <sup>39</sup>. Together, these 3 study designs were considered controlled interventions but  
193 presented data that reflect pre-post analysis. Taken together, 6 comparisons were derived from  
194 5 studies <sup>33,35-38</sup>, and are collectively described herein as controlled interventions, comprising  
195 of between participant designs. In contrast, 5 comparisons were taken from 4 separate studies  
196 <sup>11,14,39,40</sup>, and are collectively described as pre-post interventions, comprising of within  
197 participant designs.

198

199 **\*\*\*INSERT FIGURE 1 NEAR HERE\*\*\***

200

## 201 **Study characteristics, protocols, and quality**

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

205

206

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

208

## 209 **Participants**

210

211 Sample sizes ranged from 6 to 26 (N = 182) with an age range of  $52 \pm 4$  to  $64 \pm 7$  years and a  
212 mean age of 59 years. Participants were described as post-menopausal having had amenorrhoea  
213 for at least 1 year <sup>14,36,37,39,40</sup>, at least 2 years <sup>35</sup>, or in some instances, no indication as to how

214 menopausal status was confirmed <sup>11,33,38</sup>. Three studies provided information regarding the  
215 duration of time since menopause onset <sup>14,37,40</sup> (Table 1).

216

### 217 **Intervention protocol**

218

219 The exercise interventions ranged from 2 weeks<sup>39</sup> to 24 weeks<sup>11</sup>, with a mean duration of 11  
220 weeks. The mode of intervention included conventional aerobic exercise (walking, running,  
221 cycling, rowing) in 10 interventions <sup>11,14,33,35–37,39,40</sup>. Non-traditional forms of exercise training  
222 included exergaming in 1 intervention <sup>33</sup>. Exercise intensity varied across all interventions and  
223 including self-paced <sup>33</sup>, %maximum heart rate (HR<sub>max</sub>) <sup>14,35,38,40</sup>, % heart rate reserve (HRR)  
224 <sup>11,33,36,37</sup> and %peak power output <sup>39</sup>. Within these, the majority of interventions were of a  
225 moderate intensity, while some interventions progressively increased in intensity throughout  
226 the study duration <sup>11,35–38</sup>. All interventions were designed for participants to progress towards  
227 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  
228 mL.kg.min<sup>-1</sup>, with an average  $\Delta\dot{V}O_{2max}$  of  $4.6 \pm 3.7$  mL.kg.min<sup>-1</sup>.

229

### 230 **Flow Mediated Dilation Protocol**

231 All studies reported a 5-minute cuff inflation period of the forearm in the assessment of brachial  
232 artery FMD, with inflation pressures described as 180-200 mmHg <sup>33</sup>, 50 mmHg above SBP  
233 <sup>35,36,38,39</sup>, >200 mmHg <sup>11,37</sup> and 250 mmHg <sup>14,40</sup>. Images were recorded after cuff deflation for  
234 40-60 seconds <sup>33</sup>, 2 minutes <sup>14,38,40</sup> and 3-minutes <sup>11,36,37,39</sup>. Eight studies <sup>11,14,33,35–37,39,40</sup> referred  
235 to published guidelines or cited methodological justifications for image acquisition and/or  
236 analysis of the FMD protocol (see Table 2).

237

### 238 **Study Quality Assessment**

239

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

243 intervention arms <sup>41</sup>. Study quality for the only pre-post study design was considered to be  
244 ‘good’ <sup>11</sup>. See Supplementary Digital Content 2 for full evaluation of study quality.

## 245 **Flow Mediated Dilatation**

246 Figure 2 illustrates the overall effect of aerobic exercise on  $\Delta$ FMD% including all 11  
247 interventions. The meta-analysis identified that there was a significant improvement in the  
248  $\Delta$ FMD% following exercise training when combining all study designs ( $N = 9$  studies,  $N = 182$   
249 women; MD: 0.99, 95% CI: 0.46 – 1.52,  $P < 0.001$ ). However, separate to the meta-analysis,  
250 the overall between-study heterogeneity was large and statistically significant ( $I^2 = 93.8\%$ ,  $P <$   
251  $0.001$ ). Egger’s regression identified no significant publication bias (intercept: -0.72, 95% CI:  
252 -5.79 – 4.34,  $P > 0.05$ ).

253 Posthoc analysis that compared controlled interventions and pre-post interventions, identified  
254 significant heterogeneity in the mean difference in  $\Delta$ FMD% ( $P < 0.05$ ). Both controlled  
255 interventions (MD: 3.62, 95% CI: 1.55 – 5.69,  $P < 0.01$ ), and pre-post interventions (MD: 0.81,  
256 95% CI: 0.26 – 1.36,  $P < 0.01$ ) demonstrated that  $\Delta$ FMD% significantly increased after  
257 exercise training. Nonetheless, between-study heterogeneity was significant and large for  
258 controlled interventions ( $I^2 = 92.02\%$ ,  $P < 0.001$ ), yet small and non-significant for pre-post  
259 interventions ( $I^2 = 0.00\%$ ,  $P > 0.05$ ).

260

261 **\*\*\*INSERT FIGURE 2 NEAR HERE\*\*\***

## 262 **Meta-Regression (s)**

### 263 **Univariate meta-regression**

264

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

270 SBP together represent blood pressure, these covariates were linked and found to be  
271 significantly associated with  $\Delta\text{FMD}\%$  ( $P < 0.001$ ).

272 Daily or weekly METs were not significantly associated with  $\Delta\text{FMD}\%$  ( $\beta = -7.5$ , 95% CI -  
273 21.14 – 6.05,  $P > 0.05$ ;  $\beta = 2.13$ , 95% CI -0.34 – 4.59,  $P > 0.05$  respectively). Total intervention  
274 METs were also not significantly associated with  $\Delta\text{FMD}\%$  ( $\beta = 0.14$ , 95% CI: -3.72 – 3.99,  $P$   
275  $> 0.05$ ). All other covariates including baseline FMD ( $\beta = 0.30$ , 95% CI: -0.85 – 1.45,  $P >$   
276 0.05), age ( $\beta = -0.10$ , 95% CI: -0.61 – 0.42,  $P > 0.05$ ) and intervention duration ( $\beta = 0.17$ ,  
277 95% CI: -0.17 – 0.50,  $P > 0.05$ ) were not significantly associated with  $\Delta\text{FMD}\%$ .

278

### 279 **Multiple meta-regression**

280

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

### 284 **Discussion**

285

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

## 299 **Influence of aerobic exercise on FMD**

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

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

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

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

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

344 To allude further, we extracted aerobic capacity as a surrogate for the exercise intervention.  
345 Through meta-regression analysis, our observations corroborate those from Ashor *et al.*, (2015)  
346 <sup>22</sup>, whereby the greatest increases in aerobic capacity were associated with subsequent  
347 increased FMD. For example, the largest increase in  $\dot{V}O_{2peak}$  (11.0 – 11.2 mL.kg.min<sup>-1</sup>) was  
348 accompanied by the largest increase in FMD (7.5 – 10.5 percent points) <sup>33</sup>. In contrast, studies  
349 with a minimal change in  $\dot{V}O_{2peak}$  (<1 mL.kg.min<sup>-1</sup>) reported negligible changes in FMD (-1.2  
350 – 0.8 percent points) <sup>14,47</sup>. This highlights the interrelationship between FMD and aerobic  
351 capacity and implies increased relative aerobic capacity may be necessary to improve FMD, at  
352 least at the brachial artery. While these data encouragingly suggests that improved  
353 cardiorespiratory fitness may have an indirect and positive impact on endothelial function, our  
354 findings can only be generalized to upper limbs. Hoier *et al.*, (2021) <sup>46</sup> reported an 18% increase  
355 in oxygen uptake following 8-weeks of high intensity aerobic exercise training although  
356 demonstrated no improvements in popliteal FMD. Since this could imply limb specific  
357 endothelial adaptation to improved cardiorespiratory fitness, this data warrants to be interpreted  
358 with caution.

359 Systolic and diastolic blood pressure is commonly reported to be higher in postmenopausal  
360 compared to premenopausal individuals <sup>48,49</sup>. In this analysis, studies with participants that had

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

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

### 383 **Heterogeneity of the Research**

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



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

### 397 **Study Limitations**

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

### 407 **Future recommendations**

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

410

#### 411 *Exercise protocol*

412

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

420

421 *FMD assessment*

422

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

429

430 *Study quality*

431

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

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

## 443 **Conclusion**

444

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

452 high quality and conform to best practice FMD guidelines. In doing so, we can develop existing  
453 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  $\Delta$ FMD%, 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](#)

