

## Impact of 2-Weeks Continuous Increase in Retrograde Shear Stress on Brachial Artery Vasomotor Function in Young and Older Men

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**Background**—Although acute elevation in retrograde shear rate (SR) impairs endothelial function, no previous study has explored the effect of prolonged elevation of retrograde SR on conduit artery vascular function. We examined the effect of 2-weeks elevation of retrograde SR on brachial artery endothelial function in young and in older men.

**Methods and Results**—Thirteen healthy young ( $23\pm 2$  years) and 13 older men ( $61\pm 5$  years) were instructed to continuously wear a compression sleeve around the right forearm to chronically (2 weeks) elevate brachial artery retrograde SR in 1 arm. We assessed SR, diameter, and flow-mediated dilation in both the sleeve and contralateral control arms at baseline and after 30 minutes and 2 weeks of continuous sleeve application. The sleeve intervention increased retrograde SR after 30 minutes and 2 weeks in both young and older men ( $P=0.03$  and  $0.001$ , respectively). In young men, brachial artery flow-mediated dilation % was lower after 30 minutes and 2 weeks ( $P=0.004$ ), while resting artery diameter was reduced after 2 weeks ( $P=0.005$ ). The contralateral arm showed no change in retrograde SR or flow-mediated dilation % ( $P=0.32$  and  $0.26$ , respectively), but a decrease in diameter ( $P=0.035$ ). In older men, flow-mediated dilation % and diameter did not change in either arm (all  $P>0.05$ ).

**Conclusions**—Thirty-minute elevation in retrograde SR in young men caused impaired endothelial function, while 2-week exposure to elevated levels of retrograde SR was associated with a comparable decrease in endothelial function. Interestingly, these vascular changes were not present in older men, suggesting age-related vascular changes to elevation in retrograde SR. (*J Am Heart Assoc.* 2015;4:e001968 doi: 10.1161/JAHA.115.001968)

**Key Words:** atherosclerosis • echo-Doppler • endothelial function • retrograde shear stress • shear stress pattern

Shear stress, the frictional force of blood on the arterial wall, is an important hemodynamic stimulus for arterial adaptation.<sup>1–3</sup> Typically, shear stress follows a cyclic pattern, directed towards the periphery during systole (antegrade shear) and, under some circumstances, directed backwards to the heart during diastole (retrograde shear).<sup>4</sup> Elevations in antegrade shear stress are associated with potentially

beneficial effects on the vessel wall.<sup>1,5–10</sup> In contrast, elevation in retrograde shear stress may be potentially detrimental.<sup>2,10,11</sup> For example, an inverse and dose-dependent relationship is described between acute increases in retrograde shear rate (SR) and brachial artery endothelial function in young men.<sup>2,12</sup>

Several studies have explored the impact of repeated exposure to elevations in antegrade shear stress via training, heating, or in vitro manipulation of antegrade shear.<sup>7,13–17</sup> These studies consistently report a dose-dependent increase in arterial caliber and improvement in endothelial function, possibly through upregulation of endothelial nitric oxide synthase.<sup>1,18</sup> In contrast, relatively little is known about the impact of prolonged retrograde shear on the vasculature in healthy young individuals.

Advanced age is associated with progressive loss of endothelial function<sup>19–21</sup> and reduced arterial compliance.<sup>22,23</sup> These changes may contribute to chronically elevated levels of retrograde shear stress with older age,<sup>24,25</sup> and may also impact upon the ability of the vasculature to respond to changes in retrograde shear. Interestingly, previous studies in animals reported distinct adaptations to shear stress between young and older

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individuals.<sup>26–29</sup> However, no previous study has explored whether prolonged exposure to elevated retrograde shear leads to distinct adaptations in brachial artery vasomotor function between healthy young and older humans.

We examined the impact of prolonged (ie, 2 weeks) exposure to elevated levels of retrograde shear in the brachial artery of healthy young and healthy older men in vivo. For this purpose, we have customized a compression sleeve that subjects can wear continuously. By applying compression to the forearm, we hypothesized that the upstream brachial artery would be exposed to increased levels of resting retrograde shear stress. This approach allows us to examine the impact of chronic elevation in brachial artery retrograde shear stress on brachial artery vasomotor function. To this end, we examined the brachial artery before and after 30 minutes and 2 weeks of forearm compression. We expected that chronic elevation of retrograde shear, unopposed by elevations in antegrade shear, would cause a decrease in upstream brachial endothelial function in young men, but not in healthy older men.

## Methods

### Subjects

Thirteen young men ( $23 \pm 2$  years) and 13 older men ( $61 \pm 5$  years) were recruited from the community. We excluded men who had been diagnosed with cardiovascular disease or possessed risk factors such as hypercholesterolemia, hypertension, diabetes mellitus, or smoking. Men using vasoactive or antihypertensive medication or lipid-lowering drugs were excluded from participation. After registration, the participants received an information letter and had 2 weeks to decide to participate. Written informed consent was obtained from all subjects before participation. The procedures were approved by the local Ethics Committee and adhered to the Declaration of Helsinki.

### Study Design

All subjects reported to the Department of Physiology on 2 separate occasions. At the first visit, subjects were asked to complete a medical history questionnaire for inclusion/exclusion purposes, followed by assessment of height, weight, and forearm volume. Subjects were positioned on a bed in the supine position for at least 15 minutes in order to facilitate assessment of baseline blood pressure, circumference of the wrist, and circumference of the widest part of the forearm. Forearm volumes were measured by using the water displacement technique with a calibrated cylinder. Subsequently, we measured baseline diameter, SR pattern, and

flow-mediated dilation (FMD) of the right brachial artery. This was followed by the application of a compression sleeve (5 cm Tensofast™ double stretch) around the right forearm, which was kept on during testing. All subjects were right-handed. To prevent skin responses to the compression sleeve, we first applied a single layer of Tubiton™56 as inner layer of the sleeve. After 30 minutes, we reassessed brachial artery diameter, SR pattern, and FMD. Finally, we examined brachial artery diameter, SR pattern, and FMD in the contralateral left arm, which acted as a noncompression control. Subjects were instructed to wear the sleeve continuously for 2 weeks and could only remove the compression sleeve for hygiene purposes. Subjects reported to our laboratory every 3 to 4 days for a general check-up and replacement of the compression sleeve (based on pilot work) to prevent losing the efficacy of the compression. At day 14, bilateral assessment of brachial artery diameter, SR patterns, and FMD was again performed.

## Experimental Procedures

Assessment of vascular function and resting diameter were conducted in a quiet, temperature-controlled environment, according to recent expert consensus guidelines.<sup>30</sup> Repeated laboratory visits were conducted at the same time of day to control for diurnal variation.<sup>31</sup> Before each test, subjects were instructed to fast for at least 6 hours, abstain from alcohol and caffeine for 18 hours, and avoid any exercise for 24 hours.

### Retrograde shear intervention

Immediately after the initial FMD assessment, a compression sleeve was applied on the right forearm. To achieve sufficient pressure, the sleeve was folded 2 or 3 times, depending on the circumference of the forearm. Based on pilot work, a 2-fold sleeve was used for subjects with a circumference of  $>27$  cm and a 3-fold sleeve for subjects with a circumference of  $<27$  cm. Based on subjective feedback from the subject on convenience and/or complaints of venous congestion of the hand, we adjusted the times the sleeve was folded. Thirty minutes after wearing the compression sleeve, brachial artery FMD was re-assessed. After 2 weeks, subjects reported back to the Department for a final assessment of brachial artery diameter, SR pattern, and FMD%, as described above with all postintervention tests performed with the compression sleeve applied to the forearms.<sup>32</sup>

### Assessment of brachial artery flow-mediated dilation (FMD%)

Before, 30 minutes following, and 2 weeks after sleeve application, endothelium-dependent,<sup>33</sup> mainly NO-mediated,<sup>34</sup>

vasodilator function was examined using the flow-mediated dilation (FMD%). First, subjects rested in the supine position for at least 15 minutes to facilitate baseline assessment of heart rate and blood flow. Heart rate, systolic, diastolic, and mean arterial pressure were measured twice by an experienced researcher from the left brachial artery using a manual sphygmomanometer. To examine brachial artery FMD%, a 10-MHz multifrequency linear-array probe attached to a high-resolution ultrasound machine (T3000; Terason, Burlington, MA) was used to image the brachial arteries in the distal one third of the upper arm. Details for these procedures can be found elsewhere.<sup>32</sup> Baseline diameter of the brachial artery was assessed across the 1-minute period preceding the 5-minute cuff inflation.

## Data Analysis

Analysis of brachial artery diameters and SR before, during, and after the intervention was performed using custom-designed edge-detection and wall-tracking software, which is largely independent of investigator bias.<sup>32,35</sup> From the synchronized diameter and velocity data, blood flow (the product of lumen cross-sectional area and Doppler velocity [ $v$ ]) and SR (4 times velocity divided by diameter)<sup>36,37</sup> were calculated at 30 Hz. Baseline data were acquired across the 1 minute preceding the cuff inflation period. The software also allowed for the separate analysis of positive velocities (ie, red blood cells flowing in the antegrade direction) and negative velocities (ie, red blood cells flowing in the retrograde direction) derived from simultaneously acquired velocity and diameter at 30 Hz for calculation of antegrade and retrograde SR, respectively. Peak diameter following cuff deflation was automatically detected according to an algorithm.<sup>32</sup> The SR stimulus, responsible for endothelium-dependent FMD, was calculated after cuff deflation.<sup>32,37</sup> Based on a previously collected population of 65 healthy men ( $24 \pm 3$  years), we found a median coefficient of variation for the repeated assessment of resting brachial artery diameter (3.1%) and FMD% (12.9%).

## Statistics

Statistical analyses were performed using SPSS 20.0 (SPSS, Chicago, IL). All data are reported as mean (SD) unless stated otherwise, while statistical significance was assumed at  $P \leq 0.05$ . Separate statistical models were applied for young and older men. Changes in body characteristics between the pre- and postintervention measurement were tested using paired  $t$  tests. For diameter, SR, and FMD analysis, we adopted a 1-way ANOVA to determine the impact of the sleeve intervention across time (baseline, 30 minutes, and 2 weeks; single factor) in both young and older men (as

separate analyses). Post-hoc  $t$  tests (with Least Square Difference correction for multiple comparisons) were performed when a main effect was found. Changes in brachial artery vascular function and diameter of the contralateral arm were examined using a paired  $t$  test since we have collected data before and after the 2-week intervention only. According to a recent study, inadequate scaling for FMD% would be present if the upper confidence limit of the regression slope of the relationship between logarithmically transformed base diameter and peak diameter is  $< 1$ .<sup>38</sup> Accordingly, for the FMD% data only (both in young and older men), we performed an allometric modeling solution,<sup>38</sup> which employs a linear mixed-models approach with “time” as fixed factor (baseline, 30 minutes, and 2 weeks), and the natural logarithm of the baseline diameter as a covariate. Baseline differences between groups were tested using unpaired  $t$  tests. Finally, to explore whether the (compression-sleeve-induced) changes in brachial artery FMD% related to a priori levels of FMD%, we have performed a Pearson’s correlation coefficient between the change in FMD% versus baseline FMD% in both young and older men.

## Results

Baseline characteristics for young ( $n=13$ ) and older ( $n=13$ ) men are presented in Table 1. Weight, body mass index, systolic and diastolic blood pressure, mean arterial pressure, wrist and forearm circumference, and hand and forearm volume were all significantly larger in older men compared to young men, while older subjects showed a lower height (Table 1).

### Impact of Retrograde Shear: Young Men

#### *Retrograde shear intervention*

In the intervention arm, we found a significant “time”-effect for retrograde SR, with post-hoc analysis revealing an increase in retrograde SR after 30 minutes and 2 weeks ( $P=0.004$ ). Changes in antegrade SR across time in the intervention arm did not reach statistical significance ( $P=0.068$ , Table 2).

#### *Brachial artery FMD% and diameter*

We also observed that, as a result of the increase in retrograde SR, brachial artery FMD% was significantly lower after both 30 minutes and 2 weeks in the intervention arm ( $P=0.004$ , Figure). While the 30-minute compression sleeve intervention did not alter resting diameter, we observed a significant decrease in brachial artery diameter after 2 weeks of wearing the compression sleeve ( $P=0.035$ , Figure). The decrease in FMD% after 2 weeks wearing the compression

**Table 1.** Characteristics of Young (n=13) and Older Humans (n=13), Before (Pre) and After (Post) the 2-Week Forearm Compression Intervention

	Young Men		P Value	Older Men		P Value
	Pre	Post		Pre	Post	
Age, y	23±2			61±5*		
Height, cm	182±8			177±4*		
Weight, kg	71.7±9.5			89.9±17.3*		
BMI, kg/m <sup>2</sup>	21.7±2.3			29.2±5.6*		
Systolic BP, mm Hg	115±5	113±5	0.263	123±12*	121±11	0.075
Diastolic BP, mm Hg	72±6	70±8	0.271	79±6*	78±5	0.266
Mean BP, mm Hg	86±4	84±6	0.431	94±7*	92±6	0.119
Circumference wrist, cm	16.4±0.9	16.9±1.1	0.036	18.4±1.8*	18.8±1.8	0.029
Circumference forearm, cm	26.3±1.8	25.9±1.9	0.017	28.5±2.5*	28.2±2.8	0.266
Volume hand, L	0.39±0.06	0.42±0.10	0.005	0.50±0.16*	0.52±0.13	0.418
Volume forearm, L	1.40±0.10	1.41±0.17	0.276	1.66±0.46*	1.69±0.35	0.683

Data are mean±SD. P-values represent paired *t* tests. BMI indicates body mass index; BP, blood pressure.

\*Significantly different between from young men at  $P<0.05$  (unpaired *t* test).

sleeve showed a weak but significant correlation with a priori FMD% in young participants ( $r=-0.59$ ,  $P=0.03$ ). In the contralateral arm, we observed no change in brachial artery antegrade or retrograde SR or FMD% ( $P=0.19$ ,  $0.32$ , and  $0.26$ , respectively, Table 2). Diameter showed a small, significant decrease after 2 weeks of wearing the compression sleeve in the intervention arm ( $P=0.005$ , Table 2).

## Impact of Retrograde Shear: Older Men

### Retrograde shear intervention

Brachial artery retrograde SR in the intervention arm increased significantly as a result of the compression sleeve intervention ( $P=0.001$ ). Post-hoc analysis revealed a significantly larger retrograde shear after 30 minutes and after 2

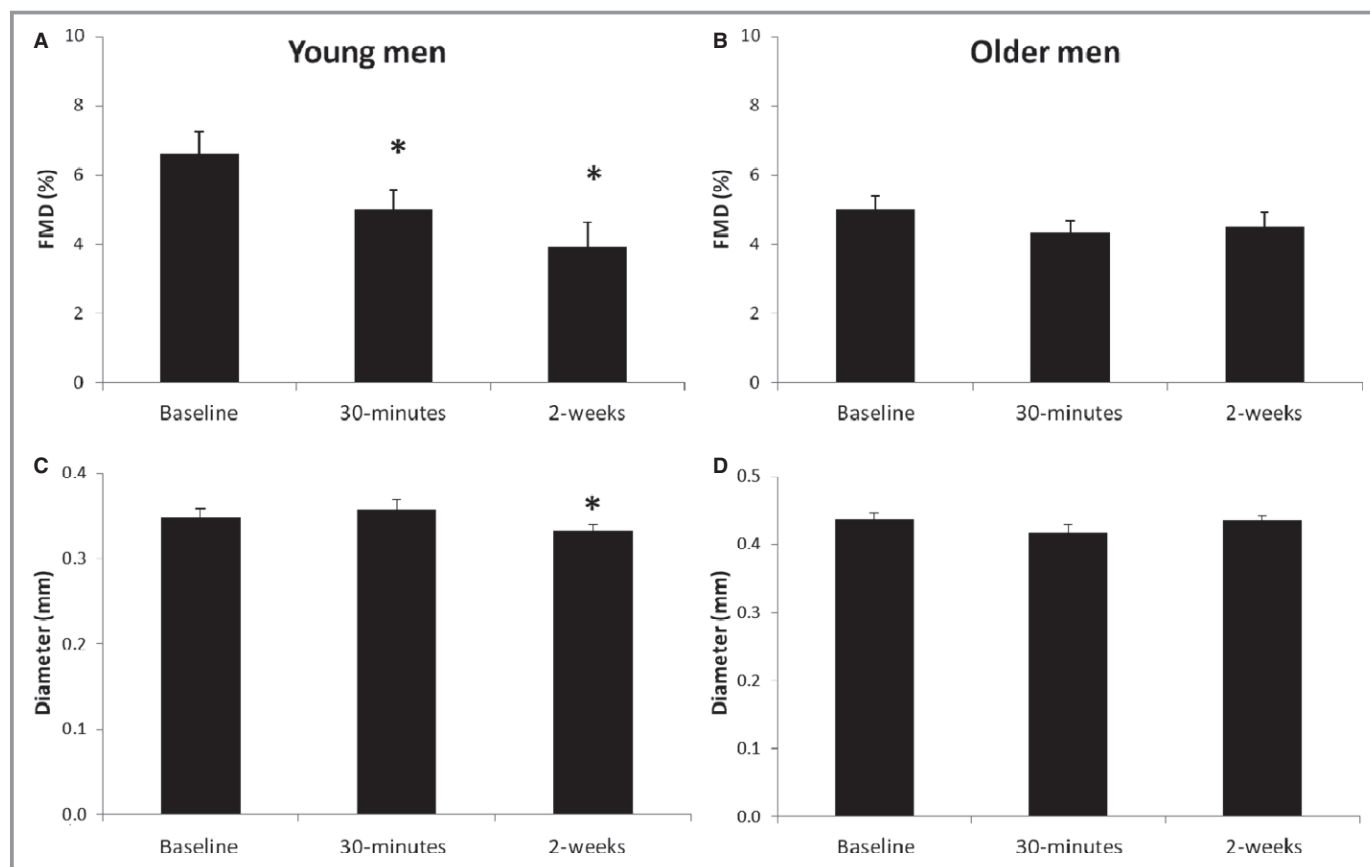
**Table 2.** Brachial Artery SR, Diameter, and FMD% at Baseline and After 30 Minutes and 2 Weeks of Forearm Compression in Young Healthy Men (n=13)

	Baseline	30 Minutes	2 Weeks	P Value
Intervention arm				
Resting diameter, mm	3.5±0.4	3.6±0.4	3.3±0.3*	0.005
FMD, %	6.6±2.3	5.0±2.1*	3.9±2.7*	0.004
FMD (scaled, %)	6.6±2.3	5.1±2.3	3.7±2.3*	0.002
SR <sub>AUC</sub> (s) 10 <sup>3</sup>	30±6	25±5	28±10	0.102
SR antegrade, s <sup>-1</sup>	96±31 <sup>†</sup>	97±30	118±36	0.068
SR retrograde, s <sup>-1</sup>	-27±21	-41±33*	-47±30*	0.030
Contralateral arm				
Resting diameter, mm	3.5±0.5		3.4±0.4	0.035
FMD, %	7.2±2.2		6.3±2.3	0.264
FMD (scaled, %)	7.3±2.1		6.2±2.1	0.170
SR <sub>AUC</sub> (s) 10 <sup>3</sup>	35±9		35±10	0.982
SR antegrade, s <sup>-1</sup>	119±38		105±32	0.192
SR retrograde, s <sup>-1</sup>	-24±20		-20±15	0.318

Data are mean±SD. P-value refers to a 1-way ANOVA (ie, intervention-arm) or paired *t* test (ie, contralateral arm) that was performed to assess changes in the outcome parameters across time within the young men. FMD indicates flow-mediated dilation; SR, shear rate.

\*Significant difference from baseline at  $P\leq0.05$ .

<sup>†</sup>Significant difference at baseline from contralateral arm.



**Figure.** Brachial artery flow-mediated dilation (FMD%, A and B) and diameter (C and D) in young ( $n=13$ , A through C) and older men ( $n=13$ , B through D) before and after 30 minutes and 2 weeks of wearing a forearm compression sleeve in the intervention arm (black bars). Error bars represent standard error. \*Post-hoc significantly different from baseline at  $P<0.05$ .

weeks of wearing the compression sleeve ( $P<0.05$ , Table 3). The change in antegrade SR, induced by the compression sleeve, did not reach statistical significance ( $P=0.08$ , Table 3).

### Brachial artery FMD% and diameter

Despite these effects of the compression sleeve on brachial artery shear patterns, we found no change in brachial artery diameter or FMD% after 30 minutes or after 2 weeks in the intervention arm ( $P=0.14$ , Figure). We also observed no change in brachial artery antegrade or retrograde SR, diameter, or FMD% in the contralateral arm ( $P=0.23$ ,  $0.09$ ,  $0.27$ , and  $0.15$ , respectively, Table 3). In older participants, the individual change in FMD% after 2 weeks wearing the compression sleeve was not related to a priori FMD% ( $r=-0.34$ ,  $P=0.26$ ).

## Discussion

Previous studies provide strong evidence that (repeated) elevations in antegrade or mean SR represent a potent hemodynamic stimulus that acutely and chronically alters vascular function and structure.<sup>1,5–10</sup> As a logical follow-up

study from initial findings that acute elevation in retrograde shear induces impairment in endothelial function,<sup>2</sup> this study aimed to assess the effect of chronic (ie, 2 weeks) manipulation of retrograde SR in humans in vivo. First, we successfully introduced a compression sleeve in young and older men, as a simple and easily applied tool to elevate resting retrograde SR in the brachial artery for 2 weeks. Our findings indicate that 30 minutes of wearing this compression sleeve in young, healthy men significantly decreased brachial artery endothelial function. Impaired brachial artery endothelial function in young men remained present after 2 weeks of exposure to elevated levels of retrograde SR. Finally, and in marked contrast with young men, we found no acute (30-minute) or chronic (2-weeks) impact of elevating retrograde SR levels on brachial artery endothelial function in healthy older men. These unique in vivo data in humans reveal the impact of increased retrograde SR on brachial artery vasculature, while this effect may differ based on age.

In previous studies, we used pneumatic cuffs to manipulate retrograde shear acutely.<sup>2,12</sup> For chronic shear manipulation, however, blood pressure cuffs are impractical. Therefore, in the present study we have introduced customized compres-

**Table 3.** Brachial Artery SR, Diameter, and FMD% at Baseline and After 30 Minutes and 2 Weeks of Forearm Compression in 13 Healthy Older Men

	Baseline	30 Minutes	2 Weeks	P Value
<b>Intervention arm</b>				
Resting diameter, mm	4.4±0.8	4.2±0.8	4.3±0.8	0.133
FMD, %	5.0±1.4	4.3±1.4	4.5±1.5	0.139
FMD (scaled, %)	5.0±1.4	4.2±1.4	4.5±1.4	0.097
SR <sub>AUC</sub> (s) 10 <sup>3</sup>	24±8	24±9	22±8	0.717
SR antegrade, s <sup>-1</sup>	78±37	75±24	93±44	0.081
SR retrograde, s <sup>-1</sup>	-11±9	-28±24*	-17±15*	0.001
<b>Contralateral arm</b>				
Resting diameter, mm	4.1±0.8		4.2±0.8	0.265
FMD, %	5.2±1.9		6.6±3.5	0.147
FMD (scaled, %)	5.1±2.6		6.6±2.6	0.133
SR <sub>AUC</sub> (s) 10 <sup>3</sup>	26±8		30±12	0.319
SR antegrade, s <sup>-1</sup>	85±24		100±43	0.228
SR retrograde, s <sup>-1</sup>	-10±9		-6±7	0.092

Data are presented as mean±SD. *P*-value refers to a 1-way ANOVA (ie, intervention arm) or paired *t* test (ie, contralateral arm) that was performed to assess changes in the outcome parameters across time within the older men. FMD indicates flow-mediated dilation; SR, shear rate.

\*Post-hoc significantly different from baseline at *P*<0.05.

sion sleeves as a novel method to chronically manipulate levels of retrograde SR. In both young and older men, retrograde SR significantly increased after both 30 minutes and 2 weeks of wearing the compression sleeve. In this manner, this study successfully introduced compression sleeves as a method to manipulate retrograde SR, both acutely and chronically in men in vivo. Although a nonsignificant increase was found in antegrade SR in both young and older men, the main observation was that we (in line with our previous work) successfully manipulated retrograde shear.<sup>2,12,39</sup> Although we only explored the impact of the sleeve intervention on resting levels of retrograde shear, we are confident that increased levels of retrograde SR were also present during various activities of daily living. In a series of previous studies, we have demonstrated that various types of physical activity (cycling, walking, handgrip exercise), performed at low and moderate intensity, lead to increases in brachial artery retrograde SR.<sup>4,40</sup> Therefore, physical activities of daily living unlikely altered the exposure to increased levels of retrograde SR in our study.

Overall, we found no impact of the compression sleeve on mean blood flow or SR. Previous work on this topic found that external compression on a limb leads to a decrease (40 to 84 mm Hg compression),<sup>41</sup> no change ( $\approx$ 37 mm Hg compression),<sup>42</sup> or increase (13 to 23 mm Hg compression)<sup>43</sup> in resting limb blood flow. The conflicting results may relate to differences in techniques, protocols, populations, types of garment, and the range and duration of compression levels. The

level of compression may also importantly alter the magnitude and direction of blood flow change. Although these previous results do not provide definitive answers to the effects of compression in these pressure ranges on blood flow to the limb, these data suggest that the effect of external compression on a limb may differ, dependent on the protocol adopted.

In line with previous findings,<sup>12</sup> we observed a decrease in FMD after 30 minutes of exposure to elevated levels of retrograde SR in young men. We extend this knowledge by the finding that after 2 weeks of chronic exposure to elevated levels of retrograde SR, FMD was still impaired. However, the impairment of endothelial function after 30 minutes showed no further decrease when the compression sleeve was worn for 2 weeks. This finding of a decrease in endothelial function after prolonged exposure to retrograde SR is in agreement with a previous ex vivo study in pig carotid arteries, where 3 days of increased retrograde SR induced a decrease in endothelial function, accompanied with a decrease in eNOS expression.<sup>44</sup> During the 2-weeks postintervention measurements of endothelial function, we did not remove the compression sleeve (because of practical reasons). This might have influenced our measurements. However, since the sleeve did not acutely alter the SR area-under-the-curve and/or resting diameter (Tables 2 and 3), we believe it unlikely that the compression sleeve impacted our main findings.

FMD in older men did not change in response to 30 minutes of increased retrograde SR, a finding that reinforces recent work.<sup>39</sup> Furthermore, 2 weeks of increased retrograde SR did

not affect FMD in older men. Despite retrograde shear being lower in old versus young men, there was no significant correlation between baseline levels of retrograde SR and changes in FMD%, and the magnitude of elevation in retrograde SR in older men was substantial and comparable to levels observed in young men. Although direct comparisons were not made, vessels of older men may be less responsive to changes in retrograde shear. In rats, older age is associated with an attenuated activation of eNOS in response to elevation in SR compared to younger rats<sup>28</sup> and it is generally accepted that aged arteries become stiffer and less functionally responsive.<sup>45</sup> When extrapolating these findings, the endothelium in older men in our study may also be less responsive to SR stimuli. Somewhat in keeping with this explanation, a priori impairment in FMD in our older men may contribute to the absence of an effect of retrograde SR. Indeed, we recently reported that a larger decrease in FMD in response to elevations in retrograde shear was observed in those with higher a priori FMD values.<sup>39</sup> We also observed in the present study that a priori FMD values were inversely correlated with the decrease in FMD after 2 weeks in young men.

### Clinical Relevance

The shear stimulus related to atherosclerosis is complex and only partly understood. Exposure to (unidirectional) retrograde SR relates to upregulation of pro-atherogenic factors.<sup>9,10</sup> Interestingly, recent observations from Peiffer et al, using a computational technique to describe that multidirectional wall shear stress, suggested that multidirectional shear may be relevant to the development of atherosclerosis.<sup>46</sup> Future studies should therefore aim to assess the importance of (in vivo) multidirectional wall shear in the development of atherosclerosis.

### Limitations

A potential limitation is that we were unable to determine the pressure generated by the compression sleeve on the vasculature. Retrospective testing performed in all participants suggest that the compression sleeve induced a compression equivalent to 16 to 28 mm Hg. Differences in circumference of the forearm between individuals likely lead to some variation in the pressure applied to the vessel, thereby manipulating the level of retrograde SR between individuals. Nonetheless, our experiment was effective in its utilization of a compression sleeve to manipulate retrograde shear. Another limitation is that adherence to the intervention was self-reported. While we measured the impact of the sleeve on retrograde shear both acutely and after 2 weeks, and this effect was persistent and sustained, we did not assess effects during activities of daily living. It is also pertinent that all subjects reported to our laboratory after 3 to 4 days to replace the compression sleeve,

which automatically served as a moment to check adherence to the intervention. This study does not provide any biomolecular insight into the mechanisms behind the responses to retrograde shear. However, it is important to emphasize that this was not the purpose of our study, especially since this is the first study to introduce and evaluate an in vivo procedure to chronically manipulate retrograde shear. Finally, we did not perform assessment of endothelium-independent dilation. Nonetheless, our primary finding of change in arterial function after prolonged retrograde SR in young, but not in older men, remains robust.

### Perspectives

Our compression-sleeve intervention successfully increased retrograde shear both acutely (30 minutes) and chronically (2 weeks) in both the younger and the older group. In young men, the increased retrograde shear produced by the compression sleeve impaired brachial artery endothelial function at 30 minutes, and chronic manipulation of retrograde shear was associated with a decrease in endothelial function comparable to that observed after 30 minutes. These findings highlight the potential detrimental impact of chronic elevations in retrograde SR. In marked contrast, older men demonstrate no change in endothelial function in response to this method of producing acute or chronic exposure to retrograde SR. This suggests advanced age is associated with impaired or attenuated ability of the vasculature to adapt in response to increases in retrograde SR in men in vivo, possibly due to blunted endothelial function as reflected in lower FMD.

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### Disclosures

None.

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