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RESEARCH ARTICLE

Cardiac perfusion and function after high-intensity exercise training in late premenopausal and recent postmenopausal women: an MRI study

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Egelund J, Nyberg M, Mandrup CM, Abdulla J, Stallknecht B, Bangsbo J, Hellsten Y, Larsson HB. Cardiac perfusion and function after high-intensity exercise training in late premenopausal and recent postmenopausal women: an MRI study. *J Appl Physiol* 126: 1272–1280, 2019. First published March 14, 2019; doi:10.1152/japplphysiol.01089.2017.—We examined the influence of recent menopause and aerobic exercise training in women on myocardial perfusion, left ventricular (LV) dimension, and function. Two groups ($n = 14$ each) of healthy late premenopausal (50.2 ± 2.1 yr) and recent postmenopausal (54.2 ± 2.8 yr) women underwent cardiac magnetic resonance imaging (cMRI) at baseline and after 12 wk of high-intensity aerobic training. Measurements included LV morphology, systolic function, and myocardial perfusion at rest and during an adenosine stress test. At baseline, resting myocardial perfusion was lower in the postmenopausal than the premenopausal group (77 ± 3 vs. 89 ± 3 ml·100 g^{-1}·min^{-1}; $P = 0.01$), 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·100 g^{-1}·min^{-1}; $P = 0.03$). The adenosine-induced change in myocardial perfusion was lower in the groups combined (by 402 ± 17 ml·100 g^{-1}·min^{-1}; $P = 0.02$), and the adenosine-induced increase in heart rate was 10 ± 2 beats/min lower ($P < 0.0001$) in both groups after training. Normalization of myocardial perfusion using an estimate of cardiac work eliminated the differences in perfusion between the premenopausal and postmenopausal groups and the effect of training. Left ventricle mass was higher in both groups ($P = 0.03$; $P = 0.006$), whereas LV end-diastolic ($P = 0.02$) and stroke ($P = 0.045$) volumes were higher in the postmenopausal group after training. Twelve weeks of exercise training increased left ventricle mass and lowered resting and adenosine-induced myocardial perfusion, an effect that was likely related to cardiac work. The current data also suggest that the early menopausal transition has limited impact on cardiac function and structure.

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

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adenosine; cardiac MRI; exercise; menopause; myocardial perfusion

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 sex 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 analogs. 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 noninvasive imaging modality, is being increasingly used for assessment and quantification of myocardial perfusion (41, 42). Using an MRI contrast agent, Hamirani and Kramer (15) showed that quantitative stress myocardial perfusion measurements with cMRI is reproducible and has similar diagnostic accuracy as PET (15). Additional advantages of cMRI are accurate measurements of cardiac morphology and function (3).

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

METHODS

Study Design

The study was a substudy 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-wk 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 was 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 yr, 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 informed consent before participation in the study. The study was registered at ClinicalTrials.gov (NCT02135575).

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

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

Exercise Intervention

The training was performed on a spinning cycle (Body Bike, Frederikshavn, Denmark). Instructors from the research group supervised two training sessions per week, and instructors from a local fitness 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 ~50 min. 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 ($\dot{V}O_{2peak}$) 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 10-min warm-up, and thereafter, the test was initiated with a start load of 50 W and increased by 25 W/min until volitional fatigue. Criteria for determination of $\dot{V}O_{2peak}$ were a plateau in $\dot{V}O_2$ (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 $\dot{V}O_{2peak}$ test was recorded.

The $\dot{V}O_{2peak}$ 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 interslice gaps covered the left ventricle. Field of view was 400 × 400 mm^2, with a matrix size of 155 × 208. Each slice of the left ventricle was obtained over ~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°; bandwidth (BW), 1,149 Hz/pixel; and 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°; time domain (TD), 130 ms (time from the 90 prepulse and the first read-out pulse); field of view 300 mm × 400 mm, matrix, 96 × 160; linear phase encoding; slice thickness, 10 mm, GRAPPA acceleration factor 2, and BW: 651 Hz/pixel. The MRI contrast agent (Gadovist; Bayer Schering Pharma, Berlin, Germany) was administrated as a bolus of 0.1 mmol/kg body wt 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 min of adenosine infusion (140 $\mu g \cdot kg^{-1} \cdot min^{-1}$). Adenosine infusion was stopped immediately after image acquisition, and the total duration of adenosine infusion was ~4 min. Rest perfusion images were obtained at least 15 min 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 on the basis of 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), we annotated the LV endomyocardial and epimyocardial borders, and we calculated the left ventricle volume 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. Left ventricle diameter and wall thickness in diastole were obtained using three-chamber images. The wall thickness and the endocardial borders were manually measured on two different images: three-chamber and four-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 endomyocardial and epimyocardial 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 five measurements and HR (RPP = SBP × 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 semimanually annotated, especially avoiding inclusion of the ventricular volume, and the magnetic resonance (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 left ventricle (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 ~10 frames, 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 relaxation in tissue and blood, a model-free deconvolution, based on the Tikhonov approach with L-curve regularization, was applied to estimate perfusion (26, 27). Perfusion was reported in milliliters per 100 g per minute, 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 modeled 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 ± SE unless otherwise stated. Subject characteristics were evaluated and compared by use of a Student's *t*-test (Excel 2010, Microsoft). An α 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, 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 yr. Systolic blood pressure was similar in the two groups before and after the exercise intervention. Diastolic blood pressure was the same in the premenopausal 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 premenopausal 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 premenopausal and postmenopausal groups, respectively (Table 1).

Myocardial Perfusion

Resting myocardial perfusion was lower in the postmenopausal compared with 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 (premenopausal by 9%, $P = 0.036$ and postmenopausal 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

Table 1. *Subject characteristics*

	Premenopausal			Postmenopausal			Group Comparison	
	Baseline (n = 14)	12 wk (n = 14)	Training Effect P	Baseline (n = 14)	12 wk (n = 14)	Training Effect P	Baseline P	12 wk 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
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	7,901 ± 327	7,327 ± 327	**0.001**	7,187 ± 327	6,699 ± 327	0.006	**0.12**	0.17
Cardiorespiratory fitness								
$\dot{V}O_{2peak}$, ml O_2/min	2,079 ± 271	2,284 ± 276	**0.009**	1,921 ± 216	2,060 ± 187	**0.007**	0.10	**0.02**
Cycling watt end-$\dot{V}O_{2peak}$ 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	

Data are presented as means ± SD. BMI, body mass index; HR_{max}, maximal heart rate during test; $\dot{V}O_{2peak}$, peak oxygen consumption. Bolded values indicate significant difference.

the training period, adenosine-induced myocardial perfusion was lower for all of the subjects combined (by 402 ± 17 ml·100 g^{-1}·min^{-1}; 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 ± 3 beats per minute, P = 0.001; postmenopausal by 11 ± 3 beats/min, P = 0.0004; Table 2). When resting and adenosine-induced perfusion were normalized to CO × 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 with the premenopausal women after training.

Cardiac Dimensions

Left ventricle 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 and 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 premenopausal and postmenopausal groups, whereas resting HR and cardiac output (CO) were lower in the postmenopausal compared with 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 with 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 with 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 wk 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 *1*) baseline resting myocardial perfusion was lower in the postmenopausal compared with the premenopausal women, whereas adenosine-induced stress perfusion was similar in both groups; *2*) after exercise training, myocardial perfusion at rest was lower in both groups of women, and adenosine-induced stress perfusion was decreased in both of the groups. *3*) 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. *4*) Left ventricle mass was similar between the two groups at baseline and increased similarly in both groups after training. *5*) Cardiac systolic function was similar between the two groups and was not altered by training.

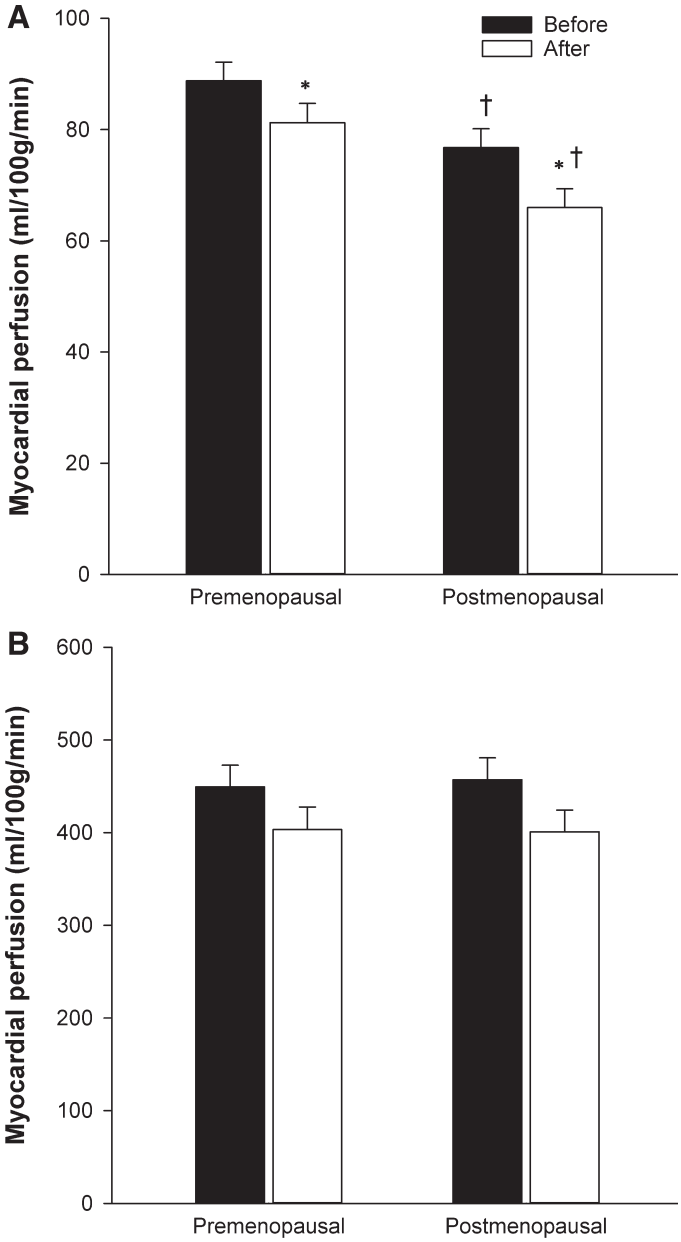

Fig. 1. Myocardial perfusion at rest (*A*) and during adenosine stress perfusion ($n = 14$) (*B*) in late premenopausal and recent postmenopausal women ($n = 13$ and $n = 14$) before and after 12 wk of intense aerobic cycle exercise training. Differences between groups at measurement point, †$P < 0.05$. Changes from baseline to 12 wk within group, *$P < 0.05$.

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 with 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 the 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 sensitivity to prostacyclin (32, 39).

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 with the premenopausal women, as reported in other studies (1, 31). Further studies are required to 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 toward less resting myocardial oxygen consumption.

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 ~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 ~30 beats/min 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 et al. (11), the effects of a 2-wk moderate- and high-intensity training regimes

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
Left ventricle 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
Left ventricle 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·100 g^{-1}·min^{-1}	89 ± 3	81 ± 3[a]	**0.036**	77 ± 3	66 ± 23	**0.002**	**0.01**	**0.001**
$Perf_{Ado}$, ml·100 g^{-1}·min^{-1}	449 ± 23	402 ± 24[a]	0.17	457 ± 23	401 ± 23	0.09	0.81	0.94
Heart rate at rest, beats/min	73 ± 2	69 ± 2	**0.002**	64 ± 2	61 ± 2	**0.04**	**0.002**	**0.017**
Heart rate during adenosine, beats/min	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
rCO×MAP, mmHg·l·min^{-1}	524 ± 27	463 ± 27	**0.004**	420 ± 27	405 ± 27	0.50	**0.006**	0.13
$Perf_{Rest}$/rCO×MAP, 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·100 g	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

Data are presented as means ± SE. AU, arbitrary units; CO, cardiac output; IVS, interventricular septum; LVEDD, left ventricular end-diastolic diameter; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; LVPWD, left ventricular posterior wall thickness; Perfusion index, adenosine perfusion/rest perfusion; rCO×MAP, resting cardiac output × mean arterial pressure; SV, stroke volume; $Perf_{Ado}$, perfusion with ADO infusion; $Perf_{Rest}$, perfusion at rest. [a]n = 13; [b]n = 11. Bolded values indicate significant difference.

were compared, and they found that a period of high-intensity training lowered adenosine-induced myocardial perfusion, whereas training at moderate intensity had no significant effect. In addition, several studies have reported that myocardial perfusion is lower in well-trained individuals compared with 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 with 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

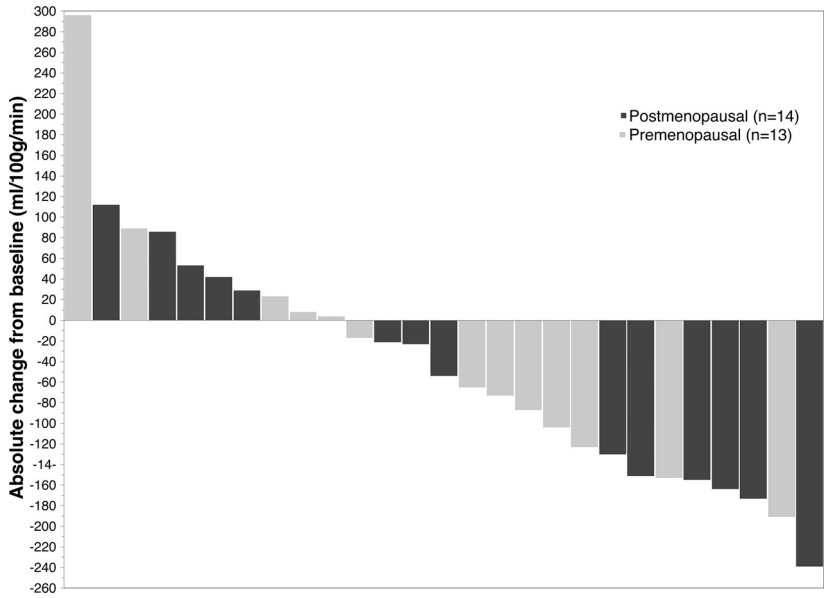

Fig. 2. Individual myocardial adenosine stress perfusion responses to 12 wk of intense aerobic cycle exercise training for the late premenopausal and postmenopausal (n = 13 and n = 14) women expressed as absolute change from baseline.

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 with untrained individuals (8, 23).

Cardiac Dimensions and Function

Before training, the premenopausal and postmenopausal groups had similar cardiac dimensions and function with the exception that the postmenopausal women had ~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 left ventricle mass in both groups and an improvement in end-diastolic volume 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 mid-life women. However, the change in left ventricle mass in our study was relatively small compared with that observed with 1 yr 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 left ventricle mass in the above-mentioned 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 left ventricle mass adaptation in the current study was similar in the premenopausal 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 multicenter study comprising 234 patients (46). We performed two comparisons 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) was RCA: 0.89, LAD: 0.88, and 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 premenopausal 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 (12). 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 premenopausal and postmenopausal women and a longitudinal design in which premenopausal 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 with late premenopausal women and that a 12-wk period of intense aerobic interval training results in a decrease in resting and adenosine-induced myocardial perfusion in the premenopausal 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 premenopausal 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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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.E., M.N., C.M.M., B.S., J.B., and Y.H. conceived and designed research; J.E., M.N., C.M.M., Y.H., and H.B.W.L. performed experiments; J.E., M.N., C.M.M., J.A., Y.H., and H.B.W.L. analyzed data; J.E., M.N., C.M.M., J.A., B.S., J.B., Y.H., and H.B.W.L. interpreted results of experiments; J.E., M.N., C.M.M., and Y.H. prepared figures; J.E., M.N., C.M.M., J.A., Y.H., and H.B.W.L. drafted manuscript; J.E., M.N., C.M.M., J.A., B.S., J.B., Y.H., and H.B.W.L. edited and revised manuscript; J.E., M.N., C.M.M., J.A., B.S., J.B., Y.H., and H.B.W.L. approved final version of manuscript.

REFERENCES

1. **Abildgaard J, Pedersen AT, Green CJ, Harder-Lauridsen NM, Solomon TP, Thomsen C. Juul A, Pedersen M, Pedersen JT, Mortensen OH, Pilegaard H, Pedersen BK, Lindegaard B.** Menopause is associated with decreased whole body fat oxidation during exercise. *Am J Physiol Endocrinol Metab* 304: E1227–E1236, 2013. doi:10.1152/ajpendo.00492.2012.
2. **Arbab-Zadeh A, Perhonen M, Howden E, Peshock RM, Zhang R, Adams-Huet B, Haykowsky MJ, Levine BD.** Cardiac remodeling in response to 1 year of intensive endurance training. *Circulation* 130: 2152–2161, 2014. doi:10.1161/CIRCULATIONAHA.114.010775.
3. **Bellenger NG, Davies LC, Francis JM, Coats AJ, Pennell DJ.** Reduction in sample size for studies of remodeling in heart failure by the use of cardiovascular magnetic resonance. *J Cardiovasc Magn Reson* 2: 271–278, 2000. doi:10.3109/10976640009148691.
4. **Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, de Ferranti SD, Floyd J, Fornage M, Gillespie C, Isasi CR, Jiménez MC, Jordan LC, Judd SE, Lackland D, Lichtman JH, Lisabeth L, Liu S, Longenecker CT, Mackey RH, Matsushita K, Mozaffarian D, Mussolino ME, Nasir K, Neumar RW, Palaniappan L, Pandey DK, Thiagarajan RR, Reeves MJ, Ritchey M, Rodriguez CJ, Roth GA, Rosamond WD, Sasson C, Towfighi A, Tsao CW, Turner MB, Virani SS, Voeks JH, Willey JZ, Wilkins JT, Wu JH, Alger HM, Wong SS, Muntner P; American Heart Association Statistics Committee and Stroke Statistics Subcommittee.** Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. *Circulation* 135: e146–e603, 2017. doi:10.1161/CIR.0000000000000485.
5. **Bergmann SR, Fox KA, Rand AL, McElvany KD, Welch MJ, Markham J, Sobel BE.** Quantification of regional myocardial blood flow in vivo with H215O. *Circulation* 70: 724–733, 1984. doi:10.1161/01.CIR.70.4.724.
6. **Bittner V.** Menopause, age, and cardiovascular risk: a complex relationship. *J Am Coll Cardiol* 54: 2374–2375, 2009. doi:10.1016/j.jacc.2009.10.008.
7. **Brown MD, Davies MK, Hudlicka O.** Angiogenesis in ischaemic and hypertrophic hearts induced by long-term bradycardia. *Angiogenesis* 8: 253–262, 2005. doi:10.1007/s10456-005-9012-y.
8. **Czernin J, Barnard RJ, Sun KT, Krivokapich J, Nitzsche E, Dorsey D, Phelps ME, Schelbert HR.** Effect of short-term cardiovascular conditioning and low-fat diet on myocardial blood flow and flow reserve. *Circulation* 92: 197–204, 1995. doi:10.1161/01.CIR.92.2.197.
9. **Dhalla AK, Wong M-Y, Wang W-Q, Biaggioni I, Belardinelli L.** Tachycardia caused by A2A adenosine receptor agonists is mediated by direct sympathoexcitation in awake rats. *J Pharmacol Exp Ther* 316: 695–702, 2006. doi:10.1124/jpet.105.095323.
10. **Daan NMP, Fauser BCJM.** Menopause prediction and potential implications. *Maturitas* 82: 257–265, 2015. doi:10.1016/j.maturitas.2015.07.019.
11. **Eskelinen J-J, Heinonen I, Löyttyniemi E, Hakala J, Heiskanen MA, Motiani KK, Virtanen K, Pärkkä JP, Knuuti J, Hannukainen JC, Kalliokoski KK.** Left ventricular vascular and metabolic adaptations to high-intensity interval and moderate intensity continuous training: a randomized trial in healthy middle-aged men. *J Physiol* 594: 7127–7140, 2016. doi:10.1113/JP273089.
12. **Fritz-Hansen T, Hove JD, Kofoed KF, Kelbaek H, Larsson HBW.** Quantification of MRI measured myocardial perfusion reserve in healthy humans: a comparison with positron emission tomography. *J Magn Reson Imaging* 27: 818–824, 2008. doi:10.1002/jmri.21306.
14. **Gilligan DM, Quyyumi AA, Cannon RO III.** Effects of physiological levels of estrogen on coronary vasomotor function in postmenopausal women. *Circulation* 89: 2545–2551, 1994. doi:10.1161/01.CIR.89.6.2545.
15. **Hamirani YS, Kramer CM.** Cardiac MRI assessment of myocardial perfusion. *Future Cardiol* 10: 349–358, 2014. doi:10.2217/fca.14.18.
16. **Heinonen I, Nesterov SV, Liukko K, Kemppainen J, Någren K, Luotolahti M, Virsu P, Oikonen V, Nuutila P, Kujala UM, Kainulainen H, Boushel R, Knuuti J, Kalliokoski KK.** Myocardial blood flow and adenosine A2A receptor density in endurance athletes and untrained men. *J Physiol* 586: 5193–5202, 2008. doi:10.1113/jphysiol.2008.158113.
17. **Hellsten Y, Jensen L, Thaning P, Nyberg M, Mortensen S.** Impaired formation of vasodilators in peripheral tissue in essential hypertension is normalized by exercise training: role of adenosine and prostacyclin. *J Hypertens* 30: 2007–2014, 2012. doi:10.1097/HJH.0b013e328356dd57.
18. **Hellsten Y, Nyberg M.** Cardiovascular adaptations to exercise training. *Compr Physiol* 6: 1-32, 2015.1002/cphy.c140080.
19. **Howden EJ, Perhonen M, Peshock RM, Zhang R, Arbab-Zadeh A, Adams-Huet B, Levine BD.** Females have a blunted cardiovascular response to one year of intensive supervised endurance training. *J Appl Physiol (1985)* 119: 37–46, 2015. doi:10.1152/japplphysiol.00092.2015.
20. **Huang A, Kaley G.** Gender-specific regulation of cardiovascular function: estrogen as key player. *Microcirculation* 11: 9–38, 2004. doi:10.1080/10739680490266162.
21. **Janssen I, Powell LH, Crawford S, Lasley B, Sutton-Tyrrell K.** Menopause and the metabolic syndrome: the Study of Women's Health Across the Nation. *Arch Intern Med* 168: 1568–1575, 2008. doi:10.1001/archinte.168.14.1568.
22. **Joyner MJ, Green DJ.** Exercise protects the cardiovascular system: effects beyond traditional risk factors. *J Physiol* 587: 5551–5558, 2009. doi:10.1113/jphysiol.2009.179432.
23. **Kjaer A, Meyer C, Wachtell K, Olsen MH, Ibsen H, Opie L, Holm S, Hesse B.** Positron emission tomographic evaluation of regulation of myocardial perfusion in physiological (elite athletes) and pathological (systemic hypertension) left ventricular hypertrophy. *Am J Cardiol* 96: 1692–1698, 2005. doi:10.1016/j.amjcard.2005.07.090.
24. **Knuuti J, Nuutila P.** PET as a cardiovascular and metabolic research tool. *Ann Med* 31: 450–456, 1999. doi:10.3109/07853899908998804.
25. **Koh AS, Lye WK, Chia SY, Salunat-Flores J, Sim LL, Keng FYJ, Tan RS, Chua TSJ.** Long-Term Prognostic Value of Appropriate Myocardial Perfusion Imaging. *Am J Cardiol* 119: 1957–1962, 2017. doi:10.1016/j.amjcard.2017.03.026.
26. **Larsson HBW, Courivaud F, Rostrup E, Hansen AE.** Measurement of brain perfusion, blood volume, and blood-brain barrier permeability, using dynamic contrast-enhanced $T_{(1)}$-weighted MRI at 3 tesla. *Magn Reson Med* 62: 1270–1281, 2009. doi:10.1002/mrm.22136.
27. **Larsson HBW, Hansen AE, Berg HK, Rostrup E, Haraldseth O.** Dynamic contrast-enhanced quantitative perfusion measurement of the brain using T1-weighted MRI at 3T. *J Magn Reson Imaging* 27: 754–762, 2008. doi:10.1002/jmri.21328.
28. **Larsson HBW, Rosenbaum S, Fritz-Hansen T.** Quantification of the effect of water exchange in dynamic contrast MRI perfusion measurements in the brain and heart. *Magn Reson Med* 46: 272–281, 2001. doi:10.1002/mrm.1188.
29. **Larsson HBW, Vestergaard MB, Lindberg U, Iversen HK, Cramer SP.** Brain capillary transit time heterogeneity in healthy volunteers measured by dynamic contrast-enhanced T_1 -weighted perfusion MRI. *J Magn Reson Imaging* 45: 1809–1820, 2017. doi:10.1002/jmri.25488.
30. **Laughlin MH, McAllister RM.** Exercise training-induced coronary vascular adaptation. *J Appl Physiol (1985)* 73: 2209–2225, 1992. doi:10.1152/jappl.1992.73.6.2209.
31. **Lovejoy JC, Champagne CM, de Jonge L, Xie H, Smith SR.** Increased visceral fat and decreased energy expenditure during the menopausal transition. *Int J Obes* 32: 949–958, 2008. doi:10.1038/ijo.2008.25.
32. **Laaksonen MS, Heinonen I, Luotolahti M, Knuuti J, Kalliokoski KKV.** VO(2peak), myocardial hypertrophy, and myocardial blood flow in endurance-trained men. *Med Sci Sports Exerc* 46: 1498–1505, 2014. doi:10.1249/MSS.0000000000000264.
33. **Mandrup CM, Egelund J, Nyberg M, Lundberg Slingsby MH, Andersen C, Løgstrup S, Bangsbo J, Suetta C, Stallknecht B, Hellsten Y.** Effects of high-intensity training on cardiovascular risk factors in pre-

menopausal and postmenopausal women. *Am J Obstet Gynecol* 216: 384.e1–384.e11, 2017. doi:10.1016/j.ajog.2016.12.017.

34. **McQueen DS, Ribeiro JA.** Pharmacological characterization of the receptor involved in chemoexcitation induced by adenosine. *Br J Pharmacol* 88: 615–620, 1986. doi:10.1111/j.1476-5381.1986.tb10242.x.
35. **Moreau KL, Hildreth KL, Meditz AL, Deane KD, Kohrt WM.** Endothelial function is impaired across the stages of the menopause transition in healthy women. *J Clin Endocrinol Metab* 97: 4692–4700, 2012. doi:10.1210/jc.2012-2244.
36. **Moreau KL, Stauffer BL, Kohrt WM, Seals DR.** Essential role of estrogen for improvements in vascular endothelial function with endurance exercise in postmenopausal women. *J Clin Endocrinol Metab* 98: 4507–4515, 2013. doi:10.1210/jc.2013-2183.
37. **Naya M, Tamaki N, Tsutsui H.** Coronary flow reserve estimated by positron emission tomography to diagnose significant coronary artery disease and predict cardiac events. *Circ J* 79: 15–23, 2015. doi:10.1253/circj.CJ-14-1060.
38. **Nyberg M, Egelund J, Mandrup CM, Andersen CB, Hansen KMBE, Hergel IF, Valbak-Andersen N, Frikke-Schmidt R, Stallknecht B, Bangsbo J, Hellsten Y.** Leg vascular and skeletal muscle mitochondrial adaptations to aerobic high-intensity exercise training are enhanced in the early postmenopausal phase. *J Physiol* 595: 2969–2983, 2017. doi:10.1113/JP273871.
39. **Nyberg M, Egelund J, Mandrup CM, Nielsen MB, Mogensen AS, Stallknecht B, Bangsbo J, Hellsten Y.** Early Postmenopausal Phase Is Associated With Reduced Prostacyclin-Induced Vasodilation That Is Reversed by Exercise Training: The Copenhagen Women Study. *Hypertension* 68: 1011–1020, 2016. doi:10.1161/HYPERTENSIONAHA.116.07866.
40. **Peterson LR, Eyster D, Dávila-Román VG, Stephens AL, Schechtman KB, Herrero P, Gropler RJ.** Short-term oral estrogen replacement therapy does not augment endothelium-independent myocardial perfusion in postmenopausal women. *Am Heart J* 142: 641–647, 2001. doi:10.1067/mhj.2001.118111.
41. **Qayyum AA, Hasbak P, Larsson HBW, Christensen TE, Ghotbi AA, Mathiasen AB, Vejlstrup NG, Kjaer A, Kastrup J.** Quantification of myocardial perfusion using cardiac magnetic resonance imaging correlates significantly to rubidium-82 positron emission tomography in patients with severe coronary artery disease: a preliminary study. *Eur J Radiol* 83: 1120–1128, 2014. doi:10.1016/j.ejrad.2014.04.004.
42. **Qayyum AA, Qayyum F, Larsson HBW, Kjaer A, Hasbak P, Vejlstrup NG, Kastrup J.** Comparison of rest and adenosine stress quantitative and semi-quantitative myocardial perfusion using magnetic resonance in patients with ischemic heart disease. *Clin Imaging* 41: 149–156, 2017. doi:10.1016/j.clinimag.2016.11.001.
43. **Rongen GA, Brooks SC, Pollard MJ, Ando S, Dajani HR, Notarius CF, Floras JS.** Effect of adenosine on heart rate variability in humans. *Clin Sci (Lond)* 96: 597–604, 1999. doi:10.1042/cs0960597.
44. **Rongen GA, Floras JS, Lenders JWM, Thien T, Smits P.** Cardiovascular pharmacology of purines. *Clin Sci (Lond)* 92: 13–24, 1997. doi:10.1042/cs0920013.
45. **Santos-Parker JR, Strahler TR, Vorwald VM, Pierce GL, Seals DR.** Habitual aerobic exercise does not protect against micro- or macrovascular endothelial dysfunction in healthy estrogen-deficient postmenopausal women. *J Appl Physiol (1985)* 122: 11–19, 2017. doi:10.1152/japplphysiol.00732.2016.
46. **Schwitter J, Wacker CM, van Rossum AC, Lombardi M, Al-Saadi N, Ahlstrom H, Dill T, Larsson HBW, Flamm SD, Marquardt M, Johansson L.** MR-IMPACT: comparison of perfusion-cardiac magnetic resonance with single-photon emission computed tomography for the detection of coronary artery disease in a multicentre, multivendor, randomized trial. *Eur Heart J* 29: 480–489, 2008. doi:10.1093/eurheartj/ehm617.
47. **Spence AL, Carter HH, Naylor LH, Green DJ.** A prospective randomized longitudinal study involving 6 months of endurance or resistance exercise. Conduit artery adaptation in humans. *J Physiol* 591: 1265–1275, 2013. doi:10.1113/jphysiol.2012.247387.
48. **Stolen KQ, Kemppainen J, Kalliokoski KK, Hällsten K, Luotolahti M, Karanko H, Lehikoinen P, Viljanen T, Salo T, Airaksinen KE, Nuutila P, Knuuti J.** Myocardial perfusion reserve and oxidative metabolism contribute to exercise capacity in patients with dilated cardiomyopathy. *J Card Fail* 10: 132–140, 2004. doi:10.1016/j.cardfail.2003.08.009.
49. **Wells GL.** Cardiovascular Risk Factors: Does Sex Matter? *Curr Vasc Pharmacol* 14: 452–457, 2016. doi:10.2174/1570161114666160722113116.
50. **Wittnich C, Tan L, Wallen J, Belanger M.** Sex differences in myocardial metabolism and cardiac function: an emerging concept. *Pflügers Arch* 465: 719–729, 2013. doi:10.1007/s00424-013-1232-1.