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

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Scholten RR, Spaanderman ME, Green DJ, Hopman MT, Thijssen DH. Retrograde shear rate in formerly preeclamptic and healthy women before and after exercise training: relationship with endothelial function. Am J Physiol Heart Circ Physiol 307: H418–H425, 2014. First published June 6, 2014; doi:10.1152/ajpheart.00128.2014.—Blood flow patterns in conduit arterioles characterized by high levels of retrograde shear stress can be detrimental for vascular health. In this study we examined whether retrograde shear rate and endothelial function are related in healthy and formerly preeclamptic (PE) women and whether this relationship is altered by exercise training. Formerly PE women (32 ± 4 yr, n = 20) and controls (32 ± 4 yr, n = 20), all 6–12 mo postpartum, performed 12-wk aerobic exercise training. We measured brachial artery shear rate (SR) and endothelial function by flow-mediated dilation (FMD, echo-Doppler). We additionally performed power spectral analysis of heart rate variability and calculated low-frequency/high-frequency (LF/HF) ratio. Antegrade SR was not different between groups, while retrograde SR was significantly higher and FMD% lower in PE women compared with controls (both P < 0.05). Retrograde shear correlated strongly with FMD% in PE women and controls (P < 0.05). LF/HF ratio inversely correlated with brachial artery retrograde SR and FMD% (both P < 0.05) in PE women and controls. Exercise training reduced retrograde shear, improved FMD%, and reduced LF/HF ratios similarly in both groups (all P < 0.05). Training-induced changes in retrograde SR correlated with changes in FMD% and LF/HF ratio. A higher brachial artery retrograde SR relates to lower brachial artery endothelial function, in both controls and formerly PE women. Exercise training improves retrograde SR, while the magnitude of this change correlated strongly with improvements in FMD and reductions in LF/HF ratio. Therefore, the impact of PE and exercise training on endothelial health may, at least partly, be related to retrograde shear rate.

retrograde shear stress; cardiovascular risk; endothelial function; preeclampsia; blood flow pattern; exercise

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 \( \geq 140/90 \text{ mmHg} \), measured twice, 6 h or more apart, and consistent proteinuria of \( \geq 300 \text{ mg} / 24 \text{ h} \) 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: NCT00900458.

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 tests were performed under standardized conditions in a temperature-controlled room (\( 22 \pm 0.5^\circ \text{C} \)). Subjects then rested in a supine position for 20 min, followed by assessment of blood pressure. Blood pressure and heart rate were measured oscillometrically (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 \( \sim 80^\circ \) 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 insolation angle (always \( <60^\circ \)). First, we performed a 1-min baseline recording of brachial artery diameter and velocity to calculate the mean, anastegrade, and retrograde shear rate. Shear rate was defined as \( 4 \times \text{velocity/diameter} \) (26). Oscillatory shear index, an indicator of the magnitude of shear oscillation, was defined as area under the curve (retrograde shear)/(antegrade shear + retrograde shear) (24, 25). The mean brachial artery blood flow was calculated based on continuous measurement of blood velocity and diameter of brachial artery (flow = \( \pi \times \text{radius}^2 \times \text{velocity} \)). 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 (\( >200 \text{ mmHg} \)) 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 \( \sim 0.0065 \text{ cm} \) for retrograde shear rate and endothelial function.
diameter and ~1 cm/s for velocity. Reproducibility of FMD using this semiautomated software possessed a CV of 6.7–10.5% (33). The postdilation shear rate data, derived from simultaneously acquired velocity and diameter measures, was used to calculate the area under the shear rate curve (SR_{AUC}) for data up to the point of maximal postdilation 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 (V_{O2,max}, 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 spiroergometric equipment (Quark CPET, Cosmed, Italy). Heart rate and rhythm were continuously 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 V_{O2} (ml) 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, CO_{2}/O_{2}) 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 (BPs) 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 V_{O2,max} 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 = HRR_{max} – HRR_{rest}, where HRR_{max} is the maximal HR measured during the fitness test at study entry and HRR_{rest} is the HR determined at rest. Training consisted of 40 min of cycling at between 70 and 80% of the individual HRR above HRR_{rest}. 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 found 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 V_{O2,max} 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% \( (r = -0.61, P < 0.001, \text{Fig. 4A}) \), but also with reduction in LF/HF ratio \( (r = 0.60, P < 0.001, \text{Fig. 4B}) \). 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.
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.

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, ms(^2)</td>
<td>1,005 ± 329</td>
<td>911 ± 315</td>
<td>1,387 ± 563</td>
</tr>
<tr>
<td>High Frequency power, ms(^2)</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 between resting autonomic balance (LF/HF ratio) 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.

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.

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).
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**

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**GRANTS**

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


