Title: The menopause alters aerobic adaptations to high-intensity interval training

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Running title: LV mechanics in post-menopausal women

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Abstract

Introduction: Post-menopausal women have lower resting cardiac function than pre-menopausal women, but whether the menopause influences maximal cardiac output and hence exercise capacity is unclear. It is possible that pre- and post-menopausal women achieve similar improvements in peak aerobic capacity ($\dot{V}O_{2peak}$) and cardiac output with exercise training via different regional left ventricular muscle function ("LV mechanics"), as suggested by *in vitro* and animal studies. The aim of this study was to investigate the effects of the menopause on LV mechanics and adaptations to exercise training. **Methods:** Twenty-five healthy untrained middle-aged women (age 45–58 years; 11 pre-menopausal, 14 post-menopausal) completed 12 weeks of exercise training. Before and after exercise training, (i) $\dot{V}O_{2peak}$ and blood volume were determined, and (ii) LV mechanics were assessed using echocardiography at rest and during two submaximal physiological tests — lower body negative pressure (LBNP) and supine cycling. **Results:** The increase in relative $\dot{V}O_{2peak}$ after exercise training was 9% smaller in post-menopausal than pre-menopausal women, concomitant with a smaller increase in blood volume ($P < 0.05$). However, cardiac output and LV volumes were not different between pre- and post-menopausal women ($P > 0.05$) despite altered regional LV muscle function, as indicated by higher basal mechanics in pre-menopausal women during the physiological tests after exercise training ($P < 0.05$). **Conclusion:** These findings are the first to confirm altered LV mechanics in post-menopausal women. In addition, the reduced aerobic adaptability to exercise training in post-menopausal women does not appear to be a central cardiac limitation, and may be due to altered blood volume distribution and lower peripheral adaptations.

Introduction

Menopause is a normal part of a woman's lifespan (1), and has been associated with a decline in resting cardiovascular function (2). These menopause-related effects include a concentric remodelling of the left ventricle (LV), lower diastolic function and higher blood pressure (3–7). However, there has been no evidence in the existing literature that the menopause influences cardiovascular *capacity*, such as that during maximal exercise (8, 9). It is therefore probable that pre- and post-menopausal women achieve similar cardiac outputs during daily activities that depend on cardiovascular capacity, but that they do so via different underlying cardiac function. This may be underpinned by a menopause-related surge in cardiac sympathetic nerve activity (10) interacting with a greater density of sympathetic nerve endings at the base of the LV than at the apex (11). Such differences may in turn result in different cardiac adaptations to exercise training in pre- and post-menopausal women, but these effects remain to be elucidated.

Traditionally, assessments of cardiac function have focused on heart rate, cardiac output and Doppler-derived indices of loading. However, the regional effects of sympathetic drive on the LV (11, 12) suggest that differences in cardiac function between pre- and post-menopausal women may potentially manifest as differences in regional LV muscle function ("LV mechanics"). In the LV, myofibre alignment varies transmurally from a right-handed helix in the endocardium to a left-handed helix in the epicardium (13). This complex spiral architecture gives rise to opposing rotations at the LV base and apex during systole and diastole, enabling the *in vivo* measurement of LV mechanics. In addition to the influence of sympathetic drive on cardiac function, the withdrawal of oestrogen after the menopause may also specifically affect regional LV muscle function. For example, in female rabbits, oestrogen has been shown to selectively increase the L-type calcium current and the sodium-calcium exchange current in epicardial myocytes excised from the base of the LV, but not in endocardial myocytes excised from the base, nor from the apex (14, 15). Since these calcium currents influence the plateau phase of the cardiac action potential (14), it is likely that the menopause influences both contraction and relaxation

of the *basal epicardium*, but the effects *in vivo* are not known. These previously proposed effects of the menopause on regional LV muscle function may therefore manifest *in vivo* as differences in rotation at the base but not at the apex.

To determine the functional relevance of altered regional LV muscle function due to the menopause, physiological tests that probe cardiovascular function and capacity are required. In this study, lower body negative pressure (LBNP) and supine cycling were used as physiological tests to investigate the effects of exercise training on LV function and mechanics in pre- and post-menopausal women. Peak aerobic capacity and blood volume were assessed to demonstrate conventional adaptations to exercise training. We hypothesised that pre- and post-menopausal women would show similar increases in peak aerobic capacity after exercise training, but with differences in underlying regional LV muscle function.

Methods

Ethical approval

All experimental procedures were approved by the Cardiff Metropolitan University's School of Sport Research Ethics Committee and conformed to the ethical principles in the Declaration of Helsinki, except for registration in a database. Prior to the start of any experimental procedures, all participants provided written and verbal informed consent.

Study design

[..[1]]Thirty-four healthy middle-aged (age 45–58 years) women [..[2]]were recruited for a longitudinal study to investigate the effects of the menopause on LV adaptations to exercise training (15 pre-menopausal, 19 post-menopausal). Only non-smoking, non-diabetic

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(self-reported) and normotensive healthy volunteers who were not taking any cardiovascular or lipid-lowering medications were recruited. These study participants were a subset sample of our previous work investigating age-related differences in resting LV structure, function and mechanics in healthy men and women (6). Nine participants did not complete this study: one participant withdrew from the study citing discomfort from the ultrasound transducer pressing on her chest during echocardiographic imaging, six participants withdrew because of personal commitments or the onset of illnesses not related to this study, and two participants were referred to a cardiologist upon observation of ectopics and were excluded from further tests as a precautionary measure. Twenty-five women thereby completed the exercise training intervention and all the laboratory tests (11 pre-menopausal and 14-post-menopausal; 74% of the initial 34 participants who enrolled in this study). Power analyses (G*Power, Version 3.1.9.2; 16) and previous studies indicated that this sample size would be sufficient to detect an increase in peak oxygen consumption following similar exercise training interventions (17, 18) with at least 0.8 statistical power at a significance level of 0.05.

Baseline cardiorespiratory fitness. Participants were sedentary or recreationally-active (<3 days vigorous exercise/week; 19) at the start of the study prior to the exercise training intervention. Although lifelong history of physical activity was not assessed, we calculated each participant's predicted maximal oxygen uptake ($\dot{V}O_{2max}$) using their age, sex and body mass in the FRIEND equation (derived from the Fitness Registry and the Importance of Exercise National Database; 20), as a reference comparison for their measured peak oxygen uptake ($\dot{V}O_{2peak}$). The two-way ANOVA was used to confirm that cardiorespiratory fitness levels of both the pre- and post-menopausal groups recruited for this study were not different from predicted values typical of the normal population.

Menopausal criteria. Our recruitment was targeted to include only distinctly pre- or post-menopausal women[..[3]], and peri-menopausal women were excluded from this study. The pre-menopausal women were characterised as having regular menstrual cycles ranging

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from 21–35 days in length without a persistent difference of more than seven days between consecutive cycles (1), and had not used oral contraceptives in the preceding four months. Post-menopausal women were identified by at least 12 consecutive months of amenorrhoea (1), which had not been induced by surgery (e.g. hysterectomy). None of the post-menopausal women had used hormone replacement therapy (HRT) in the preceding six months. Post-menopausal women were, on average, 6 years older than the pre-menopausal women (Table 1), and thus we adjusted for age in our statistical analyses (by using age as a covariate).

Overview of laboratory tests. Participants visited the laboratory for a series of physiological tests before and after 12 weeks of high-intensity aerobic interval training (Figure 1). Separated by at least 24 h, these laboratory tests consisted of (i) an aerobic capacity test on an upright cycle ergometer, (ii) an aerobic capacity test on a supine cycle ergometer, (iii) the measurement of total haemoglobin mass and blood volume using the 2-min carbon monoxide (CO)-rebreathing method, and (iv) echocardiographic images for LV function and mechanics at rest, during −15 and −30 mmHg LBNP, and during 20, 40 and 60% peak supine cycling.

Exercise training intervention

High-intensity aerobic intervals on an upright cycle ergometer (Monark 824E, Varberg, Sweden) were used in this study, to maximise the likelihood of cardiorespiratory adaptations to exercise training (17, 21). The exercise training intervention was supervised by a schedule of trained exercise researchers. Each exercise session consisted of a 10-min warm-up, 4 × 4-min intervals at 90–95% maximum heart rate (HR_{max}; RS400, Polar Electro, Kempele, Finland) separated by 3-min active recovery at >60% HR_{max}, and a 5-min cool-down (total duration 40 min) (21). Individualized HR_{max} was determined from the aerobic capacity test on an upright cycle ergometer. The researcher on-site encouraged participants to reach 90% HR_{max} within the first 2 min of each 4-min interval. There

were 1–6 participants in each exercise session, and pre- and post-menopausal women trained together. Three exercise sessions per week were strongly recommended, over a consecutive period of 12 weeks. All participants undertook at least 70% of the total number of sessions, equivalent to at least 8 weeks of exercise training to improve aerobic fitness (22) (number of sessions attended: Pre-M 33 ± 3 vs. Post-M 33 ± 4, *t*-test $P = 0.96$; time ≥90% HR_{max} per session: Pre-M 9.2 ± 1.7 min vs. Post-M 8.3 ± 1.5 min, *t*-test $P = 0.14$). The exercise training intervention was generally well-tolerated with no adverse events.

Aerobic capacity tests

To ensure that participants were euhydrated and well-rested for all of the physiological tests, they were asked to abstain from caffeine, alcohol and strenuous exercise for 24 h, and to drink 500 mL of water 90 min before arrival at the laboratory. Participants' height and body mass (Model 770, Seca, Hamburg, Germany) were measured (Table 1). Participants completed continuous ramp tests to volitional exhaustion on upright (Corival, Lode, Groningen, The Netherlands) and supine cycle ergometers (Angio 2003, Lode, Groningen, The Netherlands) on separate days to determine peak aerobic capacity ($\dot{V}O_{2peak}$) and peak power output (W_{peak}). In line with American College of Sports Medicine (ACSM) guidelines, we refer to $\dot{V}O_{2peak}$ instead of $\dot{V}O_{2max}$ in this study, as a plateau in $\dot{V}O_2$ was not used as a criterion for a valid aerobic capacity test (23). However, all participants achieved a peak respiratory exchange ratio (RER_{peak}) of ≥1.15 on the upright cycle ergometer and ≥1.05 on the supine cycle ergometer, with no evidence of differences between pre- and post-menopausal women ($P > 0.05$).

The aerobic capacity test on the upright cycle ergometer was individualised using age, height and body mass (24), with the test workload programmed to increase from 0 W to predicted W_{peak} in 10 min. Respiratory gas exchange (Oxycon Pro, Viasys Healthcare, Basingstoke, UK) and heart rate were monitored and recorded throughout the test. Following a self-selected recovery period, participants were familiarised with the supine cycle ergometer. On a separate day, participants completed another aerobic capacity test,

but on the supine cycle ergometer. The test workload on the supine cycle ergometer was programmed to increase from 0 W to 80% of each individual's measured upright W_{peak} in 10 min.

After 12 weeks of exercise training, participants' $\dot{V}O_{2peak}$ were reassessed on both upright and supine cycle ergometers. The increments in workload during the aerobic capacity tests were increased so that participants would still achieve their W_{peak} in approximately 10 min, based on an expected 18% improvement in $\dot{V}O_{2peak}$ after exercise training (17).

Total haemoglobin mass and blood volume

After 15 min of seated rest, total haemoglobin mass and blood volume were measured using the optimised 2-min CO-rebreathing technique (SpiCO®, Blood tec GbR, Bayreuth, Germany; 25, 26). Participants were familiarised with the protocol and equipment before starting the procedure. Our percentage typical error was 1.0% for measuring total haemoglobin mass, and 1.3% for blood volume (assessed separately in 10 volunteers who completed the 2-min CO-rebreathing protocol on two different days; calculated as standard deviation of the percentage difference of two repeated measurements on 10 volunteers divided by $\sqrt{2}$).

Measures of cardiovascular function

Blood pressure (FinometerPRO, FMS, Finapres Measurement Systems, Arnhem, Netherlands) and echocardiographic images were recorded at 0, −15 and −30 mmHg LBNP, and at 0, 20, 40 and 60% peak supine cycling, with 30 min of rest between the end of LBNP and the start of supine cycling. Participants lay supine at a 30° left lateral tilt for all measurements. Echocardiographic images were acquired in accordance with guidelines at the start of data collection for this study (January 2013), at end-expiration and by the same trained sonographer (27, 28). A phased array transducer (4V 1.7–3.3 MHz) was used on a commercially-available ultrasound system (Vivid E9, GE Vingmed Ultrasound AS, Horten, Norway), and images were analyzed offline for LV function and mechanics

(EchoPAC, Version 112, GE Healthcare, Horten, Norway). Transducer positions during resting measurements were temporarily marked on the participant's chest to assist the rapid relocation of similar acoustic windows during LBNP and supine cycling, during which images were further optimised and confirmed with anatomic landmarks. Three consecutive cardiac cycles were analyzed for each variable and the mean was used for statistical analyses.

Left ventricular structure and function. End-diastolic and end-systolic volumes (EDV and ESV, respectively) were determined from triplane images of the same heartbeats. Heart rate was determined from the ECG inherent to the ultrasound. Stroke volume (SV = EDV − ESV), ejection fraction $\left(\frac{\text{SV}}{\text{EDV}} \times 100\%\right)$, cardiac output (HR × SV) and systemic vascular resistance (mean arterial pressure/cardiac output) were then calculated.

Left ventricular mechanics. Rotation and rotational velocity were assessed using 2D speckle tracking of the myocardium in the parasternal short-axis images at the LV base and apex, in line with previous methodology (29). To account for differences in heart rate between and within participants, raw data were smoothed with cubic spline interpolation to generate 1200 data points, with 600 points each for systole and diastole (2D Strain Analysis Tool 1.0β14, Stuttgart, Germany) (29). Twist and twisting velocity curves were calculated by subtracting time-aligned basal data from apical data, and peak values in systole and early diastole were extracted from interpolated curves. Due to poor image quality in some participants, data on LV mechanics during LBNP are reported for 9 pre-menopausal and 10 post-menopausal women, and data during supine cycling for 8 pre-menopausal and 10 post-menopausal women.

Physiological tests

Lower body negative pressure. Mild LBNP was used to simulate the reduced cardiac filling typical of the upright posture due to gravity (30). Participants were positioned with

a neoprene kayak skirt on their iliac crest, and with their lower body in an LBNP box (built in-house; length 126 cm, width 55 cm, height 90 cm). Two consecutive 10-min stages at −15 and −30 mmHg LBNP were applied. A variable transformer (CMV 5E-1, Carroll & Meynell Transformers Ltd, Stockton-On-Tees, UK) connected to a vacuum pump (Henry HVR200A, Numatic International Ltd, Chard, England) was used to achieve the desired negative pressure within the box, which was monitored continuously using a differential pressure meter (Testo AG, Lenzkirch, Germany). Blood pressure and echocardiographic images were recorded at rest and after 5-min exposure to each stage of LBNP (30).

Supine cycling. Upon completion of LBNP, participants relaxed for 30 min to ensure a return to a resting physiological state (30). Participants then completed three consecutive 5-min stages of supine cycling at 20, 40 and 60% supine W_{peak}. Supine cycling was used to simulate the typical physical exertion from performing activities of daily living. Blood pressure and echocardiographic images were recorded at rest with the participant lying on the supine cycle ergometer at a 30° left lateral tilt, and during the final 3 min at each exercise intensity.

Statistical analysis

Statistical analyses were performed with R (31). The two-way repeated-measures analysis of variance (ANOVA) with age as a covariate was used to examine the effects of exercise training on aerobic capacity, total haemoglobin mass and blood volume in post-menopausal women compared with pre-menopausal women. For variables with a significant menopause × training interaction effect, *post hoc* Student's *t*-tests were used to identify differences between groups.

The three-way repeated-measures ANOVA with age as a covariate was used to examine the impact of the menopause, exercise training and the physiological tests on LV function and mechanics. Figure S1 shows the flowchart for interpreting the three-way ANOVA, with a focus on the effects of the menopause as the key research question (SDC). This

approach integrated all data within one statistical test and avoided the reuse of data in multiple disparate ANOVAs. For variables with a statistically significant three-way interaction effect, individual differences with exercise training were calculated *post hoc* and Student's t-tests were used to identify differences between pre- and post-menopausal women at each LBNP and exercise stage. For variables with statistically significant two-way interaction effects from the three-way ANOVA, data were grouped *post hoc* across the non-significant factor to reduce complexity, and to enable interpretation of the two-way interaction effects. The Holm-Bonferroni correction was used to adjust for multiple comparisons across LBNP and supine cycling stages.

To examine whether the effects of the menopause on LV function and mechanics following exercise training could be detected at rest (i.e. without requiring the physiological tests), *post hoc* two-way ANOVAs were used to compare resting data if any of the menopause or training effects in the three-way ANOVA were statistically significant. Alpha was set at 0.05. Data are presented as mean and standard deviation (SD) unless stated otherwise.

Results

Menopause-related effects on peak aerobic capacity and LV function under resting conditions

Measured $\dot{V}O_{2peak}$ prior to the exercise training intervention was not different from predicted $\dot{V}O_{2max}$ in pre- or post-menopausal women ($P > 0.05$; Table 1). Exercise training elicited smaller increases in peak aerobic capacity and blood volume in post-menopausal women than pre-menopausal women ($P < 0.05$; Table 1). [..[4]]There was additionally no evidence of differences in LV function between pre- and post-menopausal women at rest[..[5]], whether they were compared before or after exercise training ($P > 0.05$).

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Menopause-related effects on LV function during lower body negative pressure

In pre- and post-menopausal women, cardiac output, end-diastolic volume and stroke volume decreased in response to LBNP, concomitant with an increase in heart rate and systemic vascular resistance ($P < 0.001$; Figure 2). There was no evidence of differences in general haemodynamics and LV volumes during LBNP between pre- and post-menopausal women ($P > 0.05$; see Figure S2, SDC, LV function and systemic vascular resistance during LBNP with pre- and post-menopausal women presented separately). However, exercise training elicited a significant difference in peak diastolic basal rotational velocity during LBNP between pre- and post-menopausal women ($P = 0.04$) — specifically, peak diastolic basal rotational velocity was maintained at resting values during LBNP after exercise training in pre-menopausal women, but decreased during LBNP in post-menopausal women (Figure 3). These distinct responses in pre- and post-menopausal women were not apparent before exercise training. There was no evidence of differences in apical mechanics between pre- and post-menopausal women during LBNP ($P > 0.05$), nor of any other changes in LV mechanics in response to LBNP ($P > 0.05$; see Table S1, SDC, peak LV mechanics during LBNP and incremental exercise tests).

Menopause-related effects on LV function during supine cycling

Heart rate, cardiac output and stroke volume increased during supine cycling in both pre- and post-menopausal women, along with a decrease in systemic vascular resistance and end-systolic volume ($P < 0.001$; Figure 4). All indices of peak LV mechanics increased in response to incremental exercise ($P < 0.001$; see Table S1, SDC). Similar to the effects of LBNP, there was no evidence of differences in general haemodynamics and LV volumes between pre- and post-menopausal women during supine cycling ($P > 0.05$; see Figure S3, SDC, LV function and systemic vascular resistance during supine cycling with pre- and post-menopausal women presented separately). However, and in line with the differences in

regional LV muscle function during LBNP, exercise training elicited a significant difference in peak systolic basal rotation between pre- and post-menopausal women during supine cycling ($P = 0.02$; Figure 5). Although peak basal rotation increased during supine cycling across all groups and conditions, a plateau became apparent at 40% peak exercise after exercise training in pre-menopausal women, but not in post-menopausal women. There was no evidence of differences in apical mechanics between pre- and post-menopausal women during supine cycling ($P > 0.05$).

Impact of exercise training on LV function during supine cycling

In line with a greater peak workload after exercise training, the increase in cardiac output and heart rate from rest to 60% peak supine cycling was greater after exercise training in pre- and post-menopausal women, concomitant with a greater decrease in systemic vascular resistance ($P < 0.05$; Figure 4). End-systolic volume during supine cycling was lower after exercise training across all exercise intensities in both groups ($P = 0.04$), but end-diastolic volume was lower only at 40% peak supine cycling ($P = 0.04$; Figure 4). There was no evidence that exercise training influenced the stroke volume response to supine cycling in either pre- or post-menopausal women ($P > 0.05$). In addition to the plateau in peak basal rotation at 40% peak supine cycling observed in pre-menopausal women after exercise training, peak diastolic apical rotational velocity at 60% peak supine cycling was greater after exercise training in both pre- and post-menopausal women ($P = 0.007$), while peak systolic twisting velocity was greater at 40% peak supine cycling ($P < 0.05$; see Figure S4, SDC, peak twisting velocity, and peak diastolic basal and apical rotational velocity in response to supine cycling before and after exercise training).

Discussion

In this study, we determined the effects of the menopause on regional LV muscle function underpinning the increase in cardiovascular capacity after 12-weeks of exercise training.

Exercise training elicited a smaller increase in peak aerobic capacity and blood volume in post-menopausal than pre-menopausal women. In addition, physiological testing revealed that post-menopausal women had lower basal mechanics during LBNP and supine cycling after exercise training, compared with pre-menopausal women. To our knowledge, this is the first study to suggest that the menopause may reduce aerobic adaptability to exercise training. Furthermore, our findings suggest that the limitation to aerobic adaptability in post-menopausal women is likely due to peripheral (arterial, skeletal muscle and/or blood volume distribution) rather than central (cardiac) factors, as we found no evidence of differences in cardiac output between pre- and post-menopausal women. Nonetheless, cardiac output during physiological testing was underpinned by differences in regional LV muscle function between pre- and post-menopausal women, as hypothesised, confirming for the first time *in vivo* the previously reported regional LV differences from *in vitro* studies.

Lower aerobic adaptability in post-menopausal women compared with pre-menopausal women

In line with previous studies (8, 9, 32), 12 weeks of exercise training evoked an increase in peak aerobic capacity in pre- and post-menopausal women in this study. However, post-menopausal women had a smaller increase in peak aerobic capacity than pre-menopausal women, concomitant with a smaller increase in blood volume. This finding refutes our *a priori* hypothesis of similar increases in peak aerobic capacity in pre- and post-menopausal women after exercise training and contradicts previous results from other research groups. For example, the multi-centre HERITAGE Family Study found no evidence that the increase in peak aerobic capacity after exercise training differed between pre- and post-menopausal women, after using statistical methods to adjust for a mean age difference of >20 years (9). It is possible that the smaller age difference in the present study (6 years) influenced the response to exercise training. More recently, the Copenhagen Women Study found that cardiorespiratory fitness increased similarly between pre- and post-menopausal

women after exercise training (mean age difference of 4 years) (8). Interestingly, the mean percentage increase in maximal/peak oxygen uptake (in L/min) across pre- and post-menopausal groups was higher in our study (16–23%) than in the Copenhagen Women Study (9–10%). This may reflect a more intense exercise training intervention in our study compared with the Copenhagen Women Study (which used a spinning exercise training intervention with gradually increasing intensities across the weeks). In addition, high-intensity aerobic interval training has been suggested to elicit greater improvements in peak aerobic capacity and LV function compared with traditional moderate continuous exercise training (21), which may have contributed to the differences observed between pre- and post-menopausal women in this study. Although a comparison between high-intensity interval training and moderate continuous training was beyond the scope of this study, future work may want to focus on a direct comparison to assess differences in cardiovascular outcomes.

Considering our results in the context of previous work, it is possible that post-menopausal women are able to match the improvement in cardiorespiratory fitness in pre-menopausal women up to 10–16%, but that [..[6]]further improvements may be limited by the menopause. Exercise training studies of a longer duration and with different intensities of exercise, such as [..[7]]that conducted by Howden and colleagues (33), will be required to determine the presence of [..[8]]a ceiling to cardiorespiratory adaptations that is explained by the menopause.

Despite a smaller increase in peak aerobic capacity and blood volume in post-menopausal women after exercise training, there was no evidence that cardiac output, heart rate or LV volumes were different between pre- and post-menopausal women, whether at rest or during the physiological tests. [..[9]]We speculate that the greater blood volume [..[10]]observed in pre-menopausal women after exercise training may have [..[11]]been buffered in the arteries and veins instead, underpinned by a greater vasodilatory capacity than in post-menopausal

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women. In turn, it is likely that the adaptations observed in this study are transitory and are precursors to a larger stroke volume and lower heart rate with further exercise training (33). The greater blood volume in pre-menopausal women after exercise training in this study may have nonetheless improved thermoregulation during exercise, via increased body fluid for sweating and heat dissipation (34). Whilst we did not measure body temperature or sweat responses, our findings suggest that the contribution of cardiac output (central) adaptations to 12 weeks of exercise training are similar in pre- and post-menopausal women, and that peripheral adaptations, such as altered blood volume distribution, arterial function or skeletal muscle [..[12]]capillarization, may have limited the improvement in cardiorespiratory fitness in post-menopausal women. This observation is in direct agreement with previous studies showing that older women are more dependent on a widened arterial-venous oxygen difference (indicative of a peripheral mechanism) to improve cardiorespiratory fitness after 12 weeks of exercise training, compared with younger women (32). Collectively, the current data indicate that the menopause does not limit the short-term cardiac output adaptation to high-intensity interval training despite different regional LV muscle function. Additionally, future studies should investigate the role of the menopause in peripheral adaptations to exercise training.

The menopause alters regional LV muscle function

In support of our *a priori* hypothesis that the menopause affects regional LV muscle function, we detected differences in LV rotation at the base between pre- and post-menopausal women during physiological testing. There was no evidence of differences in apical mechanics between the two study groups, despite greater apical changes typically occurring in response to both ageing (6, 35) and cardiovascular challenges (36, 37), supporting a dominant effect of the menopause on regional LV muscle function as previously suggested *in vitro* (14, 15). After exercise training, pre-menopausal women had greater basal mechanics during the physiological tests compared with post-menopausal women, which

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agrees with previous findings that young adult pre-menopausal women are more dependent on cardiac compensatory mechanisms to achieve filling and generate stroke volume compared with men (37) and older women (32).

Drawing upon mechanistic studies conducted using animals and *in vitro* approaches, the regional effects of the menopause on basal mechanics were first hypothesised and are now confirmed in this study in humans. It is likely that higher calcium currents due to oestrogen, which were previously observed in basal but not apical cardiomyocytes *in vitro* (14, 15), underpinned the *in vivo* contraction and relaxation patterns in post-menopausal women in this study. In addition, the menopause-related surge in cardiac sympathetic nerve activity (10) interacting with a greater density of sympathetic nerve endings at the basal epicardium than the apical endocardium (11, 12) may have also contributed to the regional differences observed. The interaction between calcium handling and sympathetic activity on cardiomyocytes may explain why differences in basal mechanics were detected during physiological testing but not at rest. Our results additionally suggest that any differences at rest are likely to be smaller than the differences during physiological testing. Although not linked with altered cardiac output in the present study, altered regional myocardial function may be linked with early afterdepolarizations originating at the base of the LV, as previously postulated (38), which may indicate a menopause-related effect on cardiac repolarization and susceptibility to arrhythmias (39). Taken together, our results begin to build a link between the effects of oestrogen and sympathetic activity observed in animal or *in vitro* studies and *in vivo* function. Future studies may examine regional myocardial fibrosis or electrical activation patterns to further discern the true implications of the menopause.

Regulation of cardiac output during exercise in middle-aged women

In line with greater absolute workloads after exercise training, the increase in cardiac output from rest to 60% peak supine cycling was greater in both pre- and post-menopausal

women, with no evidence of differences between groups. This response is likely not confined to submaximal exercise efforts, and may be extrapolated to a greater cardiac output at maximal exercise intensities after exercise training. A greater cardiac output is typically achieved via a greater stroke volume (32), but interestingly, in this study it was explained by higher heart rates. Maximum heart rates, however, did not increase after exercise training in this study, and are in fact unlikely to increase with exercise training based on the existing literature (32). Further work is thus required to clarify the cardiac output and stroke volume response from 60–100% peak aerobic exercise in middle-aged women, which may additionally provide new insight into the regulation of cardiac output in this under-represented cohort (40).

Regulation of cardiac output during orthostatic stress in middle-aged women

In line with previous work (37, 41), cardiac output and stroke volume decreased during LBNP in both pre- and post-menopausal women, with no evidence of differences between groups. Whilst the stroke volume response to LBNP did not differ before and after exercise training, an improved filling was evident at -30 mmHg after exercise training, as evidenced by greater end-diastolic and end-systolic volumes in both groups. Apart from basal mechanics, there was no evidence of other changes in LV mechanics in response to LBNP. In contrast, previous studies have shown an increase in peak untwisting velocity with LBNP in men and women, and a decrease in male athletes with more than 5 years of training (37, 42). To our knowledge, this is the first study examining LV mechanics in middle-aged women in response to LBNP. Therefore, the discrepancy between our results and previous studies may indicate that middle-aged women have different LV mechanics in response to LBNP compared with younger women, female athletes and men. In addition, the strict coupling between LV mechanical function and filling has been questioned recently, and it may be that other factors such as altered atrial function or complex geometric changes may influence preload (43).

In this study, we did not observe a greater increase in heart rate in pre-menopausal compared with post-menopausal women in response to orthostatic stress, which has been described previously (41, 44). One key difference between our study and previous studies is a smaller mean age difference of 6 years between pre- and post-menopausal women, compared with ≥26 years in previous studies (41, 44). As age has also been shown to reduce heart rate responsiveness to orthostatic stress (45), previous findings may be due to age more than the menopause, a hypothesis that warrants future investigation.

Limitations

An echocardiography-related limitation of this study is that the sonographer was not blinded to the menopausal and training statuses of participants while analyzing their images. However, the key parameters of regional LV muscle function were derived from a speckle tracking algorithm embedded in GE software and were, therefore, largely operator-independent. Although this study had a smaller age difference between pre- and post-menopausal women than some previous work (41, 44), it was not possible for us to totally eliminate it. This reflects the inherent difficulty of disentangling the effects of a naturally-occurring menopause from those of chronological ageing in the female lifespan (2). To further improve confidence in the study conclusions related to the menopause, we included age as a covariate in our statistical analyses (9). Another possible limitation is the lack of measurement of sex hormones in this study. However, we used menstrual cycle criteria to recruit our pre- and post-menopausal groups, which is the most important criteria for staging reproductive ageing in women as recommended by the Stages of Reproductive Aging Workshop + 10 (STRAW + 10), because of the known limitations in standardisation, cost and invasiveness of biomarker assays (1). Hence, we are confident that the women in the two study groups were appropriately categorized as pre- and post-menopausal. However, we did not control for menstrual cycle phase in the pre-menopausal women for the physiological tests in this study, as previous work has not found conclusive evidence that the menstrual cycle affects maximum aerobic capacity, cardiac output during orthostasis (46), or plasma volume shifts

during exercise (for reviews see 47, 48). In addition, not being constrained by menstrual cycle phase in this study ensured that the physiological tests would be repeated after 12 weeks of exercise training across all study participants. Notwithstanding, it is possible that the menstrual cycle may affect cardiovascular function via differences in temperature and fluid regulation (47), and therefore, further work is necessary to delineate the effects of the menstrual cycle on physiological function.

Implications and future directions

The main practical implication of this study is the smaller increase in peak aerobic capacity observed in middle-aged post-menopausal women after 12 weeks of high-intensity aerobic interval training, compared with middle-aged pre-menopausal women. As peak aerobic capacity is an important prognostic biomarker for cardiovascular disease (21, 22), our findings indicate that the menopause reduces a middle-aged woman's ability to modify her risk of cardiovascular disease with an exercise intervention. Building upon this work, we strongly recommend a replication study to verify the effects of high-intensity aerobic interval training on cardiorespiratory adaptations in middle-aged pre- and post-menopausal women. A better understanding of adaptations to exercise training in middle-aged women would improve public recommendations for lifestyle interventions to improve cardiorespiratory fitness, which has implications for improving health outcomes globally in the ageing population.

Beyond the influence of traditional risk factors such as blood pressure and cholesterol on cardiovascular function (49), our results begin to delineate the early cardiac changes that occur with the menopause. The menopause has itself been identified as a risk factor for cardiovascular disease, through the seminal Framingham Study, but the underlying pathophysiology is unclear (49). Future work examining vascular responsiveness, baroreflex sensitivity (4), LV pressures and myocardial properties (33, 50) in pre- and post-menopausal women will likely provide further insight into the effects of the menopause on the heart. In particular, alterations in regional electrical conduction and the consequences

on arrhythmias are warranted given the altered regional LV muscle function observed *in vivo* in this study.

Conclusion

In conclusion, post-menopausal women experienced a smaller increase in peak aerobic capacity after 12 weeks of high-intensity aerobic interval training, compared with pre-menopausal women. Cardiac output and LV volumes during LBNP and supine cycling were not different between pre- and post-menopausal women, but were underpinned by differences in regional LV muscle function. Our findings provide new insight into the effects of the menopause on aerobic fitness, cardiac adaptability and regional LV muscle function.

Additional information

Competing interests: The authors report no conflicts of interest.

Author contributions: This study was conducted in the School of Sport and Health Sciences at Cardiff Metropolitan University, Cardiff, UK. AQXN contributed to conception and design of the work, acquisition, analysis and interpretation of data, and drafting and revising the work. EJS contributed to conception and design of the work, interpretation of data, and critical revision of the work for important intellectual content. SR, RMW, VLM, JMB and MS contributed to acquisition and interpretation of data, and critical revision of the work for important intellectual content.

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The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by ACSM.

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Data accessibility: The data will be made publicly available in an online repository.

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Supplemental Digital Content

Figure S1

Figure S2

Table S1

Figure [..[13]]S3

Figure S4

Figure captions

Figure 1: Schematic representation of the experimental timeline. Echocardiography was used to assess left ventricular function and mechanics in middle-aged pre- and post-menopausal women in response to lower body negative pressure and supine cycling before and after 12 weeks of exercise training. HR_{max}: Maximum heart rate.

Figure 2: Left ventricular function and systemic vascular resistance (SVR) in [..[14]]response to lower body negative pressure (LBNP)[..[15]]. As there was no evidence of any effects related to the menopause ($P > 0.05$), data in pre- and post-menopausal women were [..[16]]grouped together (effective $n = 25$) to show the effects of LBNP and exercise training (Trg). Values are mean ± standard error of the change from rest.

Figure 3: Peak diastolic basal and apical rotational velocities (rot vel) in response to lower body negative pressure (LBNP) in pre- and post-menopausal (M) women before and after exercise training (Trg; Pre-M $n = 9$, Post-M $n = 10$). Values are mean ± standard error of the change from rest.

[13]removed: S2
[14]removed: pre- and post-menopausal women in
[15]removed: before and after exercise training (Trg). Data
[16]removed: not statistically different (menopause effects $P > 0.05$) and were grouped for clarity

Figure 4: Left ventricular function and systemic vascular resistance (SVR) in [..[17]]response to supine cycling (Ex)[..[18]]. As there was no evidence of any effects related to the menopause ($P > 0.05$), data in pre- and post-menopausal women were [..[19]]grouped together (effective $n = 25$) to show the effects of Ex and exercise training (Trg). Values are mean ± standard error of the change from rest.

Figure 5: Peak systolic basal and apical rotation (rot) in response to supine cycling (Ex) in pre- and post-menopausal (M) women before and after exercise training (Trg; Pre-M $n = 8$, Post-M $n = 10$). Values are mean ± standard error of the change from rest.

[17] removed: pre- and post-menopausal women in
[18] removed: before and after exercise training (Trg). Data from
[19] removed: not statistically different (menopause effects $P > 0.05$) and were grouped for clarity

TABLE 1: Demographics, aerobic capacity and haematological parameters in pre- (Pre-M) and post-menopausal (Post-M) women before and after exercise training (Trg).

	Pre-M ($n = 11$)		Post-M ($n = 14$)		P		
Parameter	Before	After	Before	After	M	Trg	M × Trg
Age (years)*	49 (2)	-	55 (2)	-	**<0.01**	-	-
Height (cm)*	161.1 (6.2)	-	163.3 (3.6)	-	0.27	-	-
Body mass (kg)	63.4 (10.5)	62.4 (9.9)	61.8 (8.4)	61.4 (8.3)	0.19	**0.02**	0.51
Aerobic capacity							
Upright peak power test							
W_{peak} (W)	147 (29)	179 (28) ¶	145 (26)	169 (24) ¶	0.20	**<0.01**	**0.02**
$\dot{V}O_{2peak}$ (L/min)	1.84 (0.31)	2.27 (0.31) ¶	1.80 (0.34)	2.08 (0.29)¶	0.44	**<0.01**	**<0.01**
$\dot{V}O_{2peak}$ (mL/min/kg)	29 (5)	37 (5) ¶	29 (6)	34 (5) ¶	**0.04**	**<0.01**	**0.02**
Predicted $\dot{V}O_{2max}$ (mL/min/kg)*	29 (3)	-	27 (3)	-	0.10	-	-
RER_{peak}	1.32 (0.09)	1.27 (0.06)	1.30 (0.06)	1.26 (0.06)	0.99	**<0.01**	0.46
HR_{max} (beats/min)	169 (10)	171 (9)	168 (12)	166 (9)	0.36	0.78	0.31
Supine peak power test							
W_{peak} (W)	125 (32)	162 (22) ¶	126 (23)	148 (20) ¶	0.07	**<0.01**	**0.01**
$\dot{V}O_{2peak}$ (L/min)	1.77 (0.33)	2.03 (0.27) ¶	1.72 (0.35)	1.90 (0.34)¶	0.12	**<0.01**	**0.04**
$\dot{V}O_{2peak}$ (mL/min/kg)	29 (6)	33 (6) ¶	28 (6)	31 (6) ¶	**0.01**	**<0.01**	**0.05**
RER_{peak}	1.21 (0.09)	1.21 (0.08)	1.22 (0.08)	1.19 (0.06)	0.51	0.40	0.13
HR_{max} (beats/min)	160 (17)	160 (11)	155 (13)	154 (13)	0.25	0.94	0.35
Haematological parameters							
tHb mass (g)	535 (108)	541 (105)	526 (56)	534 (61)	0.61	0.07	**0.02**
Blood volume (mL)	4401 (858)	4601 (846) ¶	4294 (445)	4367 (390)	0.97	**<0.01**	**<0.01**

Values are in mean (SD). n: sample size. W_{peak}: Peak power output. $\dot{V}O_{2peak}$: Peak oxygen uptake. Predicted $\dot{V}O_{2max}$: Maximal oxygen uptake predicted using the FRIEND equation (20). RER_{peak}: Peak respiratory exchange ratio. HR_{max}: Maximum heart rate. tHb mass: total haemoglobin mass. *Student's t-tests to compare pre- and post-menopausal women before exercise training. ¶$P < 0.05$ compared with values before training. Statistical effects with $P < 0.05$ are highlighted in **bold**.

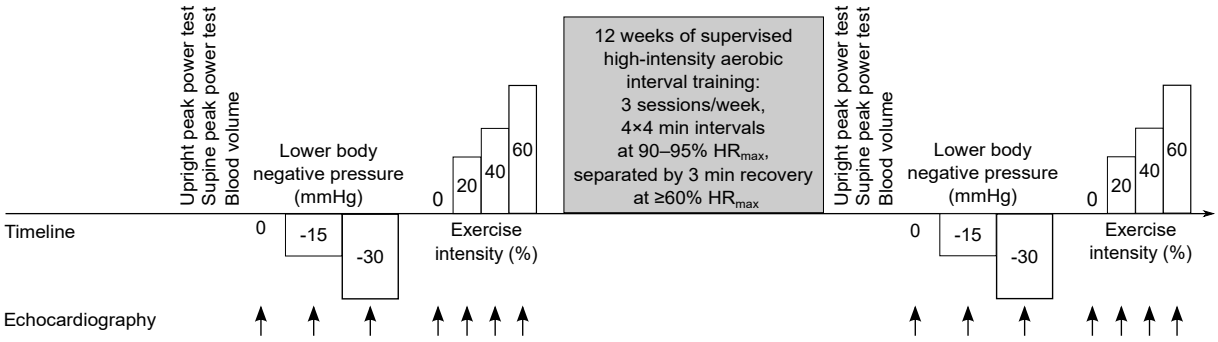

FIGURE 1: Schematic representation of the experimental timeline. Echocardiography was used to assess left ventricular function and mechanics in middle-aged pre- and post-menopausal women in response to lower body negative pressure and supine cycling before and after 12 weeks of exercise training. HR_{max}: Maximum heart rate.

[..[20]

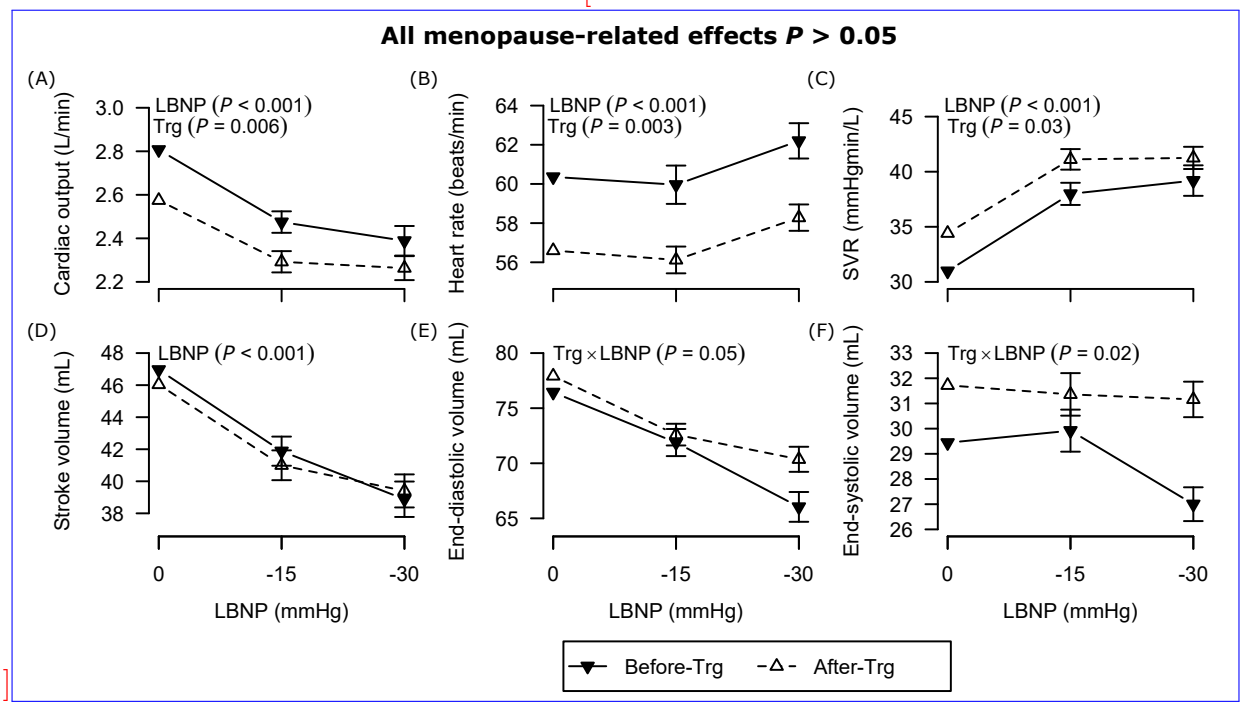

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FIGURE 2: Left ventricular function and systemic vascular resistance (SVR) in [..[21]]response to lower body negative pressure (LBNP)[..[22]]. As there was no evidence of any effects related to the menopause ([..[23]]$P > 0.05$)[..[24]], data in pre- and post-menopausal women were [..[25]]grouped together ([..[26]]effective $n = 25$) to show the effects of LBNP and [..[27]]exercise training (Trg). Values are mean ± standard error of the change from rest.

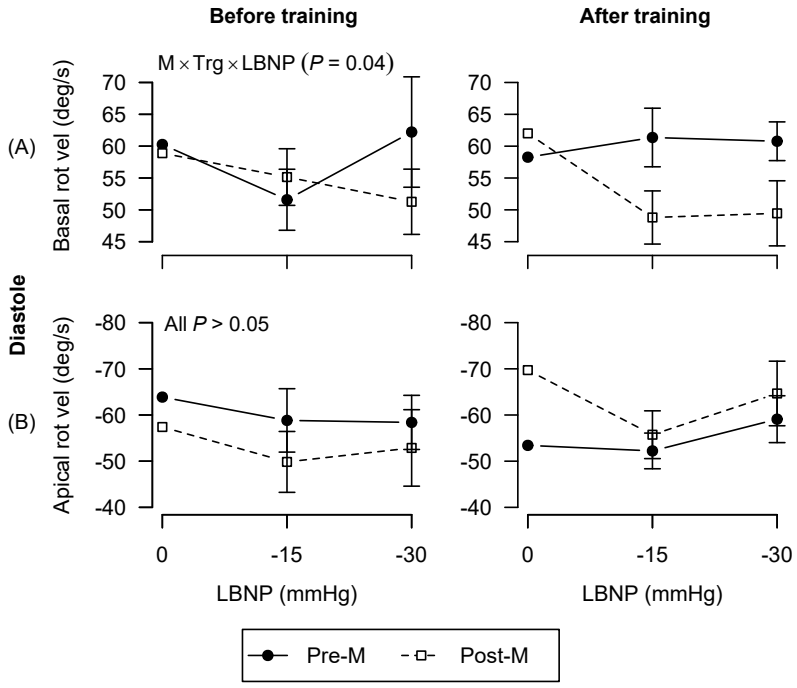

FIGURE 3: Peak diastolic basal and apical rotational velocities (rot vel) in response to lower body negative pressure (LBNP) in pre- and post-menopausal (M) women before and after exercise training (Trg; Pre-M $n = 9$, Post-M $n = 10$). Values are mean ± standard error of the change from rest.

[..[28]

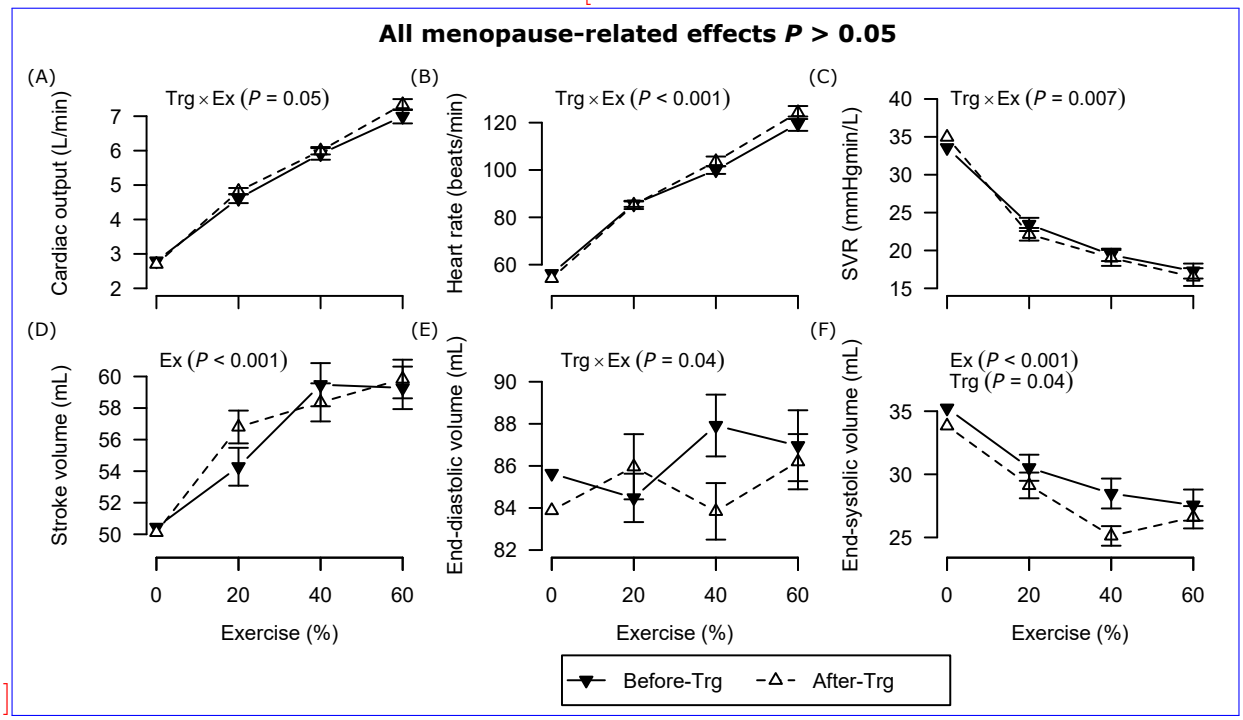

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FIGURE 4: Left ventricular function and systemic vascular resistance (SVR) in [..[29]]response to supine cycling (Ex)[..[30]]. As there was no evidence of any effects related to the menopause ([..[31]]$P > 0.05$)[..[32]], data in pre- and post-menopausal women were [..[33]]grouped together ([..[34]]effective $n = 25$) to show the effects of Ex and [..[35]]exercise training (Trg). Values are mean ± standard error of the change from rest.

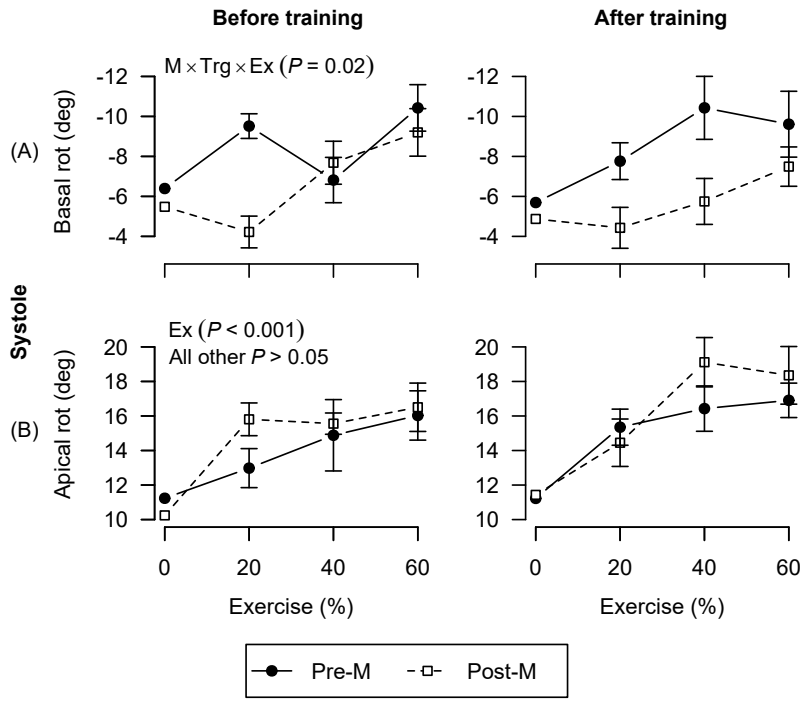

FIGURE 5: Peak systolic basal and apical rotation (rot) in response to supine cycling (Ex) in pre- and post-menopausal (M) women before and after exercise training (Trg; Pre-M $n = 8$, Post-M $n = 10$). Values are mean ± standard error of the change from rest.

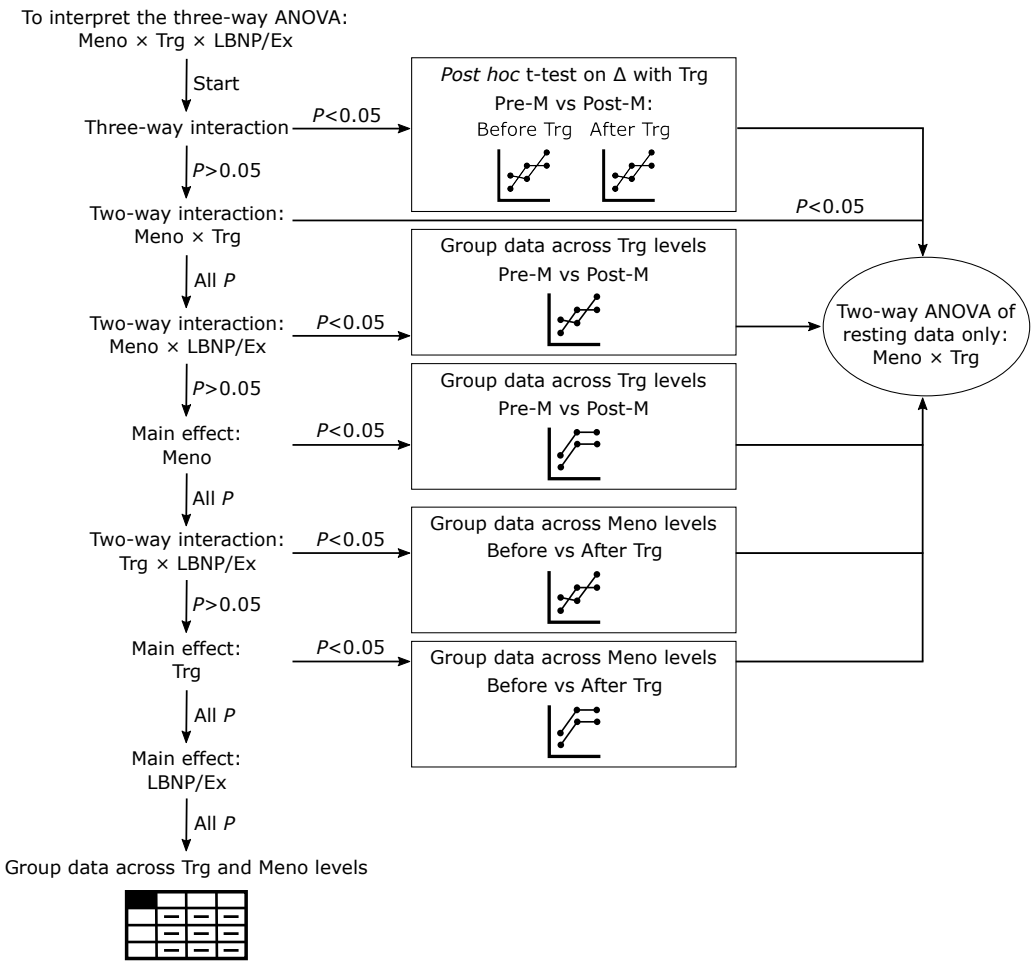

FIGURE S1: Flowchart to interpret the three-way ANOVA. The interaction and main effects of the three-way ANOVA were addressed based on their importance to our research question, which was to investigate the impact of the menopause (M/Meno) on left ventricular function and mechanics. Thus, all of the three-way ANOVA outputs that included the menopause were addressed first. Data were grouped across non-significant factors (i.e. if $P > 0.05$) to reduce complexity and aid interpretation. Graphs were used to visualise the data and to identify the source of statistically significant differences.

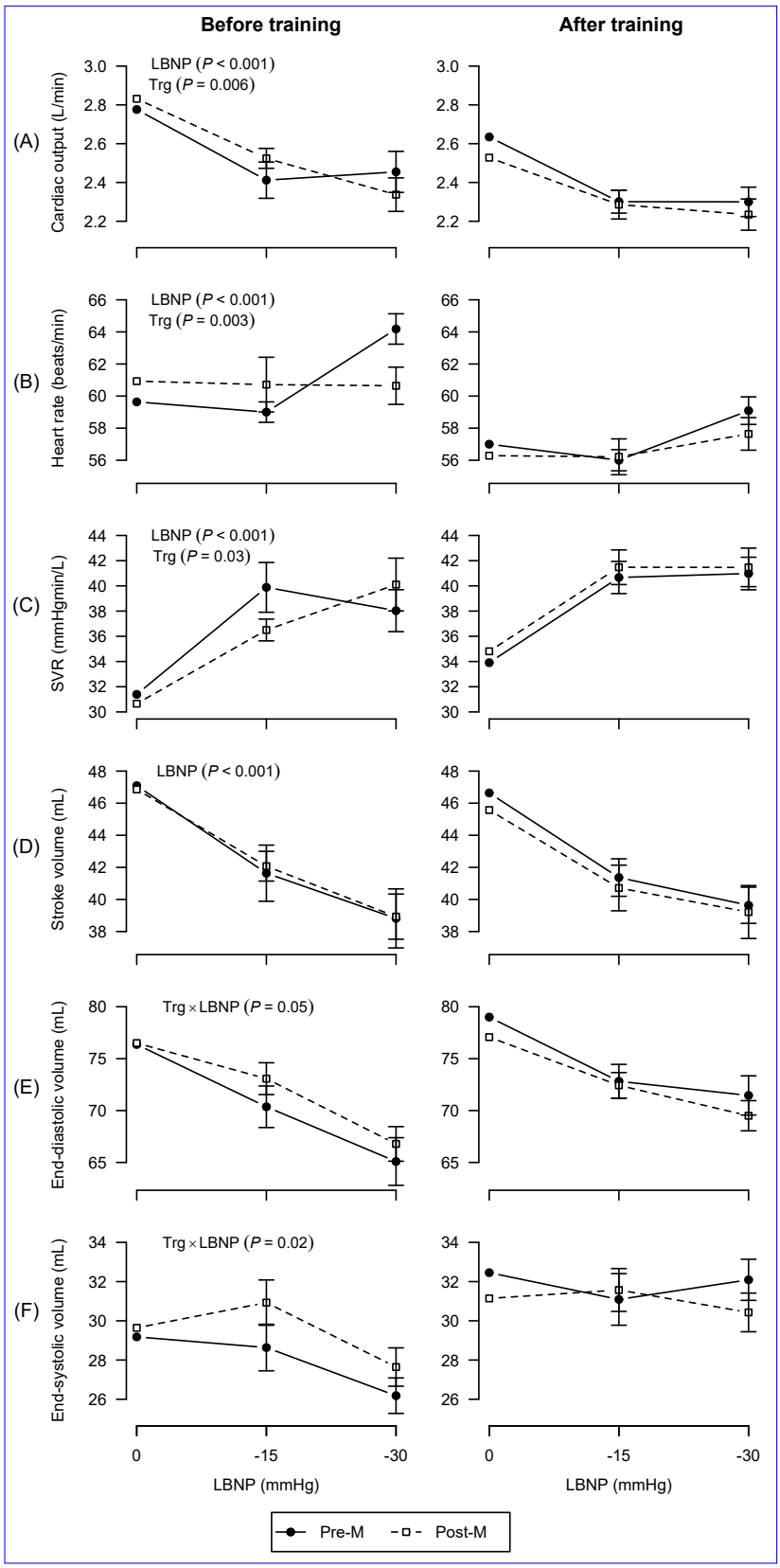

FIGURE S2: Left ventricular function and systemic vascular resistance (SVR) in pre- and post-menopausal women in response to lower body negative pressure (LBNP) before and after exercise training (Trg; Pre-M $n = 11$, Post-M $n = 14$). There was no evidence of differences between pre- and post-menopausal women (menopause effects $P > 0.05$). Values are mean $\pm$ standard error of the change from rest.

TABLE S1: Peak left ventricular (LV) mechanics during lower body negative pressure (LBNP) and supine cycling.

	LBNP (mmHg)			Exercise intensity (%)			
LV mechanics	0	-15	-30	0	20	40	60
Systolic peaks							
Twist (deg)	17.9 (4.5)	16.6 (4.5)	17.0 (4.0)	16.0 (4.7)	20.2 (4.8) †	23.3 (6.2) †‡	24.9 (6.7) †‡
Twisting velocity (deg/s)	99 (20)	98 (20)	104 (19)	88 (16)	112 (26) †	143 (37) †‡	185 (44) †‡§
Basal mechanics							
Rotation (deg)	-6.2 (2.7)	-5.6 (3.3)	-5.5 (2.7)	-5.6 (2.4)	-6.2 (3.8)	-7.6 (4.2) †	-9.1 (4.4) †‡
Rotational velocity (deg/s)	-54 (17)	-51 (14)	-53 (16)	-46 (11)	-62 (17) †	-84 (33) †‡	-118 (38) †‡§
Apical mechanics							
Rotation (deg)	12.4 (4.5)	11.8 (4.8)	12.5 (4.5)	11.0 (3.9)	14.7 (5.0) †	16.6 (5.3) †‡	17.0 (5.4) †‡
Rotational velocity (deg/s)	60 (20)	57 (22)	63 (17)	49 (13)	79 (26) †	105 (37) †‡	127 (37) †‡§
Diastolic peaks							
Untwisting velocity (deg/s)	-102 (33)	-91 (26)	-88 (22)	-93 (28)	-143 (34) †	-181 (54) †‡	-223 (65) †‡§
Basal mechanics							
Rotational velocity (deg/s)	60 (19)	54 (18)	56 (19)	55 (16)	67 (23) †	86 (23) †‡	103 (36) †‡§
Apical mechanics							
Rotational velocity (deg/s)	-61 (26)	-54 (21)	-59 (23)	-55 (23)	-92 (31) †	-116 (42) †‡	-145 (50) †‡§

Values are in mean (standard deviation). Data in pre- and post-menopausal women before and after exercise training were grouped together to show the main effects of LBNP (effective $n = 38$) and supine cycling (effective $n = 36$). †$P < 0.05$ compared with 0% exercise intensity. ‡$P < 0.05$ compared with 20% exercise intensity. §$P < 0.5$ compared with 40% exercise intensity.

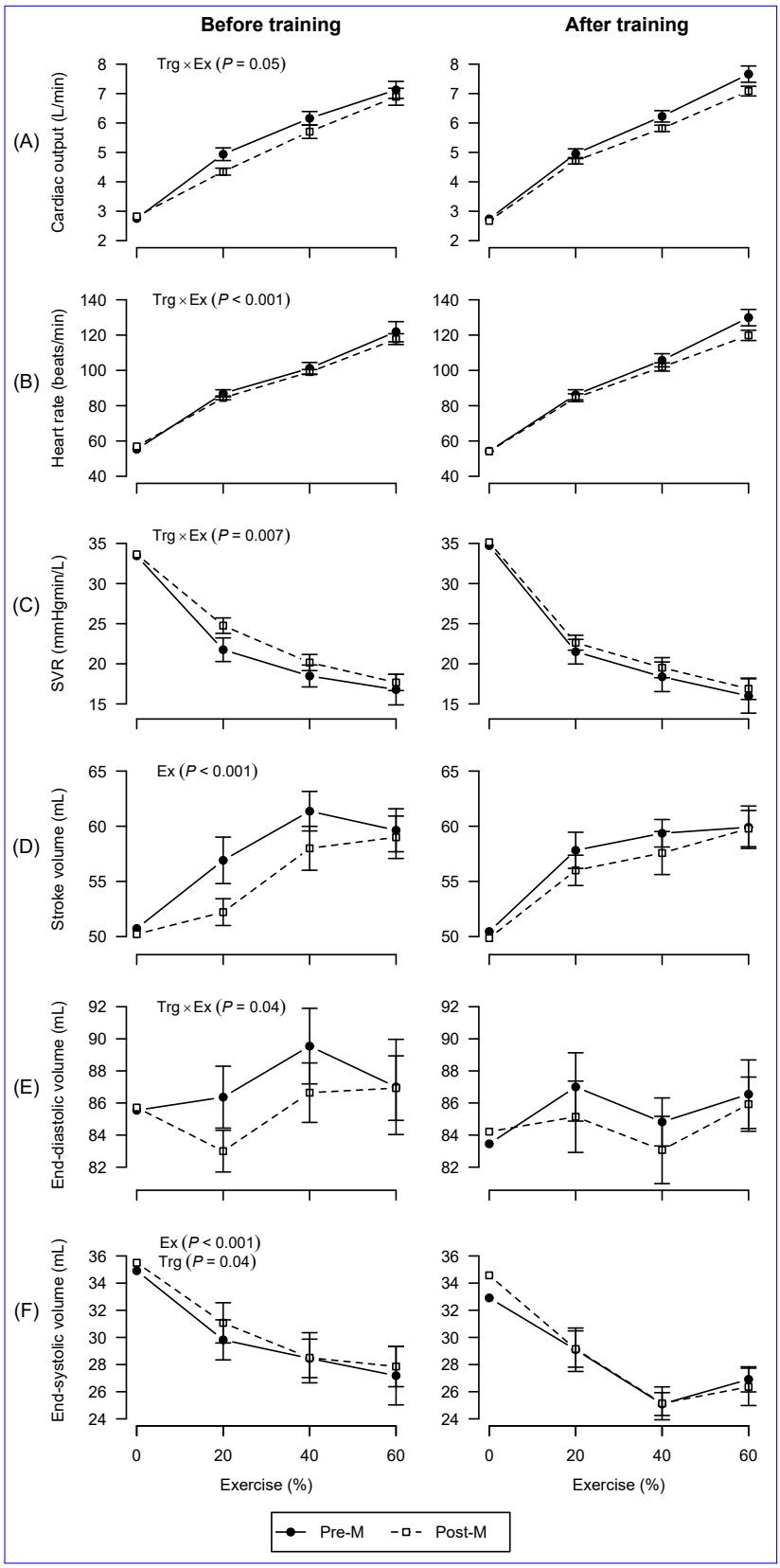

FIGURE S3: Left ventricular function and systemic vascular resistance (SVR) in pre- and post-menopausal women in response to supine cycling (Ex) before and after exercise training (Trg; Pre-M $n = 11$, Post-M $n = 14$). There was no evidence of differences between pre- and post-menopausal women (menopause effects $P > 0.05$). Values are mean $\pm$ standard error of the change from rest.

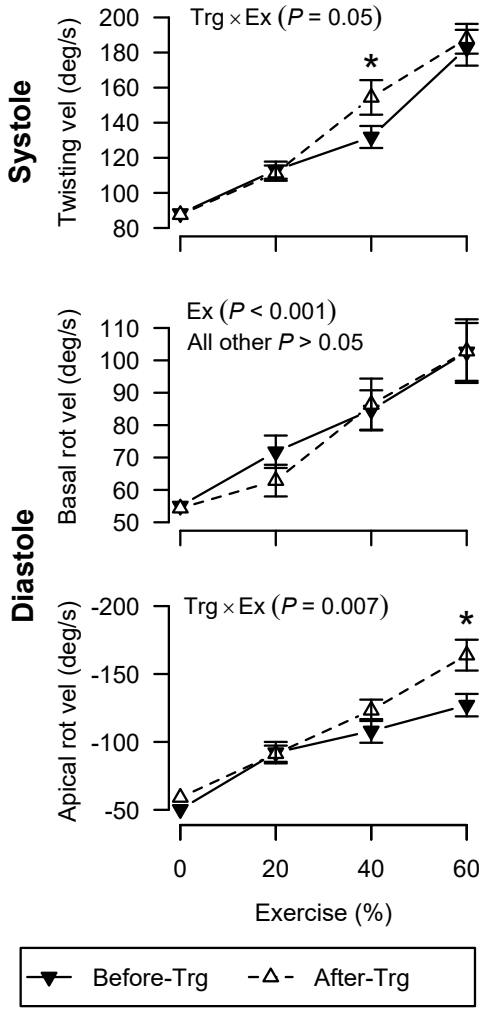

FIGURE S4: (A) Peak twisting velocity (vel), and peak diastolic (B) basal and (C) apical rotational velocity (rot vel) in response to supine cycling (Ex) before and after exercise training (Trg). Data from pre- and post-menopausal women were not statistically different (menopause effects $P > 0.05$) and were grouped for clarity. Values are mean ± standard error of the change from rest. $^*P < 0.05$ following exercise training.