Retrograde shear rate in formerly preeclamptic and healthy women before and after exercise training: relationship with endothelial function

Ralph R. Scholten,1,2 Marc E. A. Spaanderman,3 Daniel J. Green,4,5 Maria T. E. Hopman,1 and Dick H. J. Thijssen1,5

1Department of Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 2Department of Obstetrics and Gynaecology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 3Research School GROW, Maastricht University Medical Centre, Maastricht, The Netherlands; 4School of Sport Science, Exercise and Health, The University of Western Australia, Crawley, Australia; and 5Research Institute for Sports and Exercise Sciences, Liverpool John Moores University, Liverpool, United Kingdom

Submitted 24 February 2014; accepted in final form 30 May 2014

Shear stress, i.e., the frictional force of blood on the arterial wall, represents a key stimulus for adaptation in artery function and structure (19, 35). Changes in shear stress directly influence the endothelium of arteries, which plays a crucial role in the regulation of blood flow and maintenance of the quality of blood vessels. Across the cardiac cycle, shear stress demonstrates a typical pattern, flowing toward the periphery during systole (antegrade shear), and back to the heart during diastole (retrograde shear). Increases in shear stress are beneficial for arterial function, while in vitro and animal studies have demonstrated that shear stress patterns characterized by high levels of retrograde shear can increase the expression of proatherogenic genes [see reviews (14, 22, 20)] with consequent detrimental impacts for vascular health (33). We recently observed within subjects, that an increase in retrograde shear rate is followed by an immediate “dose”-dependent decrease in endothelial function (33). To date, no study has examined whether retrograde shear rate relates to endothelial dysfunction between humans and whether such relationships differ between healthy subjects and those with a priori endothelial dysfunction. We therefore measured endothelial function and resting shear pattern in healthy women and formerly preeclamptic (PE) women, the latter possessing endothelial dysfunction (13). In the current study we hypothesized that increased levels of retrograde shear rate would be associated with lower endothelial function in healthy subjects as well as in those with endothelial dysfunction. The mechanisms that account for an increased risk of cardiovascular disease in women with a history of preeclampsia are not yet well understood, but endothelial dysfunction, which has been linked to atherosclerosis, persists in formerly preeclamptic women many years after an hypertensive pregnancy. Endothelial dysfunction and cardiovascular disease after preeclampsia may be attributable to preexisting risk factors. A recent study of cardiovascular risk factors present before and after pregnancy suggests that nearly half of the elevated risk for future hypertension after preeclampsia can be explained by prepregnancy risk factors. (29) However, further investigation will be necessary to determine whether preeclampsia itself may injure the endothelium and thereby increase the risk of atherosclerosis and cardiovascular disease.

Exercise training is a stimulus that improves endothelial function in healthy subjects as well as in groups with a priori endothelial dysfunction (10, 32). To date, no study has specifically examined whether exercise training affects shear rate patterns, or also whether exercise training-induced changes in shear pattern relate to adaptations in endothelial function. Therefore, we examined the effects of 12 wk exercise training on the relationships between retrograde shear and endothelial function. We hypothesized that exercise training would reduce retrograde shear and improve endothelial function, without altering the reciprocal relationships between these factors. Previous data demonstrated that (changes in) retrograde shear is related to muscle sympathetic nerve activity (25). To further explore this relation with activity of the autonomic nerve...
system, we examined autonomic modulation by power spectral analysis of resting heart rate variability and related this outcome to retrograde shear rate levels and endothelial function (before and after training).

MATERIALS AND METHODS

Subjects

Twenty healthy female participants and 20 women with a history of preeclampsia participated in our study (Table 1). Preeclampsia was diagnosed in the prior pregnancy if women had blood pressure of ≥140/90 mmHg, measured twice, 6 h or more apart, and consistent proteinuria of ≥300 mg/24 h after gestational week 20 in previously normotensive women. All subjects were 6–12 postpartum to control for the interval between delivery and cardiovascular evaluation; all women were primiparous. None reported having been diagnosed with cardiovascular disease, diabetes mellitus, insulin resistance, or cardiovascular risk factors (such as hypercholesterolemia or hypertension). Subjects who smoked or were on medication of any type were excluded from participation. Informed consent was gained from all participants prior to the experimental procedures. The study procedures were approved by the local ethics committee of the Radboud University Nijmegen Medical Centre (CMO: 2008/226) and adhered to the Declaration of Helsinki. The study was registered at clinicaltrials.gov under ID: NCT0090458.

Experimental Design

Measurements and training were performed in the nonpregnant state, 6–12 mo after pregnancy. Subjects were tested before and after 12 wk of exercise training according to a standardized protocol. All measurements, except for VO₂max, were performed during the same visit. VO₂max was tested 1–5 days from the other visit.

Measurements

Subjects reported to the laboratory after an overnight fast and were instructed to abstain from alcohol and caffeine for 16 h, and not to perform any exercise in the 24 h preceding the measurements (25). To minimize possible endocrine influences of the sex hormones on the cardiovascular and autonomic nervous systems, all measurements were performed in the follicular phase of the menstrual cycle, between days 3 and 11 (1, 36). Upon arrival at the laboratory, body characteristics were measured. A venous blood sample was taken for fasting glucose and lipid profile and kidney function (Aeroset, Abbott Laboratories). All tests were performed under standardized conditions in a temperature-controlled room (22 ± 0.5°C). Subjects then rested in supine position for 20 min, followed by assessment of blood pressure. Blood pressure and heart rate were measured oscillographically (Dinamap, Vital Signs Monitor 1846, Critikon, Tampa, FL) at 3-min intervals for 30 min at the right upper arm. We used the median values of 9 consecutive measurements. Measurements were done with the cuff size recommended for the arm circumference (27). This was followed by assessment of autonomic balance and, subsequently, brachial artery blood flow and shear rate patterns and endothelial function via the flow-mediated dilation technique (FMD).

Brachial artery shear rate pattern and endothelium dependent, nitric oxide-mediated dilation (FMD). Vascular assessments were conducted in a quiet, temperature-controlled environment and in accordance with recent guidelines (31). To examine brachial artery shear patterns and flow-mediated dilation (FMD), the arm was extended and positioned at an angle of ~80° from the torso. A rapid inflation and deflation pneumatic cuff (D.E. Hokanson, Bellevue, WA) was positioned around the forearm to provide a stimulus to forearm ischemia. A 10-MHz multifrequency linear array probe, attached to a high-resolution ultrasound machine (T3000; Terson, Burlington, MA), was then used to image the brachial artery in the distal one-third of the upper arm. Continuous Doppler velocity assessments were also obtained using the ultrasound and were collected using the lowest possible insonation angle (always <60°). First, we performed a 1-min baseline recording of brachial artery diameter and velocity to calculate the mean, antegrade, and retrograde shear rate. Shear rate was defined as 4·velocity/diameter (26). Oscillatory shear index, an indicator of the magnitude of shear oscillation, was defined as area under the curve (retrograde shear)/(antegrade shear) (24, 25). The mean brachial artery blood flow was calculated based on continuous measurement of blood velocity and diameter of brachial artery (flow = π·radius²·velocity). Mean resting brachial blood flow of the 1-min recording was taken. Brachial vascular resistance was subsequently estimated by dividing the mean arterial pressure by brachial artery blood flow.

Subsequently, the forearm cuff was inflated (>200 mmHg) for 5 min. Diameter and flow recordings resumed 30 s prior to cuff deflation and continued for 3 min thereafter (26, 31). Posttest analysis of brachial artery diameter and velocity was performed using custom-designed edge-detection and wall-tracking software, which is independent of investigator bias. Recent papers contain detailed descriptions of our analysis approach (37). Peak diameter was automatically detected according to an algorithm and is described in detail elsewhere (2). With this technique data could be analyzed with a temporal resolution of 30 Hz, and a spatial resolution of ~0.0065 cm for

<table>
<thead>
<tr>
<th>Table 1. Clinical and biochemical characteristics of controls (n = 20) and formerly PE women (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variables</strong></td>
</tr>
<tr>
<td><strong>Pretraining</strong></td>
</tr>
<tr>
<td><strong>Age,</strong> yr</td>
</tr>
<tr>
<td><strong>BMI,</strong> kg/m²</td>
</tr>
<tr>
<td><strong>Waist-to-hip ratio</strong></td>
</tr>
<tr>
<td><strong>Systolic BP,</strong> mmHg</td>
</tr>
<tr>
<td><strong>Diastolic BP,</strong> mmHg</td>
</tr>
<tr>
<td><strong>Glucose,</strong> mmol/l</td>
</tr>
<tr>
<td><strong>Insulin,</strong> mmol/l</td>
</tr>
<tr>
<td><strong>Total cholesterol,</strong> mmol/l</td>
</tr>
<tr>
<td><strong>HDL cholesterol,</strong> mmol/l</td>
</tr>
<tr>
<td><strong>LDL cholesterol,</strong> mmol/l</td>
</tr>
<tr>
<td><strong>Triglycerides,</strong> mmol/l</td>
</tr>
<tr>
<td><strong>Creatinine,</strong> μmol/l</td>
</tr>
<tr>
<td><strong>VO₂max,</strong> ml·min⁻¹·kg⁻¹</td>
</tr>
</tbody>
</table>

Values are means ± SD. Both control and formerly preeclamptic (formerly PE) groups were 6–12 mo postpartum. BMI, body mass index; BP, blood pressure; HDL, high-density lipoproteins; LDL, low-density lipoproteins; AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00128.2014 • www.ajpheart.org
diameter and ~1 cm/s for velocity. Reproducibility of FMD using this semiautomated software possesses a CV of 6.7–10.5% (33). The postdeflation shear rate data, derived from simultaneously acquired velocity and diameter measures, was used to calculate the area under the shear rate curve (SRarea) for data up to the point of maximal postdeflation diameter for each individual (28).

Physical fitness. We examined physical fitness before and after a 12-wk training program. Fitness was defined as the peak oxygen uptake (VO2max, ml·min⁻¹·kg⁻¹) during a maximal cycling test on a cycle ergometer (Excalibur Sport, Lode BV, Groningen, Netherlands). Tests were performed in the afternoon, after a light lunch ad libitum. The initial workload was set at 10 W for 1 min, followed by 10-W increments every minute until complete exhaustion. Breath by breath oxygen uptake was measured using spirometric equipment (Quark CPET, Cosmed, Italy). Heart rate and rhythm were consistently recorded by three-lead ECG. Maximal workload (Workmax) was defined as the last completed workload before exhaustion. Test performance was considered to be adequate when 1) the increase in VO2 during Workmax was <150 ml compared with the previous workload, indicating plateau formation in oxygen uptake; 2) heart rate (HR) at Workmax was <10 beats/min from estimated maximal heart rate (220 – age); 3) respiratory exchange ratio (RER, CO2/O2) was consistently >1.1 during Workmax; and 4) capillary lactate level was >8 mmol/L 90 s after exhaustion. If the test failed to achieve these four qualifications, the test was repeated 2–3 days later. In three cases (2 formerly PE women, 1 control) the test had to be repeated; all tests were eventually considered adequate.

Autonomic function. We quantified autonomic modulation by spectral analysis from a 5-min recording of spontaneous fluctuations in HR and blood pressure (BP) (30). HR and arterial blood pressure (ABP) were measured continuously at a sampling rate of 100 Hz using a finger ABP-monitoring device attached to the 3rd digit of the right hand (Finometer, Finapres BV). Measurements were performed in a quiet, partially darkened room with subjects comfortably lying in the supine position. Posttest, these recordings were subdivided into data segments of 100 s, overlapping for 50% and resampled at 5.12 Hz. Each segment was then analyzed with fast Fourier transformation that searches for rhythmic fluctuations in systolic blood pressure (BPsys) and pulse interval with a frequency range between 0 and 2.56 Hz. The amplitude of each fluctuation determines the power at each frequency. As an indicator of autonomic modulation we used the ratio of absolute low-frequency (LF) and high-frequency (HF) powers of the pulse interval representing the cardiac autonomic balance between the sympathetic and vagal system, respectively (LF/HF) (30).

Exercise Training

Exercise training consisted of 12 wk of HR-controlled cycle training (cycle ergometer, Corival, Lode BV, Groningen, Netherlands) at 70–80% of VO2max for two to three times per week. Participants trained 2 times/wk during the first 6 wk and 3 times/wk during the last 6 wk. During each training session, HR was continuously monitored and recorded (RS800CX, Polar Electro). Each training session started with 10-min warm-up at 50% of the heart rate reserve (HRR) above the resting HR. HRR was calculated as: HRR = HRmax - HRrest, where HRmax is the maximal HR measured during the fitness test at study entry and HRrest is the HR determined at rest. Training consisted of 40 min of cycling at between 70 and 80% of the individual HR above HRrest. Within their target HR zone, participants were free to choose the number of revolutions per minute (RPM). The training was completed by cooling down for 5 min at warm-up workload.

Statistics

Statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, IL) software. All data were reported as means ± SD unless stated otherwise, while statistical significance was assumed at P < 0.05. Two-way repeated-measures ANOVA was used to examine differences between groups and to assess the effects of training. Pearson’s correlation coefficient was used to examine the relation between retrograde shear rate, FMD, and LF/HF ratios in both groups before and after exercise training.

RESULTS

Baseline Differences Between Healthy Controls vs. Formerly PE Women

Clinical and biochemical characteristics. Formerly PE women demonstrated higher systolic and diastolic blood pressure and higher triglycerides, but all were within the normal range. Although fasting blood glucose levels were comparable between groups, formerly PE women had higher fasting insulin levels. Physical fitness at baseline was comparable between groups (Table 1).

Shear pattern and endothelial function. Mean brachial artery blood flow was not different between formerly PE women and controls (31 ± 6 vs. 29 ± 5 ml/min, respectively, P = 0.09). However, when correcting for differences in blood pressure, we found a significantly higher brachial artery vascular resistance in formerly PE women compared with controls (3.1 ± 0.5 vs. 2.6 ± 0.5 ml·min⁻¹·mmHg, P = 0.01). Antegrade and mean shear rate were not different between groups (102 ± 21 vs. 90 ± 32 s⁻¹, P = 0.17, and 68 ± 20 vs. 73 ± 28 s⁻¹, P = 0.48, respectively), while retrograde shear rate was significantly higher in formerly PE women compared with healthy controls (−33 ± 13 vs. −17 ± 10 s⁻¹ P < 0.001) (Fig. 1). Oscillatory shear index was significantly higher in formerly PE women compared with controls (0.6 ± 0.3 vs. 0.3 ± 0.2, P = 0.002). A lower brachial artery FMD% was found in formerly PE women compared with controls (Table 2). A strong and significant correlation was present between brachial artery retrograde shear rate and FMD% in controls and formerly PE women, as well as for the pooled data set of controls and formerly PE women (Fig. 2).

Autonomic function. Absolute low-frequency and high-frequency power and LF/HF ratio were higher in formerly PE women compared with healthy controls (P = 0.01, P = 0.03, and P < 0.001, respectively) (Table 3). A correlation existed between the LF/HF ratio and brachial artery FMD% in the pooled data set (r = −0.62, P < 0.001), but also when examined in controls (r = −0.69, P = 0.001) and formerly PE women only (r = −0.42, P = 0.04) (Fig. 3A). In addition, a strong and significant correlation was present between the LF/HF ratio and the retrograde shear rate for the pooled data set (r = −0.79, P < 0.001) as well as in both controls (r = −0.69, P = 0.001) and formerly PE women (r = −0.73, P < 0.001) (Fig. 3B).

Impact of 12-wk Exercise Training

Healthy controls and formerly PE women. Exercise training decreased blood pressure and tended to lower body mass index in both groups (Table 1). Exercise training lowered fasting insulin levels and improved VO2max similarly in both groups (Table 1). Waist-to-hip ratio, glucose, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, and creatinine did not change after training (Table 1). Exercise training had no effect on resting mean and antegrade shear rate in both controls and formerly PE women (Fig. 1). We observed a significantly reduced retrograde shear rate in
controls after exercise training, but also in formerly PE women (Fig. 1). In both controls and formerly PE women, 12 wk exercise training improved brachial FMD% and lowered LF/HF (Tables 2 and 3, respectively). The two-way ANOVA interaction effect revealed that the magnitude of change in retrograde shear rate, FMD%, or LF/HF ratio did not differ between controls and formerly PE women.

Correlations. The pooled data set revealed that the magnitude of the reduction in retrograde shear rate correlated with the improvement in FMD% (r = -0.61, P < 0.001, Fig. 4A), but also with reduction in LF/HF ratio (r = 0.60, P < 0.001, Fig. 4B). When analyzed per group both controls (r = -0.66, P = 0.002) and patients (r = -0.62, P = 0.004) showed a significant correlation between the change in retrograde shear and the training- induced improvement of FMD%. In turn, the change in retrograde shear correlated strongly with the exercise-induced change in LF/HF ratio in patients (r = 0.82, P < 0.001), but not in controls (r = 0.37, P = 0.10) (Fig. 4B).

DISCUSSION

This study has a number of novel findings. First, we demonstrated, using a between-subjects approach, that brachial artery retrograde shear rate under resting conditions has a strong and inverse relationship with brachial artery endothelial function. This finding was observed in healthy subjects, but also in subjects with a history of preeclampsia who possessed brachial artery endothelial dysfunction. Second, in both groups, we found significant relationship between retrograde shear rate and estimated sympathetic dominance. Therefore, a higher resting retrograde shear rate correlates with the presence and magnitude of endothelial (dys)function in healthy humans as well as in formerly PE women. Third, we showed that exercise training is an effective strategy to diminish retrograde shear rate, and also to improve endothelial function and autonomic balance in healthy controls and formerly preeclamptic women. Finally, we demonstrated a strong correlation between the decrease in retrograde shear rate after training and the improve-

Table 2. Brachial artery flow-mediated dilation of controls (n = 20) and formerly PE women (n = 20)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls (n = 20)</th>
<th>Formerly PE (n = 20)</th>
<th>2-Way ANOVA, P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretraining</td>
<td>Posttraining</td>
<td>Pretraining</td>
</tr>
<tr>
<td>Baseline diameter, mm</td>
<td>2.8 ± 0.3</td>
<td>3.0 ± 0.3</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>Flow-mediated dilation, %</td>
<td>11.8 ± 3.5</td>
<td>13.3 ± 3.4</td>
<td>5.5 ± 2.2</td>
</tr>
</tbody>
</table>

Values are means ± SD. Both control and formerly PE groups were 6–12 mo postpartum.
Autonomic balance based on heart rate variability analysis of controls (n=20) and formerly PE women (n=20) in healthy women (controls, artery flow-mediated dilation (FMD%, presented as a relative change from baseline diameter) in healthy women (controls, and women with preeclampsia during their pregnancy (formerly PE, n=20). All women were 6–12 mo postpartum.

Fig. 2. Correlation between brachial artery retrograde shear rate and brachial artery flow-mediated dilation (FMD%, presented as a relative change from baseline diameter) in healthy women (controls, n=20) and women with preeclampsia during their pregnancy (formerly PE, n=20). All women were 6–12 mo postpartum.

ment after 12-wk exercise training in endothelial function. Taken together, these findings suggest that retrograde shear relates to changes in arterial function in humans in vivo, with exercise training representing an effective strategy to improve retrograde shear rate and arterial function.

We compared two distinct groups who demonstrated differences in resting shear pattern and endothelial function. Women with a history of PE showed a higher retrograde shear rate compared with healthy controls. The higher levels of resting retrograde shear rate were accompanied by a significantly lower endothelial function, a well-established finding in women with a history of PE that is believed to contribute to their increased risk for future cardiovascular disease (3, 5, 13, 38). The correlation between retrograde shear rate and endothelial function between subjects is in line with the presence of endothelial dysfunction after an acute increase in retrograde shear using a within-subjects approach (33). Another novel observation was that the correlation between retrograde shear rate and endothelial function is present in healthy controls (i.e., "normal" endothelial function) and formerly PE women (i.e., endothelial dysfunction). This suggests that the relation between retrograde shear rate and magnitude of the endothelial function is independent of a priori endothelial dysfunction in humans.

The underlying mechanisms for the higher retrograde shear rate in previously PE women may relate to increases in vascular tone in the resistance vessel beds. Indeed, formerly PE women demonstrated a significantly higher blood pressure compared with healthy controls, a finding which may be related to higher peripheral vascular tone compared with healthy controls. A potential explanation for the higher vascular resistance, and therefore retrograde shear rate, may relate to the sympathetic nervous system. Padilla et al. (25) recently found that acute elevations in muscle sympathetic nerve activity are associated with an increase in conduit artery retrograde and oscillatory shear. Increased sympathetic activity has been demonstrated in formerly PE women (7). In our study we examined LF/HF ratio, which provides an indirect measure of (cardiac) sympathetic activity pattern, and we found that increased LF/HF ratio was associated with higher retrograde shear rate. While previous studies have convincingly reported a strong relation between the sympathetic nervous system and endothelial function, these studies assessed changes in this relation after acute elevations in sympathetic nerve activity (8, 15) rather than resting levels of activity such as in the present study. It should be noted that our measurement of LF/HF ratio does not provide direct information about muscle sympathetic nerve activity. Although speculative, this provides evidence that higher retrograde shear rate may be related to activity of the sympathetic nervous system, and that subsequent increased retrograde shear rate may relate to changes in endothelial function.

Recent studies have demonstrated that an increased retrograde shear rate in healthy older men is related, at least partly, to a decreased contribution of NO to vascular tone and/or increased α-adrenergic tone (4, 23). Similarly, such explanations may relate to our study, as changes in these vasoactive substances relate to the increased retrograde shear rate (or reversal in retrograde shear rate after training) in formerly PE subjects. Previous studies have demonstrated that higher levels of vasoactive constrictors [e.g., endothelin-1 (34) or ANG II (12)] or impairment of the vasodilator pathways [e.g., NO pathway (18)] relate to an increased peripheral vascular tone, which may result in changes in shear rate patterns (or vice versa). Interestingly, activation of the endothelin-1 pathway and/or impairment of the NO pathway are both hypothesized to contribute to the detrimental vascular adaptations in pre-eclampsia. In addition, insulin may contribute to changes in shear patterns based on its ability to increase sympathetic nerve activity (17). Interestingly, we observed higher insulin levels in formerly PE women in our study. Taken together, various pathways that (in)directly alter vascular tone potentially contribute to elevated retrograde shear levels and concomitant lowering of the endothelial function.

Exercise training demonstrated a modest effect size on blood pressure and insulin, but not on any of the other parameters. This observation of a modest or even absent effect of exercise training on individual cardiovascular risk factors is reported previously. More specifically, a previous study found that only

Table 3. Autonomic balance based on heart rate variability analysis of controls (n=20) and formerly PE women (n=20)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls (n = 20)</th>
<th>Formerly PE (n = 20)</th>
<th>2-Way ANOVA, P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretraining</td>
<td>Posttraining</td>
<td>Pretraining</td>
</tr>
<tr>
<td>Low Frequency power, ms²</td>
<td>1,005 ± 329</td>
<td>911 ± 315</td>
<td>1,387 ± 563</td>
</tr>
<tr>
<td>High Frequency power, ms²</td>
<td>806 ± 259</td>
<td>965 ± 369</td>
<td>729 ± 245</td>
</tr>
<tr>
<td>LF/HF ratio</td>
<td>1.3 ± 0.4</td>
<td>0.9 ± 0.3</td>
<td>2.1 ± 0.9</td>
</tr>
</tbody>
</table>

Values are means ± SD. Both control and formerly PE groups were 6–12 mo postpartum.
40% of the beneficial effects of exercise training on cardiovascular risk can be explained by changes in traditional cardiovascular risk factors (21).

The observed effects of aerobic exercise training on blood pressure in our study in both healthy and formerly preeclamptic women are in line with a large meta-analysis that demonstrated a mean reduction of $-3$ mmHg with a more pronounced effect in a (pre-) hypertensive population (6). As this decline in blood pressure is relatively small, other effects such as direct effects on the vasculature might explain a part of the remaining cardiovascular risk reduction with exercise training. To support this notion, Green et al. (11) demonstrated that the effects of exercise on traditional cardiovascular risk factors do not relate to exercise-induced changes in endothelial function. Therefore, effects of exercise on endothelial function and shear pattern are unlikely a direct resultant of the changes in traditional cardiovascular risk factors but may represent a direct effect of exercise training on the vessels.

A potential limitation of our study is the cross-sectional nature of the assessment of the relationship between retrograde shear rate and endothelial function. However, we have extended these between-subjects observations by performance of a 12-wk exercise training study, which allowed for within-subjects comparisons. To our knowledge, our study provides the first data that indicate that exercise training in humans can reverse retrograde shear rate. A potential limitation of our study is that we presented our data as shear rate rather than shear stress. However, we expect no differences in viscosity between groups and/or after training, while the length of the brachial artery is unlikely to change after training. As these are important determinants for shear rate/stress, we believe that this did not importantly impact upon our results. Another limitation relates to the method of examining the sympathetic nervous system, as we examined cardiac sympathovagal balance (LF/HF) derived from heart rate variability. Although related to peripheral (muscle) sympathetic nerve activity, care should be taken when LF/HF ratios are translated to vascular sympathetic tone because of possible differential sympathetic outflow between heart and vasculature. A fourth limitation is that our data do not allow to directly examine the relation

![Fig. 3. Correlation between resting autonomic balance (LF/HF ratio) and brachial artery flow-mediated dilation (A, FMD%, presented as a relative change from baseline diameter) and brachial artery retrograde shear rate (B, s$^{-1}$) in healthy women (controls, n = 20) and women with preeclampsia during their pregnancy (formerly PE, n = 20). All women were 6–12 mo postpartum.](image1)

![Fig. 4. Correlation between the absolute change mediated by 12-wk exercise training in brachial artery retrograde shear rate (s$^{-1}$, a "negative" change relates to a smaller retrograde shear rate, i.e., more toward zero) and brachial artery flow-mediated dilation (A, FMD%, presented as a relative change from baseline diameter) and autonomic balance (B, LF/HF ratio) in healthy women (controls, n = 20) and women with preeclampsia during their pregnancy (formerly PE, n = 20).](image2)
between retrograde shear rate and FMD. However, our study was designed to examine the presence of such a relationship (and whether this depends on a priori endothelial dysfunction and fitness levels). Future prospective studies are warranted to further examine this relationship, including causality. In this study women were all examined in the follicular phase of the menstrual cycle when estrogen levels are lowest. We expect no different responses in male subjects; however, generalization of our data to humans is speculative until confirmed in male subjects.

**Perspectives**

Data from animal studies have demonstrated an important role for retrograde shear or oscillatory shear pattern in the upregulation of proatherogenic and downregulation of antiatherogenic genes, leading to an atherogenic endothelial phenotype (20, 33). The presence of elevated levels of retrograde shear rate may therefore be a central feature in the development of atherosclerosis and its complications, such as plaque instability or rupture. Women with a history of preeclampsia have increased risk of developing cardiovascular disease later in life (3), possibly through the presence of endothelial dysfunction (5, 13, 16). Elevated levels of retrograde shear in formerly PE women may provide a mechanistic link that explains the previously suggested relationship between endothelial dysfunction and development of cardiovascular morbidity. Furthermore, this study also highlights the potency of exercise training to improve retrograde shear rate, endothelial function, and LF/HF ratio, and therefore contribute to an improved cardiovascular risk after exercise training.

In conclusion, results from the present study demonstrate that resting levels of retrograde shear rate are strongly related to endothelial function in humans. This finding was observed in healthy women and in women with a history of preeclampsia who exhibit brachial artery endothelial dysfunction. We also found that sympathetic dominance related to retrograde shear rate in healthy subjects as well as in formerly preeclamptic women. Moreover, these differences and correlations remained significant after 12-wk exercise training, while the change in retrograde shear rate correlated significantly with the changes in FMD% and LF/HF ratio. Therefore, our data provide evidence that a higher retrograde shear rate is strongly related to the presence and magnitude of endothelial dysfunction in healthy humans and those with an increased cardiovascular risk, with exercise training being able to change these parameters (without affecting the relation between them).

**ACKNOWLEDGMENTS**

This study has Clinical Trial Registration No. NCT00900458 (http://clinicaltrials.gov/show/NCT00900458).

**GRANTS**

D. H. J. Thijssen is recipient of the E. Dekker Stipend (Netherlands Heart Foundation, 2009T064). D. J. Green received research funding support from the National Heart Foundation of Australia and the Australian Research Council.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


