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ORIGINAL ARTICLE

Relation Between Endothelial Dysfunction and Exercise Training-Mediated Adaptation in Cardiovascular Risk Factors, Cardiorespiratory Fitness, and Vascular Health in Humans: A secondary analysis

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Abstract

Purpose A priori cardiovascular (CV) health status may impact reductions in risk factors and CV mortality and morbidity following exercise training, although this is not fully understood. Therefore, the purpose of the study was to examine if endothelial function (assessed via flow mediated dilation; FMD%), predicts the magnitude of change in CV risk factors or fitness following exercise training.

Methods We pooled data from 338 individuals who underwent supervised exercise training (8–26 weeks). Using recent sex- and age-specific reference values for flow-mediated dilation (FMD%), we categorised participants as having preserved endothelial function (P-EF) (> 50th percentile of reference value, 56 females, 67 males, 46 ± 17 years) or reduced endothelial function (R-EF) (< 50th percentile of reference value, 67 females, 148 males, 48 ± 17 years). The effects of exercise training on cardiovascular risk factors (BMI, cholesterol, glucose and triglycerides), cardiorespiratory fitness (VO_{2peak}) and vascular function (FMD%) were examined using a two-way mixed design general linear model.

Results Exercise training significantly improved physical fitness ($P < 0.001$), with no difference in the magnitude of improvement between P-EF and R-EF. Modest but significant reductions were present in BMI, blood pressure and total cholesterol (all $P < 0.005$), with no difference between P-EF and R-EF groups in the magnitude of changes in these variables with training. Exercise training did not significantly alter glucose, triglycerides, high density lipoprotein (HDL) and low-density lipoprotein (LDL) (all $P > 0.05$).

Conclusion Individuals with reduced and preserved a *priori* endothelial function status can obtain benefits from exercise in terms of risk factor modification and fitness change. Therefore, exercise has the potential to be beneficial in all clinical groups.

Keywords Cardiovascular disease · Exercise · Endothelial function · Cardiorespiratory fitness · Vascular health

Introduction

Cardiovascular disease (CVD) is the leading causes of death worldwide and is one of the most serious health problems throughout the world [38, 46]. Regular exercise training and/or physical activity is a conventional and non-pharmological strategy that effectively reduces the risk for development and progression of cardiovascular disease [14]. Benefits of exercise are only partly explained through improvements in traditional cardiovascular disease risk factors, e.g., blood pressure [29], body weight [33], glucose homeostasis [34], cholesterol [13], and cardiorespiratory fitness [10]. Benefits of exercise training also relate to improvements in vascular health, including endothelial function [3, 18, 21, 58].

Daniel J. Green and Dick H. J. Thijssen have shared senior.

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Endothelial function, measured as flow mediated dilation (FMD), is largely nitric oxide (NO)-mediated [16] and predicts future CV events [19]. It is therefore considered an early marker of future atherosclerotic vascular risk and a non-invasive window into vascular health status. Recently, age and sex-specific FMD reference intervals for healthy individuals have been published [27]. It has been consistently reported that age and sex-specific differences in FMD are present [28, 50, 53, 62] with sex altering the age-related decline in FMD. [27]. FMD references intervals that account for these differences allow for a clinical interpretation of FMD.

Some epidemiological evidence suggests that individuals with CVD gain less benefit from regular physical activity, in terms of relative risk reduction for all-cause mortality and morbidity, than apparently healthy individuals [32, 41, 42]. In addition, a recent study demonstrated that cardiovascular (CV) health status alters the dose–response between moderate to vigorous physical activity (MVPA) and incident morbidity and mortality among 143,493 adults [4]. Healthy individuals and those with CV risk factors presented with a curvilinear relation, whilst those with established CVD presented a more gradual, linear relation, suggesting a smaller risk reduction from regular physical activity in those with CVD [16, 36]. This could have implications for the benefits of exercise training and interventions in these individuals.

Whilst a *priori* cardiovascular health status may impact reductions in risk factors and CV mortality and morbidity, currently, it is not known whether a *priori* endothelial dysfunction impacts upon exercise-training adaptation in CV risk factors and fitness. Therefore, the aim of this study was to investigate whether a *priori* endothelial dysfunction is associated with distinct training-induced improvement in traditional CVD risk factors, cardiopulmonary fitness, or vascular function by performing a secondary analysis in a large cohort of 338 individuals who performed supervised exercise training. Supported by our previous work on endothelial (dys)function [16, 36], it was hypothesised that those with a priori endothelial dysfunction would show smaller improvements in CV risk factors and cardiopulmonary fitness compared to those with a priori preserved endothelial function.

Methods

Participants

Endurance exercise training studies performed in our laboratories (Liverpool, Perth, Nijmegen) which met the following criteria were included in this analysis: (1) completion of moderate-intensity supervised exercise ≥ 8 weeks; (2) the exercise sessions consisted of endurance (aerobic) exercise involving large muscle groups or combined aerobic and resistance exercise (CARE) sessions; (3) exercise training was performed ≥ 2 times per week with a duration of ≥ 30 min; (4) measurements of baseline FMD and age were available to categorize a *priori* endothelial function based on [27] equations, (5) endothelial function (FMD) measurements were performed strictly adhering to expert consensus guidelines [55, 56], (6) the exercise study was approved by the local ethics committees and conformed to the standards of the Declaration Helsinki. Participants were asked not to modify their diet and lifestyle factors during the programme, no studies controlled the participants' diet throughout their study. Smokers were excluded as well as studies which included children, adolescents, or pregnant women. Additionally, any changes in brachial artery diameter or FMD that was > 3 times the overall standard deviation were removed. This led to the inclusion of 19 studies (Table 1) with 338 participants for this secondary analysis, with a wide range of different health statuses included: healthy young, older sedentary, individuals with non-alcoholic fatty liver disease (NAFLD), polycystic ovary syndrome (PCOS), type 2 diabetes, elite rowers, and individuals with CVD risk. Please see Table 1 for further details.

We performed a power calculation, to achieve a power of 80% with level of significance of 5% (two side) based on the ability to detect a 1% difference in the change in FMD over time between the two groups, with a standard deviation of the difference of 2.5%, we need to include at least 99 participants in each group.

Experimental Design

Subject characteristics and traditional CV risk factors were recorded pre and post exercise training. All physiological measures pre and post the exercise intervention were performed following an overnight fast and participants were asked to abstain from strenuous exercise for > 24 h and caffeine and alcohol for > 12 h before testing. Post measurements were completed 1–4 days following the last exercise session. Presence of pre-training endothelial dysfunction was determined using published FMD reference values [27]. Specifically, individuals were categorised as possessing "reduced endothelial function, R-EF" when pre-training FMD was lower than the age- and sex specific FMD reference values using the 50th percentile as the cut-off value [27] and preserved endothelial function (P-EF) when at or above the 50th percentile for age- and sex specific FMD reference values.

Table 1 Study characteristics of included studies. NAFLD: Non-alcoholic fatty liver disease, PCOS: polycystic ovary syndrome, T2D: type 2 diabetes

Author (year)	Group size (P-EF/R-EF)	Study population	Type of exercise	Training weeks	Frequency (days/week)	Duration (min)	Intensity
Tinken et al. (2008) [59]	12 (4/8)	Healthy young males	Aerobic	8	3	30	0.8
Black et al. (2009) [7]	11(4/7)	Older sedentary males and females	Aerobic	12	4	30	0.3
Birk et al. (2012) [6]	9 (3/6)	Healthy men, recreationally active	Aerobic	8	3	30	0.8
Pugh et al. (2016) [45]	9 (3/6)	NAFLD males and females	Aerobic	16	3.5	37.5	0.45
Sprung et al. (2013) [52]	6 (2/4)	PCOS	Aerobic	16	3.5	37.5	0.45
Buckley et al. (2018) [9]	13 (4/9)	Increased CVD risk	CARE	12	1–3		0.5–0.75
Buckley et al. (2020) [8]	33 (8/25)	Increased CVD risk	CARE	12	1–3		0.5–0.75
Miller et al. (2022) [40]	17 (7/10)	Healthy	Aerobic	8	2.5	55	0.7
Maxwell et al. (2021) [37]	10 (4/6)	CVD Risk	Aerobic	8	3	50	0.7
Thijssen et al. (2007) [57]	8 (5/3)	Older men	Aerobic	8	3	30	0.7
Schreuder et al. (2014a) [48]	13 (3/10)	Older men	CARE	8	3	60	0.725
Schreuder et al. (2014b) [49]	23 (6/17)	Healthy and T2D	CARE	8	3	60	0.725
Poelkens*	13 (12/1)	Healthy and CVD risk	Aerobic	26	3	45	0.725
Scholten et al. (2012) [47]	37 (19/18)	Pre-eclamptic women, control	Aerobic	12	3/2.5	60/55	0.725
Benda et al. (2015) [5]	14 (7/7)	Manifest CVD	Aerobic	12	2	45	0.83
Green et al. (2003) [22]	35 (8/27)	CVD risk and manifest CVD	CARE	8	3	55	0.8
Naylor et al. (2006) [43]	25 (8/17)	Elite Rowers	CARE	12	13	135	
Haynes et al. (2021) [25]	19 (8/11)	Older subjects	Aerobic	24	3	32.5	0.512
McKeown*	31 (8/23)	Healthy	CARE	12	2.5	60	0.75

*Unpublished data

Experimental Procedures

Subject Characteristics

Subject characteristics were measured pre and post the exercise training intervention. Height, weight, body mass index (BMI), waist circumference and waist to hip ratio were measured using standard methods. Body fat percentage was measured either via dual X-Ray Absorptiometry (DXA) or skin fold measurements using standard techniques. Measurements of blood pressure were conducted after ≥ 5-min rest in a seated or supine position using a manual or automated sphygmomanometer and were repeated at least twice and were averaged.

Cardiopulmonary Fitness

Peak oxygen consumption (VO_{2peak}) was measured during a maximal graded exercise test on a treadmill, a cycle ergometer or a rower. VO_{2peak} values are presented relative to body weight (ml/kg/min) (Supplementary Table 1).

Endothelial Function

FMD assessments were performed in a quiet, temperature-controlled laboratory at the same time of day to avoid diurnal effects. Participants were asked to avoid alcohol, caffeine consumption and vigorous exercise for ≥ 12 h before testing. Subjects rested for ≥ 15 min in the supine position. The participants' arm was extended and positioned at an angle ~80 degree from the torso. A rapid inflation and deflation pneumatic cuff was positioned on the forearm and inflated to suprasystolic pressure to induce ischemia. B-mode images were obtained with a ≥ 7.5 MHz multi-frequency linear array probe attached to a high-resolution ultrasound machine was used to image the brachial artery in the distal one-third of the upper arm. One study used a Megas ultrasound device (Esaote, Firenze, Italy), whilst all other studies used either the Aspen Acuson (Mountain view, CA, USA) or a Terason, t3000, (Aloka, Burlington, MA, USA) (Supplementary Table 1). Baseline diameter, flow and shear stress measurements were recorded for ≥ 1 min before the forearm cuff was inflated for 5 min. Diameter and flow recordings resumed 30 s prior to cuff deflation and continued for 3 min post deflation. FMD was calculated as peak artery diameter following hyperaemia, expressed as % increase using an allometric model. All analysis was performed using custom designed edge detection and wall tracking software which is largely independent of investigator bias (Woodman et al. 2001).

Cardiovascular Blood Parameters

Venous blood samples were taken to assess fasted glucose, total cholesterol, high density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol as well as triglycerides. The blood samples were analysed in accredited laboratory facilities.

Statistical Analysis

The effect of a *priori* endothelial function on improvements in endothelial function and CVD risk factors in response to exercise training were determined using a two-way mixed design general linear model, with a within subject factor of time (pre- versus post-training) and a between subject factor of group (endothelial function vs endothelial dysfunction). Data analysis was performed using SPSS (Version 26; SPSS Inc., Chicago, IL). Statistical significance was delimited at $P < 0.05$ and exact P values are cited (P values of '0.000' provided by the statistics package are reported as < 0.001).

Results

A total of 338 participants were included in the study. Following the age- and sex-based reference values, we classified 123 (56 female, 67 male) participants as possessing preserved endothelial function (P-EF) and 215 (69 female, 146 male) as having a *priori* reduced endothelial function (R-EF). The duration of exercise training varied, with $n = 127$ having undertaken an 8-week exercise intervention (P-EF: $n = 40$, R-EF: $n = 87$), $n = 164$ completed a 12-week intervention (P-EF: $n = 58$, R-ED: $n = 106$), $n = 15$ completed a 16-week intervention (P-EF: $n = 5$, R-EF: $n = 10$), and $n = 32$ completed a ≥ 24-week intervention (P-EF: $n = 20$, R-EF: $n = 12$). Details of the exercise training interventions are summarised in Table 1.

Baseline Characteristics

Prior to training, we found no differences between P-EF versus R-ED in general characteristics or in cardio-respiratory fitness (Table 2). Regarding traditional CVD risk factors, we found significantly higher baseline systolic blood pressure, triglycerides and fasted glucose levels in R-EF, whilst no differences were found for diastolic or mean blood pressure, cholesterol, LDL and HDL (Table 2). As a consequence of the group allocation, the R-EF-group demonstrated a significantly lower FMD%, compared to those with P-EF.

Impact of Exercise Training

Cardiovascular Risk Factors

Exercise training caused modest but significant reductions in body weight, BMI, percentage body fat, waist circumference, blood pressure (diastolic, systolic and mean) and total cholesterol (all $P < 0.05$). The magnitude of these changes were not different between preserved P-EF and R-ED (Table 3). Exercise training did not significantly alter serum levels of glucose, triglycerides, HDL and LDL (Table 3).

Cardiorespiratory Fitness

Exercise training improved cardiorespiratory fitness in those with preserved endothelial function and with reduced endothelial function [$F(1, 291) = 136.199$, $P < 0.001$]. This effect of training on VO_{2peak} was not significantly different between groups with both groups showing a significant improvement [interaction, F (1, 291) = 1.402, $P = 0.237$] (Table 3).

Table 2 Subject characteristics of the study population stratified by endothelial function into preserved endothelial function (P-EF, $n=123$) and reduced endothelial function (R-EF, $n=215$)

General characteristics	P-EF ($n=123$, 58 healthy, 46 CVD risk, 19 CVD)	R-EF ($n=215$, 88 healthy, 74 CVD risk, 19 CVD)	*P* values
Age (years)	46 ± 17	48 ± 17	0.297
Gender (% male)	54	68	**0.016**
Height (m)	1.73 ± 0.08	1.73 ± 0.10	0.815
Weight (kg, $n=115$–202)	84.6 ± 19.4	88.0 ± 87.4	0.487
Body mass index (kg/m^2, $n=115$–202)	28.2 ± 5.9	29.2 ± 5.4	0.500
Waist:Hip ($n=107$–186)	0.88 ± 0.07	0.93 ± 0.09	0.162
Waist circumference (cm, $n=45$–61)	97.37 ± 15.21	102.72 ± 15.68	0.289
VO_{2peak} (mL/kg/min, $n=107$–186)	30.36 ± 11.31	29.98 ± 12.41	0.751
Vascular function			
Flow-mediated dilation (%)	8.80 ± 2.58	3.69 ± 1.58	**< 0.001**
Baseline diameter brachial artery (mm, $n=110$–214)	3.72 ± 0.82	4.16 ± 0.80	**< 0.001**
Health Characteristics			
Systolic blood pressure (mmHg, $n=96$–166)	123 ± 14	131 ± 16	**< 0.001**
Diastolic blood pressure (mmHg, $n=96$–166)	74 ± 9	77 ± 11	0.118
Mean Arterial Pressure (mmHg, $n=113$–202)	91 ± 10	94 ± 11	0.108
Total Cholesterol (mmol/L, $n=69$–119)	4.84 ± 1.09	4.99 ± 1.18	0.192
LDL Cholesterol (mmol/L, $n=59$–83)	2.97 ± 0.96	2.98 ± 0.86	0.319
HDL Cholesterol (mmol/L, $n=66$–107)	1.26 ± 0.37	1.20 ± 0.35	0.310
Triglycerides (mmol/L, $n=59$–86)	1.20 ± 0.68	1.69 ± 1.12	**0.009**
Glucose (mmol/L, 56–69)	5.32 ± 1.43	6.18 ± 2.65	**0.002**

The preserved endothelial function group consisted of 58 healthy participants, 46 cardiovascular disease risk, 19 manifest cardiovascular disease. The reduced endothelial function group consisted of 88 healthy participants, 74 cardiovascular disease risk, 19 manifest cardiovascular disease. When n is different to P-EF, $n=123$ and R-EF, $n=215$ this has been state as $n=$P-EF, R-EF in the table. **Bold** denotes significance ($P<0.05$).

Brachial Artery Diameter and Endothelial Function

Brachial artery FMD increased over time in response to exercise training [F (1, 335) = 10.092, $P=0.002$], which differed between groups [interaction effect; F (1, 335) = 42.942, $P<0.001$] (Table 2). Post-hoc tests indicated little change in FMD% in preserved EF, whilst an increase was found in ED ($P<0.001$). Exercise training significantly increased resting brachial artery diameter [F (1, 322) = 9.334, $P=0.002$]. The magnitude of this increase in resting diameter following exercise training was not different between groups [F (1, 322) = 0.056, $P=0.813$].

Discussion

The aim of the present study was to investigate whether a *priori* endothelial function status is associated with distinct training-induced improvement in traditional CV risk factors, cardiorespiratory fitness, and vascular function. To this end, we performed analysis on 338 participants who all underwent supervised exercise training in our laboratory, with pre- and post-training evaluation of endothelial function, cardiovascular risk factors and fitness. Overall, our data suggest that exercise training improved cardiorespiratory fitness, flow-mediated dilation and some (i.e. body weight, BMI, body fat percentage, waist circumference and blood pressure) but not all (i.e. fasting glucose, HDL, LDL and triglycerides) cardiovascular risk factors. After dividing the group into a *priori* reduced endothelial function (R-ED; $n=215$) and preserved endothelial function (P-EF; $n=123$), we found comparable improvements in cardiovascular risk factors and physical fitness following exercise training in both groups. Interestingly, only those with a *priori* endothelial dysfunction demonstrated improvement in endothelial function (FMD%) after exercise training, whereas no change was found in subjects with P-EF who started with higher endothelial function, supporting previous findings [17]. Overall, our study suggests that the benefits of exercise training on many clinically important risk factors are independent of a priori endothelial dysfunction status,although a period of 8–12 weeks of moderate intensity exercise may not enhance endothelial function in those with P-EF.

Previous epidemiology research has shown that moderate to vigorous physical activity is beneficial for reducing adverse outcomes, however the shape of the association is

Table 3 Characteristics of the groups divided by endothelial function into the preserved endothelial function (P-EF) group and subjects with reduced endothelial function (R-EF) before and after exercise interventions

Parameters	P-EF		R-EF		*P*-value		
	Pre	Post	Pre	Post	Group	Time	Group*Time
Weight (kg)	85.3 ± 19.6 (81.8–88.7)	84.5 ± 19.2 (81.1–87.9)	87.6 ± 18.6 (85.0–90.3)	87.12 ± 18.4 (84.5–89.7)	0.257	**< 0.001**	0.446
Body mass index (kg/m^2)	28.5 ± 5.9 (27.5–29.6)	28.3 ± 5.8 (27.3–29.3)	29.1 ± 5.5 (28.3–29.9)	28.9 ± 5.3 (28.2–29.7)	0.334	**< 0.001**	0.367
Body Fat (%)	35 ± 8 (33–37)	34 ± 8 (32–36)	34 ± 9 (32–36)	33 ± 9 (31–35)	0.708	**< 0.001**	0.278
Waist:Hip	0.88 ± 0.07 (0.86–0.91)	0.87 ± 0.09 (0.85–0.90)	0.95 ± 0.09 (0.93–0.97)	0.92 ± 0.09 (0.90–0.95)	**0.001**	**0.001**	0.132
Waist Circumference (cm)	98.6 ± 15.4 (91.0 – 103.0)	96.77 ± 16.6 (88.0–101.0)	103.9 ± 14.3 (94.8–104.3)	101 ± 173 (93.1–103.3)	0.440	**< 0.001**	0.210
Systolic blood pressure (mmHg)	124 ± 14 (120–127)	119 ± 14 (116–121)	130 ± 16 (128–133)	126 ± 14 (124–128)	**< 0.001**	**< 0.001**	0.737
Diastolic blood pressure (mmHg)	74 ± 9 (72–77)	71 ± 9 (69–73)	76 ± 11 (75–78)	74 ± 10 (73–76)	**0.037**	**< 0.001**	0.233
Mean arterial pressure (mmHg)	91 ± 10 (89–93)	87 ± 10 (85–89)	93 ± 11 (92–95)	90 ± 10 (89–92)	**0.013**	**< 0.001**	0.309
VO_{2peak} (mL/kg/min)	30.01 ± 11.18 (27.71- 32.30)	32.84 ± 11.10 (30.52–35.16)	30.18 ± 12.52 (28.44–31.92)	32.49 ± 12.80 (30.73–34.25)	0.954	**< 0.001**	0.237
Glucose (mmol/L)	5.32 ± 1.40 (4.79–5.85)	5.30 ± 1.25 (4.87–5.73)	6.26 ± 2.53 (5.93–6.79)	6.01 ± 2.02 (5.73–6.42)	**0.005**	0.132	0.193
Triglycerides (mmol/L)	1.19 ± 0.68 (0.96–1.42)	1.18 ± 0.68 (0.98–1.34)	1.60 ± 1.0 (1.41–1.79)	1.53 ± 0.85 (1.37–1.71)	**0.006**	0.433	0.567
Total cholesterol (mmol/L)	4.80 ± 1.05 (4.57–5.05)	4.72 ± 1.1 (4.49–4.96)	4.96 ± 0.99 (4.77–5.14)	4.80 ± 0.94 (4.62–5.98)	0.460	**0.004**	0.413
High-density lipoprotein (mmol/L)	1.26 ± 0.37 (1.18–1.34)	1.27 ± 0.36 (1.19–1.35)	1.17 ± 0.31 (1.11–1.24)	1.18 ± 0.30 (1.12–1.24)	0.076	0.456	0.896
Low-density lipoprotein (mmol/L)	2.91 ± 0.94 (2.67–3.14)	2.89 ± 0.99 (2.66–3.12)	3.07 ± 0.85 (2.88–3.26)	2.95 ± 0.82 (2.75–3.14)	0.476	0.071	0.197
Flow-mediated dilation (%)	8.63 ± 2.78 (8.29–9.02)	8.21 ± 3.62 (7.71–8.72)	3.70 ± 1.51 (3.42–3.98)	4.99 ± 2.24 (4.61–5.36)	**< 0.001**	**0.002**	**< 0.001**
Diameter (mm)	3.73 ± 0.82 (3.58–3.89)	3.81 ± 0.79 (3.66–3.97)	4.16 ± 0.80 (4.05–4.27)	4.23 ± 0.85 (4.17–4.34)	**< 0.001**	**0.002**	0.813

P-values were determined using a two-way mixed design ANOVA. Data is presented as mean ± SD (95% Confidence interval). **Bold** denotes significance ($P < 0.05$)

dependent upon health. A curvilinear association between healthy and individuals with CV risk factors and PA whilst a linear association was found between individuals with CVD [4]. This supports our findings that individuals do benefit from physical activity however, the response may be different between those with have reduced endothelial function and preserved endothelial function. A unique aspect of our study was our classification of individuals into endothelial function or dysfunction by comparing individuals' FMD-values with recently published age- and sex- specific reference intervals for FMD [27]. Sex and age specific recommendations were important to consider as higher FMD in females, but also the steeper decline in FMD with age, compared with males may relate to differences in sex hormones, especially since oestrogen has been linked to cardioprotective properties [39]. Adopting this approach, we observed a relatively large population with a *priori* reduced endothelial function (63.6%), which is the direct consequence of the inclusion of a substantial number of training studies in clinical populations and those with CV risk factors (Table 1). Findings from our study are in line with our previous observations [16, 20, 35] in that regular exercise training is capable of improving endothelial function, especially in those with a priori endothelial dysfunction [16, 36, 61] (Table 3). Indeed, endothelial dysfunction is related to chronic exposure to CV risk factors [12, 15] and CVDs [1, 26]. Furthermore, findings from our study are in line with our previous observations [35], Green, Maiorana et al. [20], Green, Eijsvogels et al. [16] in that regular exercise training is capable of improving endothelial function,

especially in those with a *priori* endothelial dysfunction [16, 36, 61] (Table 3).

The key finding from this study is that exercise training is associated with improved CV risk factors and enhanced physical fitness; an observation that is not related to a *priori* endothelial status. Our previous work found that improvements in cardiovascular risk factors also occur irrespective of changes in physical fitness [24]. In fact, lower pre-training values for fitness and impaired CV risk factors or vascular function, are associated with larger training induced improvements in endothelial function in both males and females [16, 20, 35]. These previous studies and the results of the present study provide evidence to support the potency of supervised exercise training interventions to improve cardiovascular risk factors in those with a priori higher risk and/or endothelial dysfunction [4]. Indeed, those with CVD and CV risk factors typically are also characterised with endothelial dysfunction [23, 31], demonstrates smaller benefits from regular physical activity in terms of relative risk reduction for all-cause mortality and cardiovascular events. Key differences relate to study design, with our study examining changes in risk factors within subjects following (supervised) exercise training in groups of strictly selected and defined groups of healthy individuals, CVD risk and CVD. Accordingly, effects from our work can be directly related to exercise training. Studies adopting an epidemiological approach are strong in the volume and number of participants, but causal links are difficult to make, whilst levels of physical activity are estimated using (subjective) questionnaires. Such differences may contribute to the distinct findings between intervention studies and epidemiological cohort observations.

Our study has potential clinical implications; it further highlights the importance and benefits of exercise in healthy and CVD populations. Although those with reduced endothelial function had more cardiovascular disease risk factors at baseline, significant increase in FMD and improvements in risk factors were found in this population. Improvements in FMD are vital due to its prognostic value [19], with a meta-analysis suggesting that per 1% higher FMD, the risk of experiencing a cardiovascular event is 13% lower [30]. Endothelial dysfunction is an independent predictor of future cardiac events in patients with and without established coronary artery disease [44, 60]. In the current study, both groups showed improvements in multiple cardiovascular disease risk factors thus highlighting the importance of exercise training in both clinical and healthy populations. Nonetheless, it is important to highlight that subjects with endothelial dysfunction, even following the comparable improvements in cardiovascular risk factors following exercise training, presented with higher cardiovascular risk factors compared to those in the P-EF group. As this difference remained present following exercise training, this may contribute to observations from epidemiological studies, but also highlight the importance to remain physically active.

Some limitations of our study must be discussed. Firstly, controversy exists about what cut-off value represents true endothelial dysfunction, especially in relation with coronary artery endothelial function [2]. However, we quantified presence of endothelial dysfunction using the recently published age- and sex-based reference value data [27]. Importantly, these reference values were constructed based on laboratories that adopted guideline-based approaches and similar methodology to evaluate the FMD [2, 11, 54]. Whilst a strength of our study is the detailed evaluation of fitness, cardiovascular risk factors and vascular health, a limitation is that we were unable to relate these changes directly to future clinical endpoints (e.g. mortality, morbidity). Finally, our analysis only included studies in which participants completed endurance exercise or combined aerobic and resistance training, therefore results from this study cannot be extrapolated to resistance training or high intensity interval training.

In conclusion, we found that regular exercise training, irrespective of the presence of a priori endothelial dysfunction, improves physical fitness and cardiovascular risk factors, whilst only those with a *priori* endothelial dysfunction demonstrated improvement in endothelial function. Therefore, this study further highlights the importance of exercise training, even in those with endothelial dysfunction, to improve clinically relevant markers of cardiovascular risk.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42978-023-00270-9.

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Declarations

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