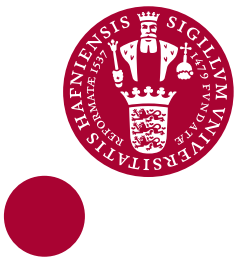

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An MRI study

Egelund, Jon; Nyberg, Michael Permin; Mandrup, Camilla M; Abdulla, Jawdat; Stallknecht, Bente; Bangsbo, Jens; Hellsten, Ylva; Larsson, Henrik Bo Wiberg

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Cardiac perfusion and function after high intensity exercise training in late pre- and recent post-menopausal women - An MRI study

Jon Egelund, MD[1], Michael Nyberg, PhD[1], Camilla M. Mandrup, MD[2], Jawdat Abdulla, MD, PhD[3], Bente Stallknecht, MD, PhD, DMSc[2], Jens Bangsbo, PhD, Dr. Sci[1], Ylva Hellsten, PhD DMSc[1], Henrik B.W. Larsson. *DMSc, MD*[4]

[1]Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark

[2]Department of Biomedical Sciences, University of Copenhagen, Denmark

[3]Department of Medicin, Division of Cardiology, Glostrup hospital, University of Copenhagen, Denmark

[4]Functional Imaging Unit, Department of Clinical Physiology, Nuclear Medicine & PET, Rigshospitalet, Faculty of Health Sciences, University of Copenhagen, Denmark

Corresponding author:

Ylva Hellsten

Universitetsparken 13

2100 Copenhagen, Denmark
Phone: 0045 35321616,

Email: yhellsten@nexs.ku.dk

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Abstract

Background: We examined the influence of recent menopause and aerobic exercise training in women on myocardial perfusion, left ventricular (LV) dimension and function.

Methods: Two groups (n=14 each) of healthy late pre- (50.2±2.1 years) and recent postmenopausal (54.2±2.8 years) women underwent cardiac magnetic resonance imaging (cMRI) at baseline and after 12-weeks of high-intensity aerobic training. Measurements included LV morphology, systolic function and myocardial perfusion at rest and during an adenosine stress test,.

Results: At baseline, resting myocardial perfusion was lower in the post- than the premenopausal group (77±3 vs. 89±3 ml/100g/min; p=0.01), while adenosine induced myocardial perfusion was not different (p=0.81). After exercise training, resting myocardial perfusion was lower in both groups (66±2; p=0.002 vs 81±3 ml/100g/min; p=0.03). The adenosine induced change in myocardial perfusion was lower in the groups combined (by 402±17 ml/100g/min; p=0.02) and the adenosine induced increase ín heart rate was 10±2 bpm lower (p<0.0001) after training in both groups. Normalization of myocardial perfusion using an estimate of cardiac work, eliminated the differences in perfusion between the pre and postmenopausal groups and the effect of training. LV mass was higher in both groups (p=0.03; p=0.006) whereas LV end-diastolic (p=0.02) and stroke (p=0.045) volume were higher in the postmenopausal group after training.

Conclusions: Twelve weeks of exercise training increased LV mass and lowered resting and adenosine induced myocardial perfusion, an effect which was likely related to cardiac work. The current data also suggests that the early menopausal transition has limited impact on cardiac function and structure.

Clinical Trial Registration information: The study was registered at ClinicalTrials.gov (NCT02135575) https://clinicaltrials.gov/show/NCT02135575

Keywords

Myocardial perfusion, Menopause, cMRI, Exercise, Adenosine

New and Noteworthy

The study provides for the first time estimates of myocardial perfusion in late pre- and recent postmenopausal women before and after a period of intense aerobic training. Resting myocardial perfusion was lower in post- than premenopausal women. Training lowered myocardial resting and stress perfusion in both groups, an effect that was likely influenced by the lower heart rate.

Introduction

Cardiovascular disease (CVD) is one of the leading causes of death globally and constitutes a major health problem in both industrialized and developing countries(4). Physical activity has been shown to be a useful strategy to reduce the risk of CVD(22), however, there is a paucity of data assessing the effect of physical activity on cardiac function and dimensions in women. The incidence of CVD rises substantially in women after menopause, which could be attributed to the marked influence of estrogen on the cardiovascular system(49). Few studies have observed a gender difference in exercise-induced cardiac adaptations and differences in vascular adaptations to exercise, depending on the female hormonal status(36, 39, 45). However, it remains unclear to what extent the hormonal changes that occur with menopause, influence cardiac adaptation to physical activity(6, 10, 21).

The magnitude of myocardial perfusion is an important functional measure of cardiac oxygen delivery. Myocardial perfusion is often assessed during resting conditions and during stress induced by infusion of adenosine or adenosine analogues. Adenosine acts as a vasodilator with potent effects on myocardial blood flow, and the cardiac response to its administration reflects the functional vasodilation capacity of the cardiac vasculature, resulting in an increased perfusion. In addition, adenosine infusion increases heart rate, and thereby the myocardial oxygen demand, an effect which may be direct or indirect. The direct activation of the sympathetic system by adenosine has been shown to be mediated primarily by A2A adenosine receptors and chemosensory excitation(9, 34), whereas the indirect effect can occur through a baroreceptor reflex in response to a concurrent fall in blood pressure (43, 44).

Impaired rest and stress induced myocardial perfusion is reported in patients with coronary heart disease and reduced myocardial perfusion has been shown to be associated with long-term prognosis for cardiovascular events(25, 37). Flow reserve, which is assessed as stress perfusion over resting perfusion, has also been found to be related to exercise capacity in patients with ischemic

cardiomyopathy(48). Although the influence of exercise training on cardiac dimensions and cardiac function are well known(18), studies on the role of physical activity on myocardial perfusion are scarce and limited to male subjects.

Traditionally, the estimation of myocardial perfusion has been conducted with positron emission tomography (PET) and single photon emission computed tomography (SPECT) (5) (24) but cardiac magnetic resonance imaging (cMRI), which is a radiation free non-invasive imaging modality, is increasingly used for assessment and quantification of myocardial perfusion(41, 42). Using an MRI contrast agent, quantitative stress myocardial perfusion measurements with cMRI has been shown to be reproducible and with similar diagnostic accuracy as PET (15). Additional advantages of cMRI are accurate measurements of cardiac morphology and function (3).

Based on findings of reduced peripheral vascular function in postmenopausal compared to premenopausal women(35, 39), we hypothesized that recent postmenopausal women have lower myocardial perfusion at rest and during adenosine induced stress, compared to premenopausal women. We also hypothesized that aerobic exercise training would increase stress induced myocardial perfusion in both pre- and postmenopausal women. To test our hypotheses, we used cMRI to examine myocardial perfusion in late pre- and recent post-menopausal women before and after a 12-week period of high intensity aerobic exercise. In addition, left ventricular (LV) dimensions and systolic function were assessed.

Methods

Study design

The study was a sub-study of a larger population study on the effect of menopause and physical activity on cardiovascular and metabolic health(33, 38, 39). It was a prospective interventional study comparing a group of late premenopausal with a group of recent postmenopausal women. The women were selected to be as close in age as possible. The included women underwent a 12-week period of high-intensity bicycle exercise training with three sessions per week. cMRI examination was conducted at rest and during intravenous adenosine infusion at baseline and after the training period. In addition, assessment of maximal oxygen uptake and blood pressure were performed.

Details of subject recruitment and procedures have been described in the previously published main study(33).

Subjects

Fifteen premenopausal and 15 postmenopausal women with a mean age of 50.2±2.1 and 54.2±2.8 years, respectively, with no reported chronic diseases, were recruited from the Copenhagen Capital region through advertisement in local newspapers as previously described(33). Before study initiation, the subjects were informed about potential risks and discomforts associated with the study. Two participants dropped out, one due to pregnancy and one due to insufficient adherence to the training program. Finally, 14 subjects in each group were included.

The study was approved by the Ethics Committee of Copenhagen and Frederiksberg municipalities and conducted in accordance with the guidelines of the *Declaration of Helsinki*. All subjects signed an

130 informed consent prior to participation in the study. The study was registered at ClinicalTrials.gov
131 (NCT02135575).

132 The menopausal status was verified by a blood sample with measured values of reproductive and
133 hypothalamic hormones. Women were excluded if the blood samples were indicative of
134 perimenopause. This assessment was based solely on hormonal level. We defined late premenopausal
135 as (regular bleedings and plasma estradiol (E_2) in the normal fertile range; follicular phase 0.05-0.51
136 nmol/l, mid cycle 0.32-1.83 nmol/l, luteal phase 0.16-0.78 nmol/l, and plasma follicle stimulating
137 hormone (FSH) <20 IU/l) and early postmenopausal as no bleeding for at least 1 year, E_2 <0.20 nmol/l
138 and FSH 22-138 IU/l. If the levels where between these values the participants were characterized as
139 perimenopausal and excluded.

140 The included premenopausal women had regular menstrual cycles. Inclusion criteria were an age
141 range of 45-57 years, BMI<30, and light to moderate physical activity <2 hours per week. Exclusion
142 criteria were smoking during the past 15 years, use of hormonal contraceptives, hormone replacement
143 treatment during the past 5 years, prescription of any medicine, any cardiovascular disease, renal
144 dysfunction, diabetes or other chronic diseases incompatible with the present study. All subjects had
145 electrocardiograms (ECG) with normal sinus rhythm and without signs of arrhythmias or ischemic
146 changes. To ensure that all subjects were normotensive, blood pressure was measured 7 times over 120
147 minutes of rest in the supine position by an automatic upper arm blood pressure monitor (M7,
148 OMRON, Vernon Hills, IL, USA) with the first measurement obtained after at least 15 min of rest. The
149 inclusion cutoff level for the blood pressure was 145/90 mmHg. Heart rate (HR) was measured during
150 the blood pressure monitoring.

151 ***Exercise intervention***

152 The training was performed on a spinning bicycle (Body Bike, Frederikshavn, Denmark). Instructors
153 from the research group supervised two training sessions per week and instructors from a local fitness
154 center supervised one weekly session. HR was monitored during all training sessions (TEAM2

Wearlink+, Polar, Kempele, Finland). The training sessions were conducted as intermittent high-intensity intervals where subjects reached HRs above 85 % of maximum HR. The length of the training sessions was estimated to be approximately 50 minutes. Detailed information on the participants' variation in exercise intensities during the training are reported elsewhere(33, 39).

Heart rate monitoring and compliance of training

The participants had an individual HR monitor (TEAM2 Wearlink+, Polar, Kempele, Finland) to record their HR during training sessions.

Determination of peak oxygen uptake

Peak oxygen uptake (VO_2peak) was measured with an Oxycon Pro (Intramedic, Denmark). The protocol was an incremental exercise test on a bicycle ergometer (Monark, E9). The participants started with a 10min warm-up and thereafter the test was initiated with a start load of 50 W and increased by 25 W per minute until volitional fatigue. Criteria for determination of VO_2peak were: A plateau in VO_2, even with increased workload and/or respiratory exchange ratio >1.1 and/or a HR > 90 % of expected value. Two out of three criteria had to be attained before the test was approved. Maximal HR (HR_{max}) during the VO_2peak test was recorded.

The VO_2peak test and the cMRI measurements were conducted in the weeks before the initiation of exercise training and between 2 and 5 days after ending the exercise intervention.

Cardiac magnetic resonance imaging

All subjects were instructed to fast overnight and abstain from caffeine-containing products for 24 h before the examination. Two venous cannulas were placed in each antecubital vein for the contrast agent and adenosine infusion, respectively. Cardiac magnetic resonance imaging (cMRI) was

performed with a clinical MAGNETOM Avanto 1.5-Tesla scanner (Siemens, Erlangen, Germany) with a 64-channel cardiac chest coil combined with back surface coils. The study subject was placed in a head-first supine position.

After obtaining initial localizing images, short-axis cine images were acquired using an ECG-gated, balanced steady-state free precession gradient-echo sequence with retrospective gating at end-expiratory breath hold providing dynamic volume images. Slice thickness was 6 mm, and a stack of 10-15 slices in the true short-axis plane with no inter-slice gaps covered the LV. Field of view was 400 x 400 mm^2, with a matrix size of 155 x 208. Each slice of the LV was obtained over about 15-30 heartbeats with ECG triggering, with a scan rate of 20 images per cardiac cycle. The following MRI parameters were used: TR, 474 ms; TE, 1.14 ms; flip angle, 80 °; BW, 1149 Hz/pixel; Grappa acceleration factor 2.

Separate automatic injectors were used to infuse the MRI contrast agent and adenosine through intravenous catheters for the perfusion measurement. Perfusion images were obtained by three short-axis slices (basal, mid-ventricular, and apical) during the first-pass of the contrast agent, using an ECG-gated, end-expiratory breath hold, single-shot gradient-echo saturation recovery TurboFlash sequence using RF spoiling. TE, 1.02 ms; TR, 191 ms; flip angle, 12°; TD, 130 ms (time from the 90 degree prepulse and the first read-out pulse); field of view 300 mm x 400 mm, matrix, 96 × 160; linear phase encoding; slice thickness, 10 mm, GRAPPA acceleration factor 2, bandwidth: 651 Hz/pixel. The MRI contrast agent (Gadovist; Bayer Schering Pharma, Berlin, Germany) was administrated as a bolus of 0.1 mmol/kg body weight at a rate of 5 mL/s, followed by 15 mL of saline at the same rate. One frame (three slices) per cardiac cycle was obtained, with a total of 60 frames of dynamic acquisitions. Stress perfusion (first perfusion measurement) was determined after 3 minutes of adenosine infusion (140 μg/kg/min). Adenosine infusion was stopped immediately after image acquisition, and the total duration of adenosine infusion was approximately 4 minutes. Rest perfusion images were obtained at least15 minutes after the adenosine infusion. Subjects were instructed to hold their breath for as long as

possible during the time of all image acquisitions, and thereafter to breathe slowly, during the scanning. The stress response was determined first as this was the most important parameter and based on our previous experience, the rest measurements were unlikely to be affected by the prior test.

Data analysis

Left ventricle morphology

Using the Argus software (Syngo MR B17 Argus, Siemens), the LV endo-myocardial and epi-myocardial borders were annotated and LV volume was calculated for both systole and diastole in addition to the LV myocardial volume. Papillary muscles were considered as part of the ventricle cavity, as commonly done. LV diameter and wall thickness in diastole were obtained using 3-chamber images. The wall thickness and the endocardial borders were manually measured on two different images a 3 chamber and a 4-chamber, which were then averaged

Left ventricle function

Stroke volume (SV), Cardiac output (CO) and ejection fraction (EF) were automatically calculated with the annotated LV endo-myocardial and epi-myocardial borders in diastole and systole, with use of the Argus software.

Rate pressure product

The rate pressure product (RPP) for the subjects at rest was calculated from the mean systolic blood pressure (SBP) of 5 measurements and HR (RPP= SBP x HR) obtained on a separate day.

Myocardial perfusion calculation

The mid-ventricular slice was used for perfusion evaluation. The slice was obtained in the systole with maximal contraction and thickness and thus without much partial volume effect. The outer and inner border of all frames of the slice were semi-manually annotated, especially avoiding inclusion of the ventricular volume, and the MR signal as a function of time for the entire slice was used. In addition, a similar MR signal was obtained from a region of interest in the LV (avoiding inclusion of any papillary

muscle) and used as an arterial input function (AIF). Both tissue MR signal and AIF were normalized to baseline frames before arrival of contrast agent, and baseline constituted about 10 frames, in order to account for coil sensitivity inhomogeneity, followed by baseline subtraction. Assuming a reasonable linearity between the baseline normalized MR signal and the contrast agent, fast water exchange between various tissue compartments (due to a short TD) (28) and equal relaxativity in tissue and blood, a model free deconvolution, based on Tikhonov approach with L-curve regularization was applied in order to estimate perfusion(26, 27). Perfusion was reported in ml/100g/min, assuming a tissue density of 1 g/ml. The AIF from the first perfusion measurement (the stress scan) was also used in the calculation of the following rest perfusion measurement, as the AIF obtained in the second scan was confounded by the previous contrast injection, resulting in a diminished AIF. Therefore, the AIF obtained during the rest scan was scaled to the same size (peak height) of the first AIF.

Resting perfusion was normalized to the product of mean arterial pressure and cardiac output. Adenosine induced perfusion was normalized to heart rate.

Statistical analysis

We used fixed-effect factors with “group” (premenopausal and postmenopausal) and “time” (before and after training), and an interaction term between group and time that was evaluated directly as differences between groups and within groups using a linear mixed model framework. Between-subject variation was modelled using random effects. Model assumptions on homogeneity of variance and normal distribution were confirmed through residual and Q–Q plots. Data are reported as means ± SEM unless otherwise stated. Subject characteristics were evaluated and compared by use of student’s t-test (Excel 2010, Microsoft). An alpha level of ≤ 0.05 was considered significant. The effect size was 14.3 for a power of 0.8 for resting myocardial perfusion.

All statistical analyses were executed using R statistical package ver. 3.2.2 (R Core Team 2015) through RStudio interface (RStudio Team 2015, Inc. Boston, MA) with the extension packages lme4 and multcomp.

Results

Subject characteristics

The baseline characteristics of subjects are shown in Table 1. The postmenopausal women had been postmenopausal for a mean of 3.1±0.5 years. Systolic blood pressure was similar in the two groups before and after the exercise intervention. Diastolic blood pressure was the same in the pre- and postmenopausal group before training, but reduced by 7% in the postmenopausal group after exercise training (p=0.02; Table 1).

The attended average number of training sessions for the pre- and postmenopausal group was 37±7 (93% compliance) and 35 ±5 (88% compliance), respectively. The average duration of the training session was 53 and 52 min for the pre- and postmenopausal groups, respectively (Table 1).

Myocardial perfusion

Resting myocardial perfusion was lower in the postmenopausal compared to the premenopausal group both at baseline (13.5%, p=0.009) and after the training period (6.5%, p=0.001; Fig. 1, Table 2). After the training period, resting myocardial perfusion was lower (pre-menopausal by 9%, p= 0.036 and post-menopausal by 14%, p=0.002) without any interaction, Fig. 1, table 2.

Adenosine induced myocardial perfusion was similar between the two groups both at baseline and after training. After the training period, adenosine induced myocardial perfusion was lower for all of the subjects combined (by 402±17 ml/ 100g/min; p=0.02; Fig. 3), but the change was not significant for the separate groups (premenopausal, p=0.16; postmenopausal; p=0.08). There was no interaction, i.e. no difference between the two groups in the effect of training (Fig. 1, Table 2).

Peak HR during adenosine infusion was lower in both groups after the training period (premenopausal by 10±3bpm, p=0.001; postmenopausal by 11±3bpm, p=0.0004; Table 2). When

resting and adenosine induced perfusion were normalized to CO x MAP or HR, respectively, there were no longer any significant differences between groups or with the training intervention. Flow reserve, expressed as adenosine induced myocardial perfusion divided by resting myocardial perfusion, was higher (p=0.01) in the postmenopausal compared to the premenopausal women after training.

Cardiac dimensions

LV mass was similar between the two groups at baseline and higher in both groups after training (4%; p=0.03; in the premenopausal and 6%; p=0.005 in the postmenopausal group) (Fig. 2, Table 2).

LV end diastolic diameter (LVEDD), interventricular septum (IVS) and LV posterior wall thickness (LVPWD) were similar between the two groups and did not change with the training period. LV end-diastolic volume (LVEDV) was similar between the groups both before and after the training period. In the postmenopausal group, LVEDV was higher (6%, p=0.02) after than before the training period (Table 2).

Cardiac function

At baseline, stroke volume at rest was similar in the pre- and postmenopausal groups, whereas resting HR and cardiac output (CO) was lower in the postmenopausal compared to the premenopausal group (7%, p=0.05 and 19%, p<0.0001, respectively). After the training period, CO at rest was lower than before the training period in the premenopausal group (10%, p=0.0007). In the postmenopausal women, stroke volume at rest was higher (5%; p=0.045) after compared to before the training period with a corresponding lower HR (5%; p=0.04) and an unaltered CO.

Resting RPP was lower in both groups (7.3 %, p=0.001 in the premenopausal and 7 %, p=0.006 in the postmenopausal group) after compared to before the training period. LVEF was similar between the two groups and did not change with the training period (Table 2).

Discussion

In the present study, quantitative stress myocardial perfusion and LV morphology and function were assessed using cMRI before and after 12 weeks of high intensity aerobic cycle exercise training in late premenopausal and recent postmenopausal women, close in age and matched for BMI. The major findings were; i) baseline resting myocardial perfusion was lower in the postmenopausal compared with the premenopausal women, whereas adenosine induced stress perfusion was similar in both groups; ii) after exercise training, myocardial perfusion at rest was lower in both groups of women and adenosine induced stress perfusion was decreased the two groups combined. iii) Normalization of myocardial perfusion using an estimate of cardiac work, abolished both the differences in myocardial perfusion between the groups and the effect of training, iv) LV mass was similar between the two groups at baseline and increased similarly in both groups after training; iv) cardiac systolic function was similar between the two groups and was not altered by training.

Myocardial perfusion

We tested the hypothesis that resting and stress induced myocardial perfusion were lower in recent postmenopausal women than in late premenopausal women and that perfusion was enhanced by exercise training. The postmenopausal women were found to have lower myocardial perfusion than the premenopausal women at rest whereas stress induced perfusion was similar between the groups. The lower resting perfusion in the postmenopausal compared to the premenopausal women would suggest an impaired cardiac microvascular function as microvascular function in skeletal muscle has been shown to be impaired after menopause (39). A potential cause of impaired microvascular function with estrogen loss could be dysfunction of two of the central vasodilator systems; nitric oxide and prostacyclin. Estrogen has been shown to have a strong impact on both the expression of endothelial nitric oxide synthase as well and on nitric oxide bioavailability (20) . Moreover, postmenopausal women present a reduced prostacyclin sensitivity (39)(32).

However, as the difference between the two groups was abolished when perfusion was related to an estimate of metabolic work, a likely explanation for the difference in perfusion was a difference in cardiac work. Lower cardiac work in the postmenopausal women could have been due to a lower basal metabolic rate compared to the premenopausal women, as reported in other studies (1, 31). Further studies are required to further evaluate this intriguing aspect.

The period of intense aerobic training led to a lower resting myocardial perfusion in both groups. This observation is, to our knowledge, the first in women but it is in line with previous observations of lower resting perfusion in middle-aged men undergoing intense exercise training (8, 11). A plausible explanation for a training induced lower myocardial perfusion is a greater oxygen diffusion capacity and thereby enhanced oxygen extraction, as a result of increased capillarization (7, 30) . Alternatively, the lower perfusion could be due to a more optimal distribution of blood flow within the cardiac tissue (29). Moreover, heart rate and RPP at rest were lower after training in both groups, and the effect of training was eliminated when perfusion was normalized to estimated cardiac work, suggesting a change towards less resting myocardial oxygen consumption.

The exercise training lowered the stress induced myocardial perfusion when the two groups were combined ($p=0.02$), although this effect was not statistically significant in the separate groups (premenopausal $p=0.16$; postmenopausal $p=0.08$). Interestingly, a substantial decrease in the heart rate response to adenosine of approximately 10 beats/min occurred in association with the lowering of stress perfusion after training. Adenosine is known to potently induce coronary vasodilation but also to increase sympathetic activity indirectly through baroreceptor mediated reflex or potentially through activation of A2A adenosine receptors (9, 34, 43, 44). During the stress perfusion before training, heart rate increased by approximately 30 bpm and thus the concurrent increase in stress-induced myocardial perfusion could reflect the corresponding increase in myocardial oxygen consumption. The observed 10 beats/min decrease in heart rate response and stress-induced myocardial perfusion after training may reflect a lower chronotropic effect of adenosine and a corresponding lesser need for oxygen delivery.

This possibility was strengthened by the finding that the difference in perfusion was abolished when normalized to an estimate of cardiac work. A lower response in the adenosine induced tachycardia could be due to a change in A2A adenosine receptors. However, in a study by Heinonen et al. (16) it was shown that the cardiac density of the A2A receptor was similar in untrained and endurance trained individuals. Nevertheless , sensitivity and distribution of the A2A receptors may still be a plausible explanation for the reduced adenosine induced tachycardia after training and the training induced lowering of adenosine responsiveness should be further examined in future studies. In a study by Eskelinen and co-workers, the effects of a two-week moderate and high-intensity training regimes were compared and the authors found that a period of high intensity training lowered adenosine induced myocardial perfusion whereas training at moderate intensity had no significant effect (11). In addition, several studies have reported that myocardial perfusion is lower in well trained individuals compared to untrained controls (16, 32). In these cross-sectional studies, it was reported that endurance trained men presented an almost 50% lower increase in heart rate with adenosine infusion compared to the untrained men whereas blood pressure did not change in any of the groups (16, 32). An intriguing parallel to the lower adenosine induced tachycardia and perfusion in trained individuals is the finding that aerobic training reduces the vasodilator response to intravenously infused adenosine in skeletal muscles (11, 17). This could indicate that adenosine desensitization may be a general phenomenon in response to training in the cardiovascular system. Taken collectively these results suggest that exercise training reduces adenosine induced myocardial perfusion, potentially due to an attenuated chronotropic effect of adenosine and a lowering of the vascular responsiveness to adenosine. It should be mentioned that not all studies have shown decreased perfusion after exercise training, for example, use of the adenosine reuptake inhibitor dipyridamole has shown a more pronounced myocardial perfusion in trained compared to untrained individuals (8, 23).

Cardiac dimensions and function

Before training, the pre- and postmenopausal groups had similar cardiac dimensions and function with the exception that the postmenopausal women had approximately 20% lower resting cardiac output. Since arterial oxygen carrying capacity, as indicated by hemoglobin concentrations, was similar in the two groups of women, the finding of a lower cardiac output may suggest a lower resting whole body energy expenditure in the postmenopausal women (1,26), as mentioned above.

The exercise training intervention, which improved maximal aerobic power, led to an increase in LV mass in both groups and an improvement in EDV and SV only in the postmenopausal women. These observations are in accordance with previous findings on the effect of a period of endurance training in younger women (2, 19, 47) and suggest that training can induce cardiac adaptations also in midlife women. However, the change in LV mass in our study was relatively small compared to that observed with one year of intense aerobic training in a group of young men and women (2) (19). Although estrogen has been shown to promote cardiac growth (50), the adaptation in LV mass in the abovementioned study was found to be lower in the young women than in the young men (2), suggesting a limited effect of estrogen. In accordance, as the LV mass adaptation in the current study was similar in the pre- and postmenopausal women, estrogen per se does not appear to have had a significant influence on the adaptation.

Cardiac perfusion assessed by MRI

Validation of our method has previously been published (12, 41, 46) Generally, MRI perfusion estimation seems better than SPECT, as shown in a multi-center study comprising 234 patients (46). We performed two comparison with PET : 13N-ammonia PET (12) and rubidium-82 PET (41). Both studies showed significant correlation between MRI and PET estimated myocardial perfusion. For example, stress minus rest correlation between the modalities were for right coronary artery (RCA) 0.78, left anterior descending artery (LAD) 0.79, and left circumflex artery (LCX) 0.88. The

corresponding correlation for myocardial perfusion reserve (stress/rest ratio) were (RCA) 0.89, (LAD) 0.88, (LCX) 0.88. A Bland-Altman analysis showed no bias of any of the modalities.

Study limitations Cardiac perfusion assessed by MRI

One of the limitations in the present study was that the pre-menopausal women were not all examined in the midfollicular phase. It has been shown that vascular function varies through the menstrual cycle, but similar studies have not been conducted for the heart. However, different physiological levels of acute estrogen supplementation as well as regular estrogen supplementation have been shown not to influence myocardial perfusion (14, 40). A large effect of the menstrual cycle on perfusion is therefore unlikely.

It cannot be excluded that there is a systematic difference between myocardial perfusion measurements as assessed by PET and the current MRI methodology as PET tracers and MRI contrast agents differ in their diffusion properties and quantification of myocardial perfusion depends on the exact tracer kinetics model being used (13). It is however, important to point out that a potential difference in absolute values between the MRI method and PET is unlikely to affect the differences before groups and with the training intervention. Also, a single observer performed the measurements in the present study and it cannot be excluded that this could have resulted in some analytical bias.

Another study limitation was that the number of participants was limited and the data should therefore be interpreted with caution. Furthermore, as the women were all healthy it is particularly not clear whether the findings are relevant for women with lifestyle-related disease. Finally, the design of the current study was cross-sectional for the pre- and post-menopausal women and a longitudinal design in which pre-menopausal women are followed over many years into menopause is warranted to provide further support for the current findings.

Conclusion

The present study shows that myocardial perfusion at rest is lower in recent postmenopausal compared to late pre-menopausal women and that a 12 week period of intense aerobic interval training results in a decrease in resting and adenosine induced myocardial perfusion in the pre- and postmenopausal women. Potential explanations for the lower resting myocardial perfusion after training could be lower myocardial work, as the difference was no longer present when perfusion was normalized to an estimate of cardiac work. An additional contributing effect could be enhanced oxygen diffusion conditions through increased capillarization (7). Moreover, as training resulted in a lowering of heart rate in response to adenosine infusion, and as the difference in adenosine stress perfusion before and after training was no longer significant after normalization to cardiac work, we propose that the lower adenosine induced myocardial perfusion, at least in part, was due to a lower chronotropic effect of adenosine and a consequent lower myocardial oxygen demand. Finally, as the late pre- and recent postmenopausal women showed only small differences in cardiac structure, function and myocardial perfusion, we suggest that aging and/or long-term inactivity may have greater influence on the heart than estrogen alone, however, the role of estrogen deficiency has to be evaluated in a study design in which estrogen is manipulated directly.

Overall, the findings of this study are important as they clearly show that a period of intense aerobic exercise training is effective in inducing clinically relevant myocardial adaptations.

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Figure captions

Figure 1. *Myocardial perfusion at rest (A) and during adenosine stress perfusion (N=14)(B) in late pre- and recent postmenopausal women (N=13 and N=14) before and after 12 weeks of intense aerobic cycle exercise training. Differences between groups at measurement point†: p<0.05. Changes from baseline to 12 weeks within group: *: p<0.05*

Figure 2. *Individual myocardial adenosine stress perfusion responses to 12 weeks of intense aerobic cycle exercise training for the late pre- and postmenopausal (N=13 and N=14) women expressed as absolute change from baseline.*

Table 1. *Subject characteristics*

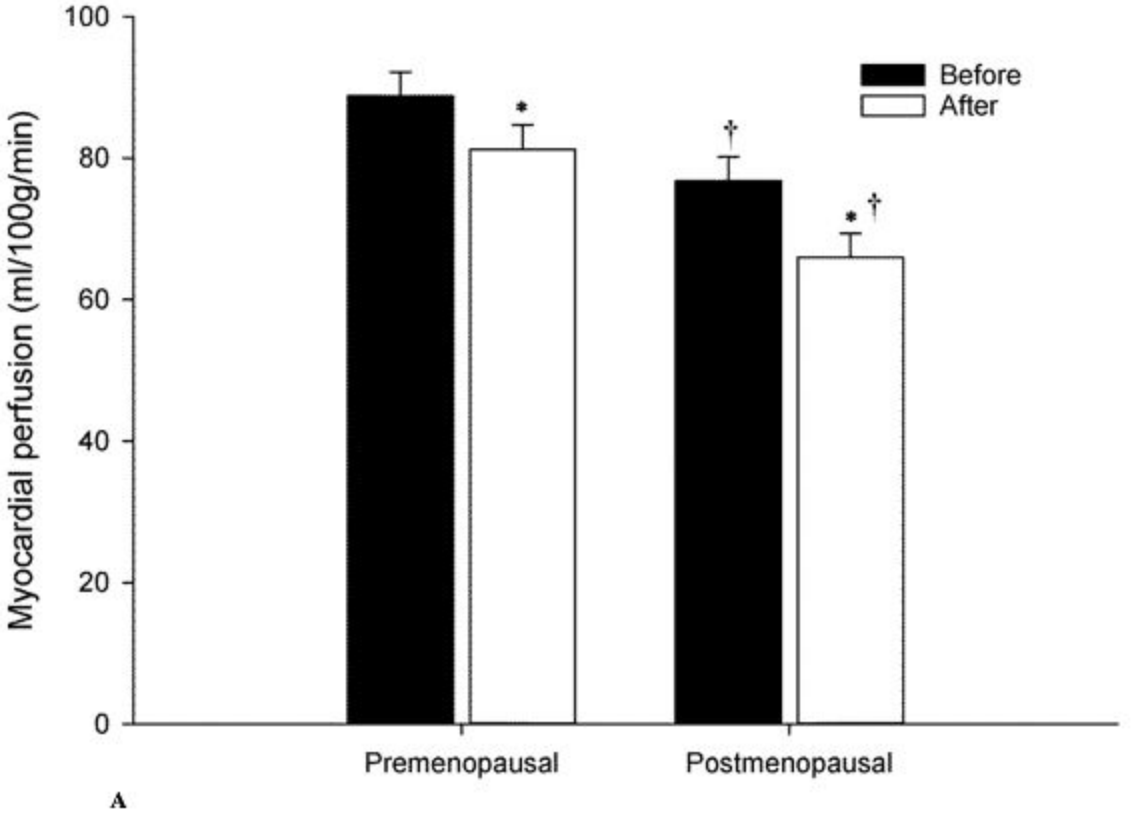
	Premenopausal			Postmenopausal			Group comparison	
	Baseline (n=14)	**12 weeks (n=14)**	**Training effect *p***	**Baseline (n=14)**	**12 weeks (n=14)**	**Training effect *p***	**Baseline *p***	**12 weeks *p***
Anthropometrics								
Age	50.2 ±2.1			54.3 ±2.8		-	**0.0002**	
Height (m)	1.69 ±0.1			1.66 ±0.1		-	0.22	
Weight (kg)	70.8 ±4.8	69.6 ±4.5	**0.019**	66.3 ±8.1	66.1 ±8.2	0.71	0.09	0.18
BMI ($kg/m^{2)}$	24.8 ±2.2	24.4 ±2.1	**0.018**	24.0 ±2.5	23.9 ±2.7	0.77	0.36	0.62
Blood Pressure								
Systolic (mmHg)	114±15	113±13	0.99	113±16	108±15	0.075	0.99	0.40
Diastolic (mmHg)	74±10	72±8	0.95	74±9	69±9	**0.02**	0.95	0.41
Mean arterial pressure (MAP) mmHg	87±12	85±10	0.27	87±11	82±1	**0.03**	0.97	0.39
Resting heart rate	69±8	65±7	**0.0005**	64±6	62±6	0.20	**0.05**	0.24
Rate pressure product	7901±327	7327±327	**0.001**	7187±327	6699±327	0.006	**0.12**	0.17
Cardiorespiratory fitness								
$VO_{2\text{-peak}}$ (ml O_2/min)	2079 ±271	2284 ±276	**0.009**	1921 ±216	2060 ±187	**0.007**	0.10	**0.02**
Cycling Watt end VO_2-peak test	196 ±24	220 ±28	**<0.0001**	186 ±16	204 ±17	**0.003**	0.18	0.08
HR_{max}	179 ±8	174 ±8	**0.003**	173 ±9	170±8	0.35	0.01	0.23
Training compliance								
Sessions	37 ±7			35 ±5			0.26	
Duration of sessions (min)	53 ±2			52 ±5			0.29	
Training intensity								
60-85 % of HR_{max} (%)	53±11			51±19			0.72	
86-100% of HR_{max} (%)	45±12			43±18			0.76	

Table 1. *Data are presented as mean (±Standard deviation). BMI: Body mass index; HR_{max}: maximal heart rate during test; $VO_{2\text{-peak}}$: Peak maximal oxygen consumption.*

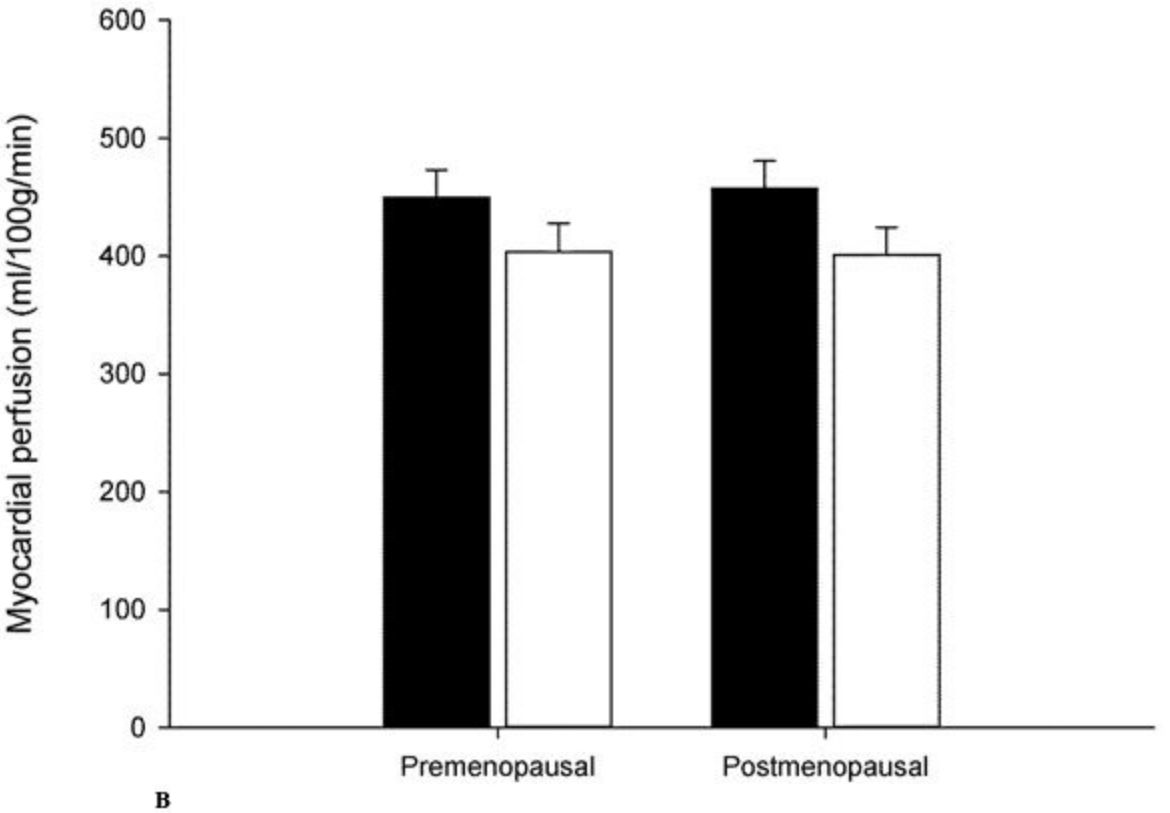
Table 2. *Cardiac morphology, function and perfusion*

	Premenopausal			Postmenopausal			Group comparison	
	Baseline (n=14)	**12 weeks** (n=14)	**Training effect** ***p***	**Baseline** (n=14)	**12 weeks** (n=14)	**Training effect** ***p***	**Baseline** ***p***	**12 weeks** ***p***
Dimensions								
LVEDD (cm)	4.75±0.1	4.78±0.1	0.52	4.88±0.1	4.92±0.1	0.42	0.29	0.26
IVS (cm)	0.82±0.03	0.87±0.03	0.12	0.84±0.03	0.84±0.03	0.37	0.73	0.56
LVPWD (cm)	0.72 ±0.02	0.74±0.02	0.23	0.70±0.02	0.71±0.02	0.52	0.58	0.37
Function								
LVEF (%)	66.8±1.2	66.0±1.2	0.31	65.0±1.2	65.1±1.2	0.84	0.27	0.58
Volume and mass								
LVEDV (ml)	129±4	131±4	0.52	120±4	127±4	**0.02**	0.14	0.48
LVEDV index (ml/m^2)	71±2	73±2	0.35	69±2	73±2	**0.01**	0.51	0.91
LVESV (ml)	43±2	44±2	0.20	42±2	44±2	0.11	0.90	0.99
LVESV index (ml/m^2)	24±1	25±1	0.15	24±1	26±1	0.08	0.71	0.60
SV (ml)	86±3	86 ±3	0.97	78 ±3	82±3	**0.045**	**0.05**	0.34
CO (l/min)	5.97±0.2	5.39 ±0.3	**0.0008**	4.85 ±0.2	4.93±0.2	0.64	**<0.0001**	0.11
LV mass (g)	91.6±3.6	95.6 ±3.6	**0.03**	84.5 ±3.6	89.6±3.6	**0.006**	0.16	0.24
LV mass index (g/m^2)	50.6±2.0	53.1 ±2.0	**0.02**	48.7 ±2.0	51.9±2.0	**0.004**	0.51	0.65
Perfusion								
$Perf_{Rest}$ (ml/100g/min)	89±3	81±3[a]	**0.036**	77±3	66±23	**0.002**	**0.01**	**0.001**
$Perf_{Ado}$ (ml/100g/min)	449±23	402±24[a]	0.17	457±23	401±23	0.09	0.81	0.94
Heart rate at rest (bpm)	73±2	69±2	**0.002**	64±2	61±2	**0.04**	**0.002**	**0.017**
Heart rate during adenosine (bpm)	100±3[b]	90±3[a]	**0.001**	98±3[a]	87±3[a]	**0.0004**	0.51	0.45
Flow reserve	5.2±0.3	5.0±0.3[a]	**0.72**	6.1±0.3	6.2±0.3	**0.81**	0.05	0.01
rCOxMAP (mmHg/l/min)	524±27	463±27	**0.004**	420±27	405±27	0.50	**0.006**	0.13
$Perf_{Rest}$/rCOxMAP (AU)	0.18±0,01	0.18±0.01[a]	0.76	0.19±0.01	0.17±0.01	0.20	0.59	0.43
$Perf_{Ado}/HR_{Ado}$ (ml/beat x100g)	4.53±0.39[b]	4.87±0.34[a]	0.45	4.79±0.34[a]	4.56±0.34[a]	0.60	0.60	0.54

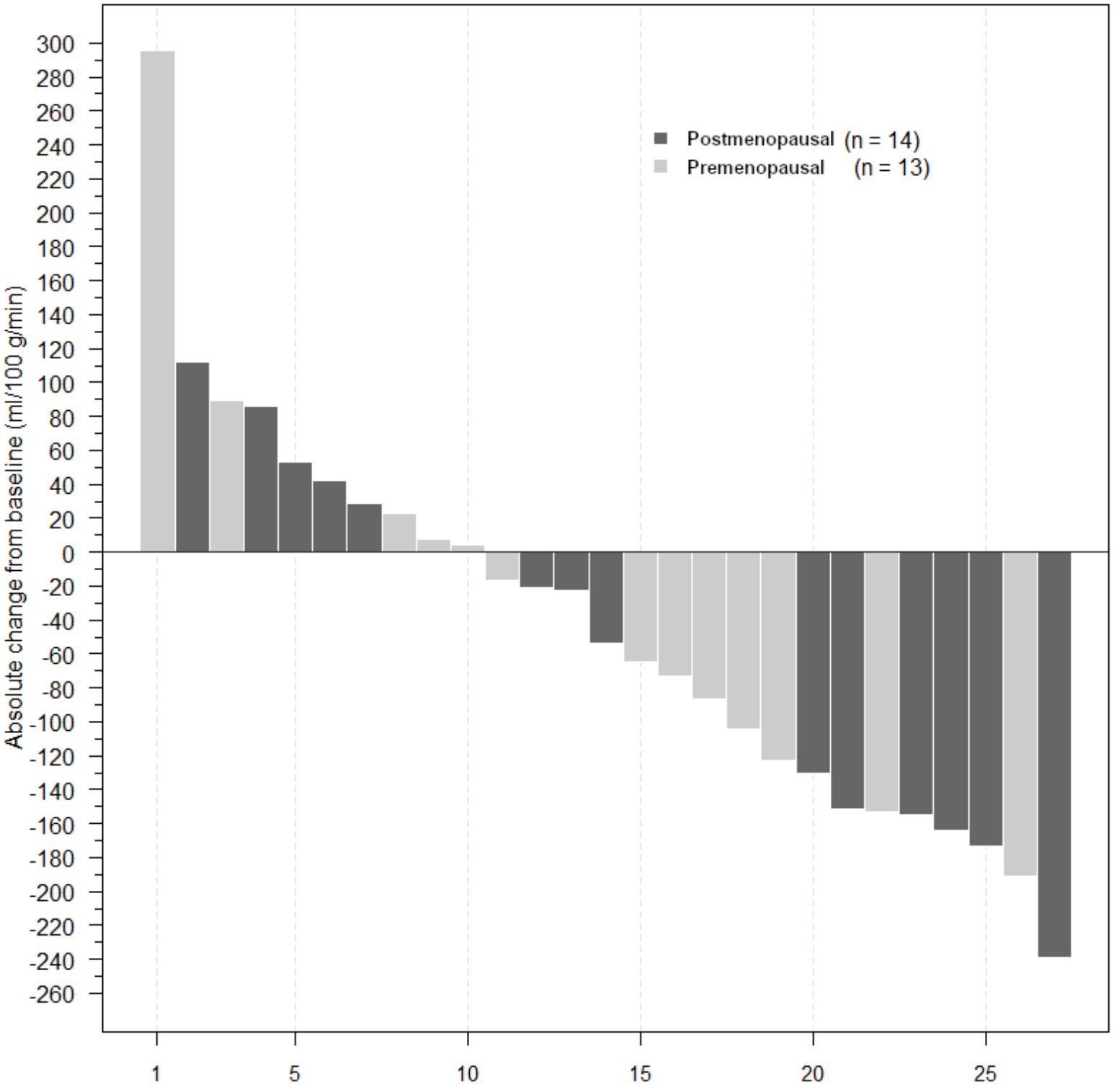
Table 2. *Data are presented as mean (±SEM). LVEDD: Left ventricular (LV) end-diastolic diameter; IVS: interventricular septum; LVPWD: LV posterior wall thickness; LVEF: LV ejection fraction; LVEDV: LV end diastolic volume; LVESV: LV end systolic volume; SV: stroke volume; CO: cardiac output; bpm: beats per minute. Perfusion index: Adenosine perfusion / rest perfusion*. rCOxMAP: resting cardiac output x mean arterial pressure. $Perf_{Rest}$: Perfusion at rest. AU: arbitraty unit. $Perf_{Ado}$.: Perfusion with ADO infusion a: n =13, b: n=11


Myocardial perfusion (ml/100g/min)
100
80
60
40
20
0
Before
After
*
†
*
†
Premenopausal
Postmenopausal

A


Myocardial perfusion (ml/100g/min)
600
500
400
300
200
100
0
Premenopausal
Postmenopausal

B


Postmenopausal (n = 14)
Premenopausal (n = 13)
Absolute change from baseline (ml/100 g/min)
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-220
-240
-260
1
5
10
15
20
25