Atherosclerosis 241 (2015) 199–204

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Impact of retrograde shear rate on brachial and superficial femoral artery flow-mediated dilation in older subjects

Tim H.A. Schreuder [a], Daniel J. Green [b, c], Maria T.E. Hopman [a], Dick H.J. Thijssen [a, b, *]

[a] Radboud Institute for Health Sciences, Radboud University Medical Center, The Netherlands
[b] Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, United Kingdom
[c] School of Sport Science, Exercise and Health, The University of Western Australia, Crawley, Western Australia, Australia

ARTICLE INFO

Article history:
Received 31 October 2014
Received in revised form
3 April 2015
Accepted 13 April 2015
Available online 18 April 2015

Keywords:
Retrograde shear stress
Endothelial function
Shear stress pattern
Echo-doppler
Atherosclerosis

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-min exposure to cuff inflation around the forearm at 0, 30 and 60 mmHg, to manipulate retrograde shear rate. Subsequently, the 30-min 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.

1. 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].

* Corresponding author. Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, United Kingdom.
E-mail address: D.Thijssen@ljmu.ac.uk (D.H.J. Thijssen).

http://dx.doi.org/10.1016/j.atherosclerosis.2015.04.017
0021-9150/

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.

2. Methods

2.1. 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 1–7 h 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.

2.2. Experimental design

Each subject reported to the laboratory three times. On each day, we examined the impact of a 30-min 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-min. Brachial and superficial femoral artery FMD was performed before and immediately after each 30-min 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].

2.3. 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 h, abstain from alcohol and caffeine for 18 h and avoid any exercise for 24 h.

2.3.1. Flow-mediated endothelium-dependent vasodilator function (FMD%)

Before and after the 30-min 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-min 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-min. The forearm cuff was then inflated (>200 mmHg) for 5-min. Diameter and blood flow recordings resumed 30 s prior to cuff deflation and continued for 3 min 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 min 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 min. Performance of the ultrasound recordings for a single subject was performed by the same experienced sonographer.

2.3.2. Interventions

Immediately after the initial FMD assessment, a 30-min 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-min 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].

2.4. 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,