IMPACT OF 2-WEEKS CONTINUOUS INCREASE IN RETROGRADE SHEAR STRESS ON BRACHIAL ARTERY VASOMOTOR FUNCTION IN YOUNG AND OLDER MEN

DICK H.J. THIJSSEN PHD[1,2]

TIM H.A. SCHREUDER PHD [1]

SEAN W. NEWCOMER PHD [4]

M. HAROLD LAUGHLIN PHD [5]

MARIA T.E. HOPMAN PHD, MD [1]

DANIEL J. GREEN PHD [2,3]

[1]*Radboud University Medical Center*,
Radboud Institute for Health Sciences, Department of Physiology, Nijmegen, The Netherlands
[2]*Research Institute for Sport and Exercise Science*,
Liverpool John Moores University, Liverpool, United Kingdom
[3]*School of Sport Science, Exercise and Health*,
The University of Western Australia, Crawley, Western Australia
[4]*Kinesiology*,
California State University San Marcos, San Marcos, California, USA
[5]*Department of Biomedical Sciences*,
University of Missouri, Columbia, MO, USA

SHORT TITLE: *Chronic retrograde shear in young and older men*

Subject code: [95] Endothelium/vascular type/nitric oxide

Total Word Count: 6,204

Word Count Abstract: 249

Total Number of Figures: 1

Total Number of Tables: 3

Author for correspondence:

Dick H.J. Thijssen, Professor in Cardiovascular Physiology and Exercise, Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, United Kingdom. Tel: +44 151 904 64 62, Email: D.Thijssen@ljmu.ac.uk

ABSTRACT

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±2yrs) and 13 older men (61±5yrs) were instructed to continuously wear a compression sleeve around the right forearm to chronically (2 weeks) elevate brachial artery retrograde SR in one arm. We assessed SR, diameter and flow-mediated dilation (FMD%) 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 FMD% was lower after 30-minutes and 2-weeks (P=0.004), whilst resting artery diameter was reduced after 2-weeks (P=0.005). The contra-lateral arm showed no change in retrograde SR or FMD% (P=0.32 and 0.26), but a decrease in diameter (P=0.035). In older men, FMD% and diameter did not change in either arm (all P>0.05). **Conclusion**. 30-minute elevation in retrograde SR in young men caused impaired endothelial function, whilst 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.

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 hemodynamic stimulus for arterial adaptation.[1-3] 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).[4] Elevations in antegrade shear stress are associated with potentially beneficial effects on the vessel wall.[1, 5-10] In contrast, elevation in retrograde shear stress may be potentially detrimental.[2, 10, 11] For example, an inverse and dose-dependent relationship is described between acute increases in retrograde shear rate and brachial artery endothelial function in young men.[2, 12]

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.[7, 13-17] These studies consistently report a dose-dependent increase in arterial caliber and improvement in endothelial function, possibly through upregulation of endothelial nitric oxide synthase.[1, 18] 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[19-21] and reduced arterial compliance.[22, 23] These changes may contribute to chronically elevated levels of retrograde shear stress with older age,[24, 25] 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 individuals.[26-29] 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 (i.e. 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±2 years) and thirteen older men (61±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 anti-hypertensive medication or lipid lowering drugs were excluded from participation. After registration, the participants received an information letter and had two 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 two 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 fifteen 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, shear rate 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, shear rate pattern, and FMD. Finally, we examined brachial artery diameter, shear rate pattern and FMD in the contra-lateral left arm, which acted as a non-compression 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 3-4 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, shear rate 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.[30] Repeated laboratory visits were conducted at the same time of day to control for diurnal variation.[31] 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 two or three 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, shear rate pattern and FMD%, as described above with all post-intervention tests performed with the compression sleeve applied to the forearms.[32]

Assessment of brachial artery flow-mediated dilation (FMD%). Before, 30 minutes following and 2-weeks after sleeve application, endothelium-dependent,[33] mainly NO-mediated,[34] 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 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. Details for these procedures can be found elsewhere.**[32]** 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 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.[32, 35] 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)[36, 37] 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 (i.e. red blood cells flowing in the antegrade direction) and negative velocities (i.e. 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.[32] The shear rate stimulus, responsible for endothelium-dependent FMD, was calculated after cuff deflation.[32, 37] Based on a previously collected population of 65 healthy men (24±3 yrs), 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, Illinois). 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 post-intervention measurement were tested using paired *t*-tests. For diameter, shear rate, and FMD analysis, we adopted a one-way ANOVA to determine the impact of the sleeve intervention across time (baseline, 30-min 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 contra-lateral 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 less than one.[38] Accordingly, for the FMD% data only (both in young and older men), we performed an allometric modelling solution,[38] 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, BMI, 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, whilst 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 shear rate, with post-hoc analysis revealing an increase in retrograde shear rate after 30 minutes and 2-weeks (P=0.004). Changes in antegrade shear rate 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 shear rate, brachial artery FMD% was significantly lower after both 30 minutes and 2-weeks in the

intervention arm (P=0.004, Figure 1). Whilst 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 1). The decrease in FMD% after 2-weeks wearing the compression 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 shear rate, 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 shear rate 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-weeks of wearing the compression sleeve (P<0.05, Table 3). The change in antegrade shear rate, 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 1). 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 shear rate represent a potent hemodynamic stimulus that acutely and chronically alters vascular function and structure.[1, 5-10] As a logical follow-up study from initial findings that acute elevation in retrograde shear induces impairment in endothelial function,[2] this study aimed to assess the effect of chronic (i.e. 2-weeks) manipulation of retrograde shear rate 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 shear rate 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 shear rate. Finally, and in marked contrast with young men, we found no acute (30-minute) or chronic (2-weeks) impact of elevating retrograde shear rate levels on brachial artery endothelial function in healthy older men. This unique *in vivo* data in humans reveals the impact of increased retrograde shear rate on brachial artery vasculature, whilst this effect may differ based on age.

In previous studies, we used pneumatic cuffs to manipulate retrograde shear acutely.[2, 12] For chronic shear manipulation, however, blood pressure cuffs are impractical. Therefore, in the present study we have introduced customized compression sleeves as a novel method to chronically manipulate levels of retrograde shear rate. In both young and older men, retrograde shear rate 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 shear rate, both acutely and chronically in men *in vivo*. Although a non-significant increase was found in antegrade shear rate in both young and older men, the main observation was that we (in line with our previous

work) successfully manipulated retrograde shear.[2, 12, 39] Although we only explored the impact of the sleeve intervention on resting levels of retrograde shear, we are confident that increased levels of retrograde shear rate 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.[4, 40] 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 shear rate. Previous work on this topic found that external compression on a limb leads to a decrease (40-84 mmHg compression),[41] no change (~37 mmHg compression)[42] or increase (13-23 mmHg compression)[43] 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,[12] 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.[44] During the 2 weeks post-intervention measurements of endothelial function we did not remove the compression sleeve (because of practical reasons). This may have influenced our measurements. However, since the sleeve did not acutely alter the SR area-under-the-curve and/or resting diameter (Tables 2-3), 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.[39] 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 shear rate 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[28] and it is generally accepted that aged arteries become stiffer and less functionally responsive.[45] 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 shear rate. 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.[39] 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.[10, 46] Interestingly, recent observations from Peiffer *et al.*, using a computational technique to describe that multidirectional wall shear stress, suggested that multi-directional shear may be relevant to the development of atherosclerosis.[47] 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 16-28mmHg. 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 shear rate between individuals. Nonetheless, our experiment was effective in its utilisation of a compression sleeve to manipulate retrograde shear. Another limitation is that adherence to the intervention was self-reported. Whilst 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 3-4 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 shear rate. In marked contrast, older men demonstrate no change in endothelial function in response to this method of producing acute or chronic exposure to retrograde shear rate. This suggests advanced age is associated with impaired or attenuated ability of the vasculature to adapt in response to increases in retrograde shear rate in men *in vivo*, possibly due to blunted endothelial function as reflected in lower FMD.

ACKNOWLEDGEMENTS & AUTHOR CONTRIBUTIONS

We would like to thank Paul van Herpt, Yvette Bouts, Janneke Bakker, Judith Heijman and Michiel Zijlker for their assistance.

DJG, SCN and DHJT designed the study. THAS and DHJT collected and analyzed the data. All authors interpreted the data. THAS, DJG and DHJT wrote the manuscript. DHJT and DJG 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.

SOURCES OF FUNDING

DHJT was supported by Netherlands Heart Foundation (E Dekker stipend, 2009T064).

DJG is funded by the Australia Research Council Grant DP1313793.

CONFLICTS OF INTEREST DISCLOSURE

None of the authors have any conflict of interest.

REFERENCES:

1. Tinken TM, Thijssen DH, Hopkins N, Black MA, Dawson EA, Minson CT, Newcomer SC, Laughlin MH, Cable NT, Green DJ. Impact of shear rate modulation on vascular function in humans. *Hypertension*. 2009;54:278-285
2. Thijssen DH, Dawson EA, Tinken TM, Cable NT, Green DJ. Retrograde flow and shear rate acutely impair endothelial function in humans. *Hypertension*. 2009;53:986-992
3. Niebauer J, Cooke JP. Cardiovascular effects of exercise: Role of endothelial shear stress. *J Am Coll Cardiol*. 1996;28:1652-1660
4. Green D, Cheetham C, Reed C, Dembo L, O'Driscoll G. Assessment of brachial artery blood flow across the cardiac cycle: Retrograde flows during cycle ergometry. *J Appl Physiol*. 2002;93:361-368
5. Chappell DC, Varner SE, Nerem RM, Medford RM, Alexander RW. Oscillatory shear stress stimulates adhesion molecule expression in cultured human endothelium. *Circ Res*. 1998;82:532-539
6. Hsiai TK, Cho SK, Wong PK, Ing M, Salazar A, Sevanian A, Navab M, Demer LL, Ho CM. Monocyte recruitment to endothelial cells in response to oscillatory shear stress. *FASEB J*. 2003;17:1648-1657

7. Tinken TM, Thijssen DH, Hopkins N, Dawson EA, Cable NT, Green DJ. Shear stress mediates endothelial adaptations to exercise training in humans. *Hypertension*. 2010;55:312-318
8. Wang C, Baker BM, Chen CS, Schwartz MA. Endothelial cell sensing of flow direction. *Arterioscler Thromb Vasc Biol*. 2013;33:2130-2136
9. Laughlin MH, Newcomer SC, Bender SB. Importance of hemodynamic forces as signals for exercise-induced changes in endothelial cell phenotype. *J Appl Physiol (1985)*. 2008;104:588-600
10. Newcomer SC, Thijssen DH, Green DJ. Effects of exercise on endothelium and endothelium/smooth muscle cross talk: Role of exercise-induced hemodynamics. *J Appl Physiol*. 2011;111:311-320
11. Widlansky ME, Gokce N, Keaney JF, Jr., Vita JA. The clinical implications of endothelial dysfunction. *J Am Coll Cardiol*. 2003;42:1149-1160
12. Schreuder THA, Green DJ, Hopman MTE, Thijssen DHJ. Acute impact of retrograde shear rate on brachial and superficial femoral artery flow-mediated dilation in humans. *Physiol Rep*. 2014;2:e00193
13. Carter HH, Spence AL, Atkinson CL, Pugh CJ, Naylor LH, Green DJ. Repeated core temperature elevation induces conduit artery adaptation in humans. *Eur J Appl Physiol*. 2014;114:859-865
14. Birk GK, Dawson EA, Atkinson C, Haynes A, Cable NT, Thijssen DH, Green DJ. Brachial artery adaptation to lower limb exercise training: Role of shear stress. *J Appl Physiol*. 2012;112:1653-1658
15. Naylor LH, Carter H, FitzSimons MG, Cable NT, Thijssen DH, Green DJ. Repeated increases in blood flow, independent of exercise, enhance conduit artery vasodilator function in humans. *Am J Physiol Heart Circ Physiol*. 2011;300:H664-669

16. Green DJ, Carter HH, Fitzsimons MG, Cable NT, Thijssen DH, Naylor LH. Obligatory role of hyperaemia and shear stress in microvascular adaptation to repeated heating in humans. *J Physiol*. 2010;588:1571-1577

17. Carter HH, Spence AL, Atkinson CL, Pugh CJ, Cable NT, Thijssen DH, Naylor LH, Green DJ. Distinct effects of blood flow and temperature on cutaneous microvascular adaptation. *Med Sci Sports Exerc*. 2014;46:2113-2121

18. Tuttle JL, Nachreiner RD, Bhuller AS, Condict KW, Connors BA, Herring BP, Dalsing MC, Unthank JL. Shear level influences resistance artery remodeling: Wall dimensions, cell density, and enos expression. *Am J Physiol Heart Circ Physiol*. 2001;281:H1380-1389

19. Seals DR, Jablonski KL, Donato AJ. Aging and vascular endothelial function in humans. *Clinical science*. 2011;120:357-375

20. Seals DR, Kaplon RE, Gioscia-Ryan RA, LaRocca TJ. You're only as old as your arteries: Translational strategies for preserving vascular endothelial function with aging. *Physiology (Bethesda)*. 2014;29:250-264

21. Taddei S, Virdis A, Mattei P, Ghiadoni L, Gennari A, Fasolo CB, Sudano I, Salvetti A. Aging and endothelial function in normotensive subjects and patients with essential hypertension. *Circulation*. 1995;91:1981-1987.

22. Himburg HA, Dowd SE, Friedman MH. Frequency-dependent response of the vascular endothelium to pulsatile shear stress. *Am J Physiol Heart Circ Physiol*. 2007;293:H645-653

23. Pepe S, Lakatta EG. Aging hearts and vessels: Masters of adaptation and survival. *Cardiovasc Res*. 2005;66:190-193

24. Casey DP, Padilla J, Joyner MJ. Alpha-adrenergic vasoconstriction contributes to the age-related increase in conduit artery retrograde and oscillatory shear. *Hypertension*. 2012;60:1016-1022

25. Padilla J, Simmons GH, Fadel PJ, Laughlin MH, Joyner MJ, Casey DP. Impact of aging on conduit artery retrograde and oscillatory shear at rest and during exercise: Role of nitric oxide. *Hypertension*. 2011;57:484-489

26. Dumont O, Pinaud F, Guihot AL, Baufreton C, Loufrani L, Henrion D. Alteration in flow (shear stress)-induced remodelling in rat resistance arteries with aging: Improvement by a treatment with hydralazine. *Cardiovasc Res*. 2008;77:600-608

27. Freidja ML, Vessieres E, Clere N, Desquiret V, Guihot AL, Toutain B, Loufrani L, Jardel A, Procaccio V, Faure S, Henrion D. Heme oxygenase-1 induction restores high-blood-flow-dependent remodeling and endothelial function in mesenteric arteries of old rats. *J Hypertens*. 2011;29:102-112

28. Sun D, Huang A, Yan EH, Wu Z, Yan C, Kaminski PM, Oury TD, Wolin MS, Kaley G. Reduced release of nitric oxide to shear stress in mesenteric arteries of aged rats. *Am J Physiol Heart Circ Physiol*. 2004;286:H2249-2256

29. Tuttle JL, Hahn TL, Sanders BM, Witzmann FA, Miller SJ, Dalsing MC, Unthank JL. Impaired collateral development in mature rats. *Am J Physiol Heart Circ Physiol*. 2002;283:H146-155

30. Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B, Widlansky ME, Tschakovsky ME, Green DJ. Assessment of flow-mediated dilation in humans: A methodological and physiological guideline. *Am J Physiol Heart Circ Physiol*. 2011;300:H2-12

31. Jones H, Lewis NC, Thompson A, Marrin K, Green DJ, Atkinson G. Diurnal variation in vascular function: Role of sleep. *Chronobiol Int*. 2012;29:271-277

32. Black MA, Cable NT, Thijssen DH, Green DJ. Importance of measuring the time course of flow-mediated dilatation in humans. *Hypertension*. 2008;51:203-210

33. Dawson EA, Rathore S, Cable NT, Wright DJ, Morris JL, Green DJ. Impact of catheter insertion using the radial approach on vasodilatation in humans. *Clinical science*. 2010;118:633-640

34. Green DJ, Dawson EA, Groenewoud HM, Jones H, Thijssen DH. Is flow-mediated dilation nitric oxide mediated?: A meta-analysis. *Hypertension*. 2014;63:376-382

35. Woodman RJ, Playford DA, Watts GF, Cheetham C, Reed C, Taylor RR, Puddey IB, Beilin LJ, Burke V, Mori TA, Green D. Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *J Appl Physiol*. 2001;91:929-937

36. Pyke KE, Dwyer EM, Tschakovsky ME. Impact of controlling shear rate on flow-mediated dilation responses in the brachial artery of humans. *J Appl Physiol*. 2004;97:499-508

37. Pyke KE, Tschakovsky ME. Peak vs. Total reactive hyperemia: Which determines the magnitude of flow-mediated dilation? *J Appl Physiol*. 2007;102:1510-1519

38. Atkinson G, Batterham AM, Thijssen DH, Green DJ. A new approach to improve the specificity of flow-mediated dilation for indicating endothelial function in cardiovascular research. *J Hypertens*. 2013;31:287-291

39. Schreuder TH, Green DJ, Hopman MT, Thijssen DH. Impact of retrograde shear rate on brachial and superficial femoral artery flow-mediated dilation in older subjects. *Atherosclerosis*. 2015;241:199-204

40. Thijssen DH, Dawson EA, Black MA, Hopman MT, Cable NT, Green DJ. Brachial artery blood flow responses to different modalities of lower limb exercise. *Med Sci Sports Exerc*. 2009;41:1072-1079

41. Thorsson O, Hemdal B, Lilja B, Westlin N. The effect of external pressure on intramuscular blood flow at rest and after running. *Med Sci Sports Exerc*. 1987;19:469-473

42. Sperlich B, Born DP, Kaskinoro K, Kalliokoski KK, Laaksonen MS. Squeezing the muscle: Compression clothing and muscle metabolism during recovery from high intensity exercise. *PLoS One*. 2013;8:e60923

43. Bochmann RP, Seibel W, Haase E, Hietschold V, Rodel H, Deussen A. External compression increases forearm perfusion. *J Appl Physiol*. 2005;99:2337-2344

44. Gambillara V, Chambaz C, Montorzi G, Roy S, Stergiopulos N, Silacci P. Plaque-prone hemodynamics impair endothelial function in pig carotid arteries. *Am J Physiol Heart Circ Physiol*. 2006;290:H2320-2328

45. Thijssen DH, Carter SE, Green DJ. Arterial structure and function in vascular ageing: "Are you as old as your arteries?". *J Physiol*. 2015 (In Press, DOI: 10.1113/270597)

46. Laughlin MH, Newcomer SC, Bender SB. Importance of hemodynamic forces as signals for exercise-induced changes in endothelial cell phenotype. *J Appl Physiol*. 2008;104:588-600

47. Peiffer V, Sherwin SJ, Weinberg PD. Computation in the rabbit aorta of a new metric - the transverse wall shear stress - to quantify the multidirectional character of disturbed blood flow. *J Biomech*. 2013;46:2651-2658

48. Naylor LH, Weisbrod CJ, O'Driscoll G, Green DJ. Measuring peripheral resistance and conduit arterial structure in humans using doppler ultrasound. *J Appl Physiol*. 2005;98:2311-2315

FIGURE LEGENDS

Figure 1. Brachial artery flow-mediated dilation (FMD%, A-B) and diameter (C-D) in young (n=13, A-C) and older **men** (n=13 B-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 $P<0.05$.

Table 1. Characteristics of young (n=13) and older humans (n=13), before (pre) and after (post) the 2-week forearm compression intervention. Data are mean±SD. P-values represent paired t-tests.

	Young men			**Older men**		
	Pre	*Post*	**P-value**	*Pre*	*Post*	**P-value**
Age (yrs)	23±2			61±5#		
Height (cm)	182±8			177±4#		
Weight (kg)	71.7±9.5			89.9±17.3#		
BMI (kg/m^2)	21.7±2.3			29.2±5.6#		
Systolic BP (mmHg)	115±5	113±5	0.263	123±12#	121±11	0.075
Diastolic BP (mmHg)	72±6	70±8	0.271	79±6#	78±5	0.266
Mean BP (mmHg)	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

BP: blood pressure, #Significantly different between from young men at $P<0.05$ (unpaired *t*-test)

Table 2. Brachial artery shear rate (SR), diameter, and flow-mediated dilation (FMD%) at baseline and after 30-minutes and 2-weeks of forearm compression in young healthy men (n=13). Data are mean±SD. P-value refers to a one-way ANOVA (i.e. intervention-arm) or paired *t*-test (i.e. contra-lateral arm) which was performed to assess changes in the outcome parameters across time within the young men.

Intervention arm	**Baseline**	**30 minutes**	**2-weeks**	**P-value**
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
Shear $Rate_{AUC}$ (s) 10^3	30±6	25±5	28±10	0.102
SR antegrade (s^{-1})	96±31¶	97±30	118±36	0.068
SR retrograde (s^{-1})	-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
Shear $Rate_{AUC}$ (s) 10^3	35±9		35±10	0.982
SR antegrade (s^{-1})	119±38		105±32	0.192
SR retrograde (s^{-1})	-24±20		-20±15	0.318

*Significant difference from baseline at P≤0.05. #Significant difference from 30 minutes at P≤0.05. ¶Significant difference at baseline from contralateral arm.

Table 3. Brachial artery shear rate, diameter, and flow-mediated dilation (FMD%) at baseline and after 30-minutes and 2-weeks of forearm compression in 13 healthy older men. Data are presented as mean±SD. P-value refers to a one-way ANOVA (i.e. intervention-arm) or paired *t*-test (i.e. contra-lateral arm) which was performed to assess changes in the outcome parameters across time within the older men.

Intervention arm	**Baseline**	**30 minutes**	**2-weeks**	**P-value**
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
Shear $Rate_{AUC}$ (s) 10^3	24±8	24±9	22±8	0.717
SR antegrade (s^{-1})	78±37	75±24	93±44	0.081
SR retrograde (s^{-1})	-11±9	-28±24*	-17±15*	0.001
Contralateral arm				
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
Shear $Rate_{AUC}$ (s) 10^3	26±8		30±12	0.319
SR antegrade (s^{-1})	85±24		100±43	0.228
SR retrograde (s^{-1})	-10±9		-6±7	0.092

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

Figure 1