IMPACT OF RETROGRADE SHEAR RATE ON BRACHIAL AND SUPERFICIAL FEMORAL ARTERY FLOW-MEDIATED DILATION IN OLDER SUBJECTS

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ABSTRACT

An inverse, dose-dependent relationship between retrograde shear rate and brachial artery endothelial function exists in young subjects. This relationship has not been investigated in older adults, who have been related to lower endothelial function, higher resting retrograde shear rate and higher risk of cardiovascular disease. **Aim** To investigate the impact of a step-wise increase in retrograde shear stress on flow-mediated dilation in older males in the upper and lower limbs. **Methods** Fifteen older (68 ± 9 years) men reported to the laboratory 3 times. We examined brachial artery flow-mediated dilation before and after 30-minutes exposure to cuff inflation around the forearm at 0, 30 and 60 mmHg, to manipulate retrograde shear rate. Subsequently, the 30-minute intervention was repeated in the superficial femoral artery. Order of testing (vessel and intervention) was randomised. **Results** Increases in cuff pressure resulted in dose-dependent increases in retrograde shear in both the brachial and superficial femoral artery in older subjects. In both the brachial and the superficial femoral artery, no change in endothelial function in response to increased retrograde shear was observed in older males ('time' $P=0.274$, 'cuff*time $P=0.791$', 'cuff*artery*time $P=0.774$'). **Conclusion** In contrast with young subjects, we found that acute elevation in retrograde shear rate does not impair endothelial function in older humans. This may suggest that subjects with *a priori* endothelial dysfunction are less responsive or requires a larger shear rate stimulus to alter endothelial function.

KEY WORDS; RETROGRADE SHEAR STRESS, ENDOTHELIAL FUNCTION, SHEAR STRESS PATTERN, ECHO-DOPPLER, ATHEROSCLEROSIS.

INTRODUCTION

Shear stress, the frictional force of blood on the arterial wall, is an important stimulus to arterial adaptation.[1-3] Under resting conditions shear stress demonstrates a typical pattern across the cardiac cycle, flowing towards the periphery during systole (antegrade shear) and back to the heart during diastole (retrograde shear).[4] Antegrade shear stress is believed to be beneficial, having anti-atherogenic effects on the endothelium.[1, 5-10] In contrast, increased levels of retrograde shear stress are thought to have *pro*-atherogenic effects.[2, 10, 11]

Previously, our group reported an inverse and dose-dependent relationship between retrograde shear rate and brachial artery endothelial function.[2] In a more recent follow-up study, we found that, in young adults, acute exposure to an increase in retrograde shear leads to comparable decreases in flow-mediated dilation in atherosclerosis-prone (i.e. superficial femoral artery (SFA)) and –resistant (i.e. brachial artery (BA)) conduit arteries in humans.[12] Since these studies were performed in healthy volunteers, these findings are difficult to extrapolate to subjects with *a priori* endothelial dysfunction. Interestingly, adaptation in endothelial function in response to 8-weeks of exercise training may differ between healthy subjects[7, 13, 14] and those with endothelial dysfunction[15] Indeed, some animal studies[16-19] suggest that acute elevations in shear rate lead to distinct responses between young and older animals or those with cardiovascular disease. In general, these studies show that older age and/or cardiovascular disease is associated with an attenuated vascular adaptation to the same shear stimuli compared to healthy young animals.

Advanced age is associated with endothelial dysfunction[20-22] as well as elevated levels of retrograde shear rate.[23, 24] Whether acute elevations in retrograde shear stress contribute to further attenuation of endothelial function is currently unknown. The primary aim of this study was to assess changes in

flow-mediated dilation (FMD), a measure of endothelial function, in response to increases of retrograde shear stress in older men. We hypothesized that retrograde shear-induced decreases in FMD would be diminished in older subjects. Secondly, we aimed to explore whether responses in FMD were similarly present in the atherosclerosis-resistant brachial and -prone superficial femoral artery. We expected that, similar to our previous observations in young subjects,[12] older subjects would demonstrate no differences between upper and lower limb conduit artery responses.

METHODS

Subjects

Fifteen older (68±9 years, BMI: 26.2±3.4 kg/m^2) recreationally active, Caucasian men were recruited from the community via advertisements in the local media. Recreationally active was defined as 1-7h of exercise training per week. Subjects who were diagnosed with overt cardiovascular disease were excluded, as were subjects with severe hypertension (systolic BP >160, diastolic BP >100). Subjects who were on medication influencing the cardiovascular system were instructed to abstain from medication during the measurement days. Smokers were excluded from this study. The study procedures were approved by the Ethics Committee of Radboud University Medical Center, adhered to the Declaration of Helsinki and all subjects gave prior written consent.

Experimental Design

Each subject reported to the laboratory three times. On each day, we examined the impact of a 30-minute intervention on brachial and femoral artery endothelial function. Measurements in the upper and lower limb arteries were performed consecutively (randomised between subjects, but kept consistent within a subject on the 3 testing days), whilst the cuff pressure was randomised between days and

between subjects. All measurements were done under standardized conditions and unilaterally (i.e.

right side). Endothelial function was examined using the flow-mediated dilation (FMD) technique,

which involves an ischemic stimulus induced by distal cuff inflation to supra-systolic level for 5-

minutes. Brachial and superficial femoral artery FMD was performed before and immediately after

each 30-minute intervention, which consisted of inflating an occlusion cuff (placed around the forearm

or thigh) to 0, 30 or 60 mmHg. All experiments adhered to the protocol of our previous study regarding

retrograde shear in young subjects.[12]

Experimental Procedures

Vascular function assessments were conducted in a quiet, temperature controlled environment,

according to recent expert consensus guidelines.[25] Repeated laboratory visits were conducted at the

same time of day to control for diurnal variation.[26] 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.

Flow-mediated endothelium-dependent vasodilator function (FMD%). Before and after the 30-minute

intervention, we assessed the flow-mediated dilation (FMD); i.e. an endothelium-dependent, partly

nitric oxide mediated dilation.[27] First, subjects rested supine for a period of 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 in

supine subjects using a manual sphygmomanometer. To examine brachial artery FMD, the right 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 on the forearm of the imaged arm,

immediately distal to the olecranon process to provide a stimulus to forearm ischaemia. A 10-MHz

multi-frequency linear array probe attached to a high resolution ultrasound machine (T3000; Terason,

Burlington, MA) was used to image the brachial arteries in the distal 1/3rd of the upper arm. When an optimal image was obtained, the probe was held stable and the ultrasound parameters were set to optimize the longitudinal, B-mode images of lumen-arterial wall interface. Doppler velocity assessment was continuously and simultaneously obtained using the ultrasound machine, and was collected using the lowest possible insonation angle (always <60°), which did not vary during each study. Baseline images were recorded for 1-minute. The forearm cuff was then inflated (>200 mmHg) for 5-minutes. Diameter and blood flow recordings resumed 30 sec prior to cuff deflation and continued for 3 minutes thereafter.

After performing the brachial artery FMD, we repeated this procedure for the superficial femoral artery. Subjects rested supine with the lower leg slightly elevated, resting on ~15 cm thick foam. The rapid inflation/deflation pneumatic cuff was positioned approximately 15 cm below the inguinal ligament to induce the 5 minute ischemic stimulus. Recording of the right superficial femoral artery was performed in the proximal third of the thigh, at least 3 cm distal from the bifurcation and above the occlusion cuff. Post-deflation recording of the superficial femoral artery was performed for 5 minutes. Performance of the ultrasound recordings for a single subject was performed by the same experienced sonographer.

Interventions. Immediately after the initial FMD assessment, a 30-minute intervention was observed. To manipulate brachial artery shear, an occlusion cuff was placed around one forearm and inflated to 0, 30 or 60 mmHg. Manipulation of shear patterns in the superficial femoral artery was performed placing the cuff around the thigh. Placement of the cuff was consistently performed around the right forearm and thigh. The order of cuff intervention (0, 30, 60 mmHg) was randomized across the 3 testing days, but similar for both arteries on a testing day. Pilot observations revealed that cuff inflation to 30 and 60

mmHg successfully altered retrograde shear rate in a dose-dependent manner, which was present in both the brachial and superficial femoral artery and both young and older humans. Brachial and superficial femoral artery mean shear rate and the pattern of shear rate (antegrade *versus* retrograde) were recorded at 10-minute intervals during each intervention. Oscillatory shear index (OSI, dimensionless) was calculated as an indicator for the magnitude of oscillation as │Retrograde shear│ / (│Antegrade shear│ + │Retrograde shear│).[24] OSI values range from 0 to 0.5, with 0 corresponding to unidirectional shear throughout the cardiac cycle, and 0.5 representing pure oscillation with time-average shear equal to 0.[24]

Data analysis

Analysis of brachial and superficial femoral artery diameters and shear rate before, during and after the intervention was performed using custom-designed edge-detection and wall-tracking software which is largely independent of investigator bias.[28] A detailed description of the technological details can be found elsewhere.[29] Ultimately, from the synchronized diameter and velocity data, blood flow (the product of lumen cross-sectional area and Doppler velocity (v)) and shear rate (4 times velocity divided by diameter)[30, 31] were calculated at 30 Hz. Baseline diameter, blood flow, and shear rate were calculated as the mean of data acquired across the 1 minute preceding the cuff inflation period. Peak diameter following cuff deflation was automatically detected according to an algorithm.[29] FMD% was calculated as the percentage rise of this peak diameter from the preceding baseline diameter.

Statistics

Statistical analyses were performed using SPSS 20.0 (SPSS, Chicago, Illinois). All data are reported as mean (± standard deviation (SD)) unless stated otherwise, while statistical significance was assumed at p<0.05. In figures 1 and 2, data is presented as mean (± standard error (SE)). All data were distributed

normally. Baseline differences between days were tested using one-way ANOVA. We performed Bonferroni correction to correct for multiple comparisons by dividing α by the number of comparisons. For shear rate analysis, we adopted a 2-way ANOVA to determine whether the impact of cuff inflation on retrograde shear rate ('cuff': 0, 30 *versus* 60 mmHg) differed between arteries ('artery': brachial *versus* superficial femoral artery). Subsequently, to explore the impact of these elevations in retrograde shear rate on endothelial function, we added the comparison of FMD before and after the 30-minute intervention ('time') into a 3-way ANOVA. Post-hoc *t*-tests were performed when a main or interaction effect was found. Pearson correlations were used to examine the relation between changes in retrograde shear rate and changes in FMD%. According to a recent study by Atkinson *et al.*, 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 less than one.[32] We checked our data for this phenomenon, and where appropriate we performed the allometric modelling solution proposed by Atkinson *et al.*,[32] which employs a linear mixed models approach with 'time', 'artery', and 'cuff pressure' as fixed factors, and the natural logarithm of the baseline diameter as a covariate. **Last, we compared pre-intervention FMDs of our older group with pre-intervention FMDs of a group of healthy younger subjects that underwent the same experimental design.[12] This data was published elsewhere[12] and were included in the present study for reasons of comparison of pre-intervention endothelial function between older and younger subjects.**
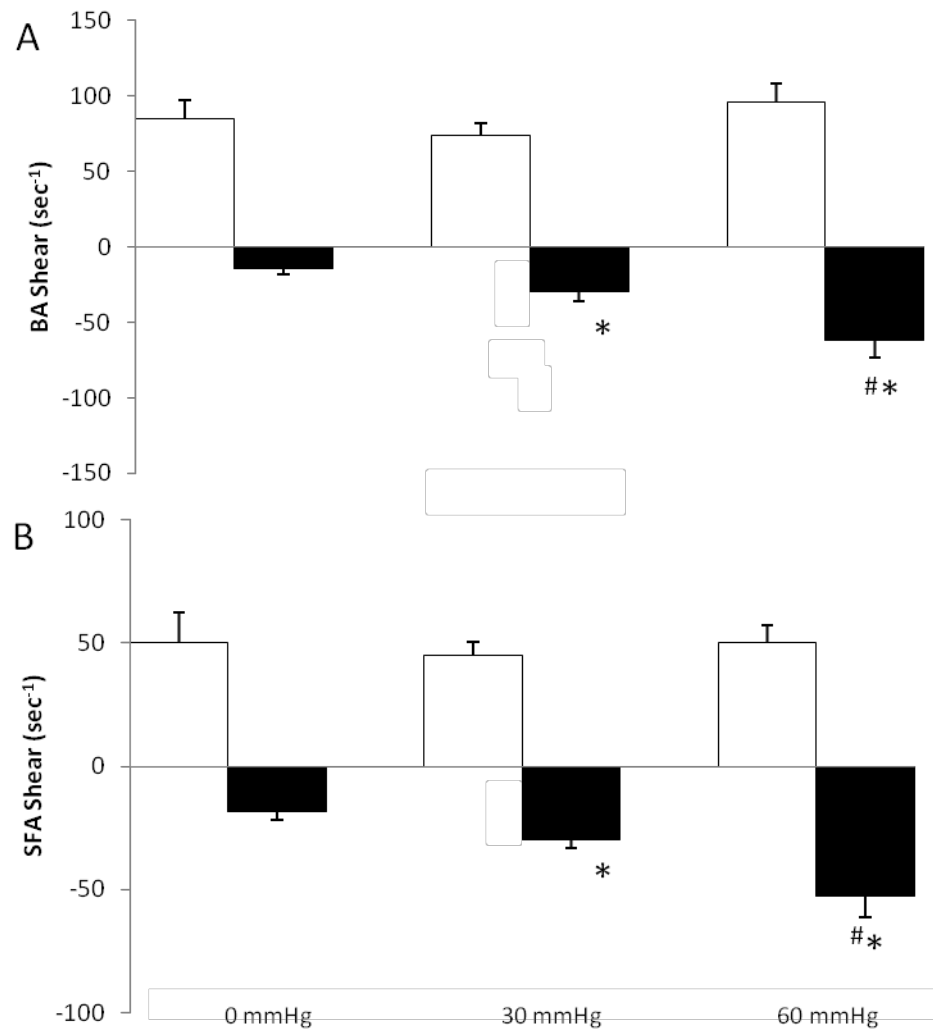
RESULTS

Baseline characteristics are described in Table 1. At baseline, we found no differences between testing days in pre-intervention systolic or diastolic blood pressure or brachial and superficial femoral artery shear rate patterns in older subjects (Table 2). **The subgroup of healthy young subjects were XX±XX years and showed a BMI of XX±XX kg/m² and were normotensive (XX±XX and XX±XX for systolic and diastolic blood pressure).**

Table 1. Subject characteristics in older subjects (N=15). Data are presented as mean±SD.

Variable	Older men (N=15)
Age (yrs)	68±9
Height (cm)	176±4
Weight (kg)	81.4±13.0
Body mass index (kg/m²)	26.2±3.4
Systolic blood pressure (mmHg)	132±11
Diastolic blood pressure (mmHg)	77±7
Mean arterial pressure (mmHg)	97±11

Table 2. Pre-intervention resting mean antegrade shear rate, retrograde shear rate, and oscillatory shear index in the brachial and superficial femoral artery of older men (N=15). Data is presented as mean ± SD. P-values represent repeated measures ANOVA.

	0 mmHg	30 mmHg	60 mmHg	P-value	0 mmHg	30 mmHg	60 mmHg	P-value
	Brachial Artery				Superficial Femoral Artery			

Antegrade shear rate (s^{-1})	80±45	75±40	86±45	0.517	43±24	47±17	48±19	0.740
Retrograde shear rate (s^{-1})	-16±16	-15±17	-13±19	0.718	-22±16	-22±12	-26±13	0.616
Oscillatory shear index	0.19±0.19	0.16±0.12	0.14±0.17	0.327	0.35±0.18	0.32±0.14	0.35±0.13	0.820

Shear rate patterns

Increases in cuff pressure resulted in step-wise increases in retrograde shear ('cuff' P<0.001). This effect was similarly present between brachial and superficial femoral arteries ('artery*cuff' P=0.203). Post-hoc *t*-tests confirmed that the effect of cuff inflation was dose-dependent (Figure 1). Antegrade shear did not change during cuff inflation to 30 and 60 mmHg in both arteries ('cuff' P=0.202, 'artery*cuff' P=0.445) (Figure 1).
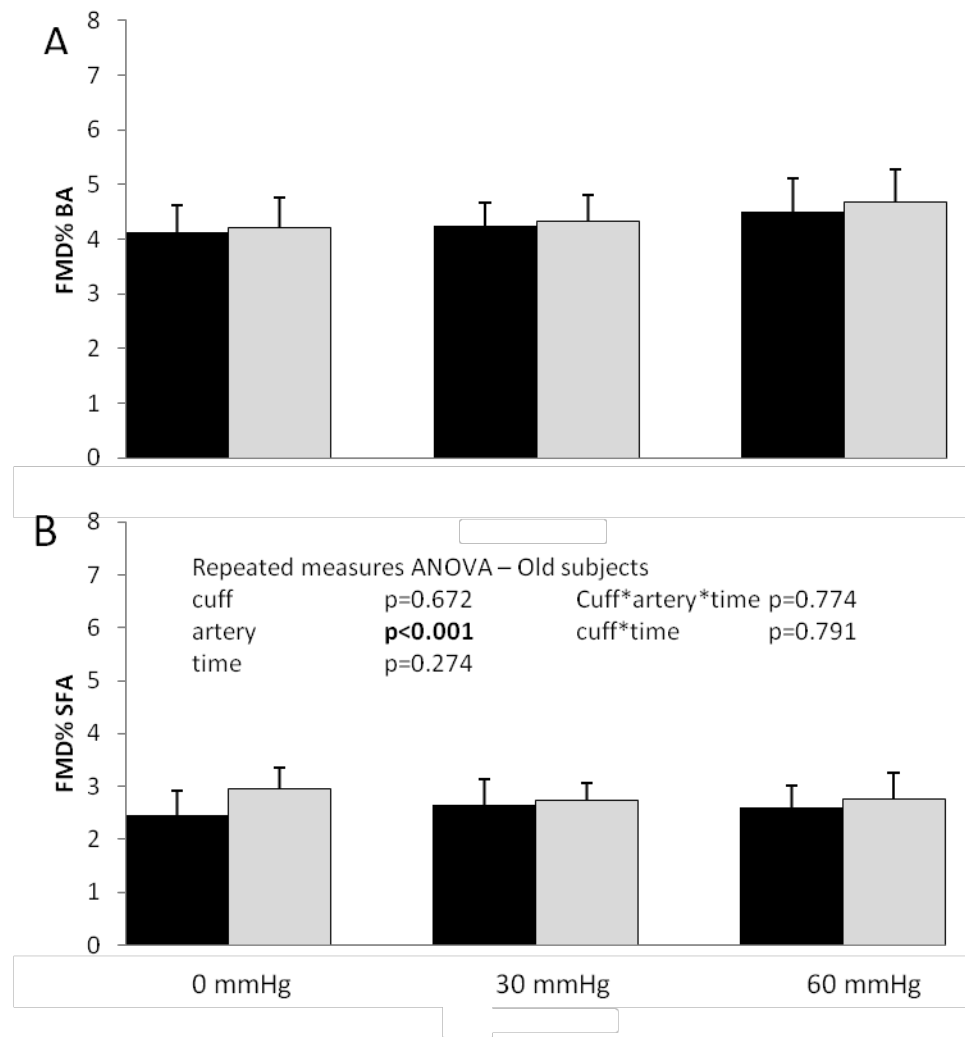
Figure 1 - Antegrade (white) and retrograde shear rate (black) in the brachial (A) and superficial femoral artery (B) during the 30-minute intervention at 0, 30, and 60 mmHg in old subjects (N=15). Error bars represent SE. *Significantly different from baseline at p<0.05. #Significantly different from 30 mmHg at p<0.05.

Flow-mediated vasodilation

Pre-intervention FMD values in older subjects (XX±XX% and XX±XX%) were significantly lower compared with younger subjects (XX±XX% and XX±XX%)[12] in both the brachial and the superficial femoral artery, respectively (P<0.001 and P=0.001). Also after allometric scaling, we found that older subjects demonstrated a significantly lower FMD for the brachial and superficial femoral artery (P=0.XX and 0.XX, respectively).

Our analysis revealed no effect of cuff inflation on FMD in older subjects in either artery ('time' P=0.274, 'cuff*time' P=0.791, 'cuff*artery*time' P=0.774, Figure 2). We also performed a linear

mixed model analysis to correct for inadequate scaling. This analysis reinforced our initial findings (all
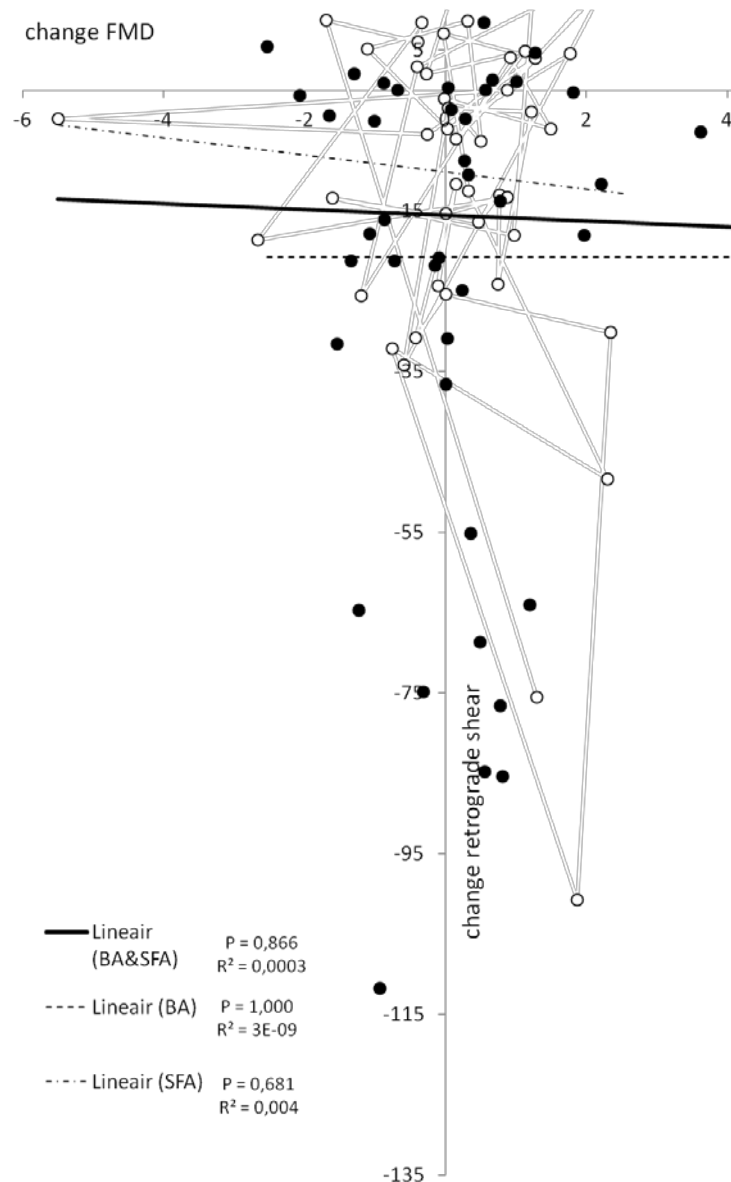
fixed factors P>0.05).

Figure 2 - Flow mediated dilation before (black) and after the 30-minute intervention (white) in the brachial (A) and superficial femoral artery (B) for interventions at 0, 30, and 60 mmHg in old subjects (N=15). Error bars represent SE. *Significantly different from pre-intervention at p<0.05. #Significantly different from young subjects at p<0.05.

Correlations

Older humans showed no correlation between the cuff-inflation induced change in retrograde shear and the change in FMD in the brachial artery (P=1.000, r<0.001), superficial femoral artery (P=0.681, r=-0.063), and the pooled data set (P=0.866, r=-0.018) (Figure 3). We found a positive correlation between baseline FMD and ΔFMD (r=0.386, P<0.001).

Figure 3 - Correlation between change in retrograde shear (y-axis) and change in flow mediated dilation (x-axis) for the brachial (black dots) and superficial femoral artery (open dots). We have included data from all three cuff pressures in this analysis as separate data points.

DISCUSSION

The aim of this study was to assess the impact of elevation in retrograde shear on endothelial function in older men and to determine whether this effect differs between arteries. We reported earlier that cuff inflation around a limb leads to an immediate elevation in retrograde shear rate, consequently leading to a dose-dependent decrease in the conduit artery endothelial function of young men.[12] We established that this effect is similarly present in the brachial and superficial femoral arteries. However, despite successful manipulation of retrograde shear rate with cuff inflation, older men did not demonstrate changes in endothelial function in either artery. **We established that pre-intervention FMD values in older subjects were significantly lower compared with younger subjects,[12] suggesting *a priori* endothelial dysfunction in our older subjects.** Our findings, therefore, indicate that distinct adaptations occur in the vasculature of young and older humans when exposed to similar hemodynamic stimuli. *A priori* endothelial dysfunction, such as often observed in healthy older humans, may be associated with an attenuated ability to adapt in response to shear rate.

Previous work in animals has reported attenuated sensitivity of both conduit and resistance vessels in aging[33], and age-related impairment for endothelial NO synthesis.[34, 35] Furthermore, the sensitivity to shear stress is decreased in resistance vessels of older rats compared to young rats.[36] Accordingly, we expected to observe an attenuated response in older humans during elevation of retrograde shear, demonstrated by less marked changes in endothelial function at the highest shear levels and a weak correlation between increases in retrograde shear and impairment in endothelial function. A potential explanation for the lack of such an impact of acute elevation in retrograde shear rate on endothelial function in healthy older humans relates to an insufficient increase in retrograde shear rate to affect FMD in older individuals, especially given the presence of a dose-response relation in young subjects.[12] However, we found elevations in retrograde shear in older humans that were of similar magnitude to

those previously observed in young subjects.[12] Furthermore, we found no correlation between the level of retrograde shear and changes in FMD in the older subjects. Therefore, the magnitude of retrograde SR is unlikely to explain our results. However, we cannot exclude the possibility that the duration of the retrograde shear stimulus was too short to elicit a response in older men.

Another potential explanation for the absence of a change in endothelial function after elevation in retrograde shear rate in older subjects relates to the sensitivity of smooth muscle cells. Depressed sensitivity to NO may contribute to attenuated or absent changes in FMD. We did not measure smooth muscle cell function in this study. However, it has been suggested that smooth muscle cell sensitivity to vasodilators is preserved with older age, despite deterioration of endothelium-dependent vasodilation.[37] However, findings on the impact of advanced age on preservation of smooth muscle cell sensitivity to vasodilators have been conflicting.[38] Other possible explanations to the absence of a change in FMD may relate to an age-related increased arterial stiffness, hyperactive sympathetic system and/or increased vascular tone. Finally, the absence of an effect of retrograde shear rate on FMD in older subjects may relate to an *a priori* lower FMD. Similarly, a recent study found that FMD improvements in response to an acute bout of exercise are dependent of resting FMD, with a larger increase observed in subjects with lower *a priori* FMD values.[39] We have performed a similar comparison and confirm the presence of a positive correlation between baseline FMD and ΔFMD (r=0.386, P<0.001). In other words, a decrease in FMD in response to elevations in retrograde shear was observed in those with higher *a priori* FMD values (i.e. preserved endothelial function). This suggests that a lower baseline FMD in older subjects could at least partly explain our findings.

Our second aim was to explore whether limb-specific differences were present. We expected that, in line with our previous observations in young subjects,[12] superficial femoral artery FMD responses after

exposure to elevation in retrograde shear rate would be comparable with brachial artery FMD responses in older subjects. We found no change in FMD to increases in retrograde shear in atherosclerosis-prone vessels or atherosclerosis-resistant vessels of older subjects. Furthermore, atherosclerosis-prone and -resistant arteries both have similar degrees of impairment at baseline in the older men, when compared with the younger subjects in our previous study.[12] Brachial artery FMD in older men is 33% lower than in young, whilst superficial femoral artery FMD in older men is 35% lower than in younger men. The comparable impairment in FMD, combined with the lack of responsiveness to retrograde shear, suggests that advanced age affects the endothelium systemically (possibly through atherosclerosis). Previously, it has been suggested that vasodilator responses are blunted in the leg compared with the arm.[40, 41] However, these studies did not investigate responsiveness to retrograde shear.

Clinical Relevance. As retrograde shear has been associated with the process of atherosclerosis, prolonged exposure to retrograde shear stress may be detrimental for vascular health.[42, 43] Previous work found that older subjects are exposed to elevated levels of retrograde shear.[24] In this light, it is important our data shows that acute increases in retrograde shear do not affect endothelial function in subjects with prior endothelial dysfunction. This impaired capacity of older vessels to alter endothelial function to changes in the endothelial milieu may prevent older humans to induce rapid vascular adaptations, both in response to potentially harmful or beneficial hemodynamic stimuli.

Limitations. Strengths of this study include the use of well-trained and experienced sonographers, the use of the automated analysis software and a tightly controlled, within-subject protocol. A potential weakness is that this study remains exploratory in nature and does not provide insight into the mechanisms explaining the absence of an effect of increases in retrograde shear on endothelial function in older humans, or whether the absence of an effect reflects (unknown) mechanism protecting the

endothelium against increases in retrograde shear rates. However, our study was not designed to answer such reductive questions, rather to describe the impact of *in vivo* changes in arterial hemodynamic conditions on arterial function in older subjects. Another limitation is that our participants were overweight in average, which may have influenced *a priori* endothelial function in our group of older humans.[44] A final limitation is that our current sample size allows us to detect changes in FMD of approximately 1%,[28] and we cannot fully rule out the possibility of type II errors yielding a false-negative result. As we have found changes in FMD exceeding the 1% threshold in our previous work on retrograde shear in younger subjects,[12] we believe that increasing the number of subjects will not alter the outcome of our current study.

In conclusion, in both the brachial and the superficial femoral artery we did not find a decrease in endothelial function in response to increased retrograde shear in older men *in vivo*. This findings contrasts with previous findings in younger men[12] and suggests that older men demonstrate attenuated adaptability to short (30-minute) exposure to elevations in retrograde shear rate *in vivo*. At least part of our results can be explained by the *a priori* lower endothelial function in older humans. Therefore, our data suggests that *a priori* endothelial dysfunction is associated with impaired adaptability of the conduit artery endothelium in response to short-term increases in retrograde shear in humans *in vivo*.

AUTHOR CONTRIBUTION

DJG and DHJT designed the study. THAS and DHJT collected and analysed the data. THAS, DJG, MTEH and DHJT interpreted the data. THAS, DJG, MTEH and DHJT wrote the manuscript. DHJT had primary responsibility for final content. DHJT is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript. No conflicts of interest exist.

CONFLICTS OF INTEREST DISCLOSURE

None of the authors have any conflict of interest.

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