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Sympathetic nervous system activation, arterial shear rate, and flow-mediated dilation

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[1]*School of Sport Science, Exercise and Health, The University of Western Australia, Crawley, Western Australia, Australia;* [2]*Research Institute for Sport and Exercise Science, Liverpool John Moores University, Liverpool, United Kingdom;* *and* [3]*Department of Physiology, Radboud University Medical Centre, Nijmegen, The Netherlands*

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Thijssen DH, Atkinson CL, Ono K, Sprung VS, Spence AL, Pugh CJ, Green DJ. Sympathetic nervous system activation, arterial shear rate, and flow-mediated dilation. *J Appl Physiol* 116: 1300–1307, 2014. First published April 3, 2014; doi:10.1152/japplphysiol.00110.2014.—The aim of this study was to examine the contribution of arterial shear to changes in flow-mediated dilation (FMD) during sympathetic nervous system (SNS) activation in healthy humans. Ten healthy men reported to our laboratory four times. Bilateral FMD, shear rate (SR), and catecholamines were examined before/after 10-min of −35-mmHg lower body negative pressure ($LBNP_{10}$). On *day 1*, localized forearm heating ($LBNP_{10}$+heat) was applied in one limb to abolish the increase in retrograde SR associated with LBNP. *Day 2* involved unilateral cuff inflation to 75 mmHg around one limb to exaggerate the LBNP-induced increase retrograde SR ($LBNP_{10}$+cuff). Tests were repeated on *days 3* and *4*, using 30-min interventions (i.e., $LBNP_{30}$+heat and $LBNP_{30}$+cuff). $LBNP_{10}$ significantly increased epinephrine levels and retrograde SR and decreased FMD (all $P < 0.05$). $LBNP_{10}$+heat prevented the increase in retrograde SR, whereas $LBNP_{10}$+cuff further increased retrograde SR ($P < 0.05$). Heating prevented the decrease in percent FMD (FMD%) after $LBNP_{10}$ (interaction effect, $P < 0.05$), whereas cuffing did not significantly exaggerate the decrease in FMD% (interaction effect, $P > 0.05$). Prolongation of the LBNP stimulus for 30-min normalized retrograde SR, catecholamine levels, and FMD (all $P > 0.05$). Attenuation of retrograde SR during 30 min ($LBNP_{30}$+heat) was associated with increased FMD% (interaction effects, $P < 0.05$), whereas increased retrograde SR ($LBNP_{30}$+cuff) diminished FMD% (interaction effects, $P < 0.05$). These data suggest that LBNP-induced SNS stimulation decreases FMD, at least in part due to the impact of LBNP on arterial shear stress. Prolonged LBNP stimulation was not associated with changes in SR or FMD%. Our data support a role for changes in SR to the impact of SNS stimulation on FMD.

sympathetic nervous system; endothelial function; cardiovascular risk; shear stress; norepinephrine

THE SYMPATHETIC NERVOUS SYSTEM (SNS) plays an important role in the regulation of vascular tone. Some previous studies have linked activation of the SNS to prognosis in cardiovascular disease (4, 14). Interestingly, physiological aging (18), as well as cardiovascular disease (16) or risk (19), have been characterized by increased levels of SNS activity, and subsequent studies have related the elevated SNS activity to vascular reactivity (5, 6). These findings highlight the importance of the SNS in the regulation of vascular health in humans.

Previous studies pertaining to endothelial function have reported that acute activation of the SNS leads to an immediate vasodilator impairment (8, 10, 17, 27), although this may depend on the type of SNS stimulus adopted (7). While the precise mechanisms underlying the relationship between SNS activation and endothelial function remain unclear, one possibility relates to the impact of SNS activation on shear stress, the frictional force that blood exerts on the endothelium of conduit arteries. A recent paper by Padilla and colleagues (22) demonstrated that activation of the SNS leads to an immediate increase in retrograde shear stress, which is associated with decreased endothelial function (15, 21, 26, 29). The typical decrease in endothelial function during acute activation of the SNS may, therefore, be mediated through elevations in retrograde shear rate.

The first aim of this experiment was to examine the role of shear patterns in the acute effect of the SNS on endothelial function, measured as brachial artery flow-mediated dilation (FMD). To examine this hypothesis, we experimentally manipulated shear during SNS stimulation in one arm. More specifically, we unilaterally abolished (using local heat application) or augmented (using subdiastolic cuff inflation) retrograde shear during SNS stimulation. FMD was examined simultaneously and bilaterally, before and after the test. We hypothesized that the decrease in FMD after a brief period of lower body negative pressure (LBNP) (10 min) would be abolished by unilateral local heat application, but exaggerated when retrograde shear was increased by cuff placement around one forearm. Second, we examined whether the acute effect of SNS activation on endothelial function is apparent after both brief (10 min) and prolonged (30 min) LBNP. Given the dose-dependent relationship between acute changes in shear and artery diameter (3) and FMD (2, 29), we hypothesized a larger impact of shear on FMD after prolonged SNS activation.

METHODS

Participants

A total of 10 healthy recreationally active men (28 ± 5 yr; 178 ± 8 cm; 76 ± 9.4 kg) volunteered to participate. Based on their medical history, participants did not have any health problems, and none was taking any medication. Before testing, all participants were informed of the methods of the study, and all provided written, informed consent. The study was approved by the University of Western Australia's institutional ethics committee and adhered to the Declaration of Helsinki (2008).

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Experimental Design

For this within-subject study, participants reported to our laboratory on four occasions. On each day, using high-resolution ultrasound, we simultaneously examined bilateral brachial artery shear rate patterns and endothelial function [i.e., FMD according to recent expert consensus guidelines (28)]. After each intervention, we reexamined brachial artery shear rate and FMD and plasma catecholamine levels. On *days 1* and *2*, the intervention consisted of exposure to −35-mmHg LBNP to activate the SNS and increase brachial artery retrograde shear rate. On *day 1*, the LBNP-mediated increase in brachial artery retrograde shear was unilaterally abolished by application of heat to one forearm (LBNP+heat) (20). On *day 2*, LBNP-induced retrograde shear was unilaterally increased by inflation of a blood pressure cuff to 75 mmHg around one forearm (LBNP+cuff) (29). We randomized the arm that received the cuff stimulus between subjects, but this remained consistent between the testing days, within subjects. We also randomized the order of the LBNP+heat and LBNP+cuff testing days. On *days 3* and *4*, these procedures were repeated, using a 30-min intervention period.

Experimental Measures

All participants attended testing sessions in a fasted state, having been asked to refrain from exercise, alcohol, and caffeine for 18 h before all experimental testing. All participants were studied at the same time of day to control for the impact of circadian variation, and tests were performed with a minimum of 1 day and a maximum of 7 days between testing sessions. On each day, instrumentation took place during the first 15 min, when the subjects were placed with their lower body in the LBNP tank in the supine position.

LBNP experiment. During the LBNP protocol, the subject was in the supine position with the lower limbs in an enclosed tank (VACU-Sport, GSA International, Aachen, Germany). Throughout the protocol (10 and 30 min), LBNP pressure was set at −35 mmHg. All subjects successfully completed the protocols.

Shear manipulation: heat. During LBNP on *day 1*, we heated one forearm. This allowed for a within-subject comparison of the impact of abolishing the increased exposure to retrograde shear rate during LBNP.

Shear manipulation: subdiastolic forearm cuff inflation. During the LBNP-protocol on *day 2*, a blood pressure cuff was inflated unilaterally around the forearm to ~75 mmHg. This allowed for a within-subject comparison of the impact of exaggerating the increased brachial artery retrograde shear rate during LBNP. This cuff pressure was maintained until cuff pressure was further increased to 220 mmHg for the postintervention assessment of FMD.

Plasma catecholamines. Subjects rested supine while a blood sample (10 ml) was drawn from an antecubital vein using a 22-gauge needle (BD Vacutainer Eclipse, Becton Dickinson) to examine blood levels of epinephrine (E) and norepinephrine (NE). Blood was drawn immediately before, and at the end of, the 10- and 30-min LBNP stimuli (i.e., after performance of the FMD procedures). Plasma was extracted and transferred to a microtube and frozen at −80°C. Posttest analysis of E and NE were performed in a four-step procedure. Catecholamines were adsorbed onto alumina at a pH of 8.6, washed and then eluted with a dilute acid, and analyzed by high-performance liquid chromatography with electrochemical detection.

Endothelial function. To examine brachial artery FMD, the arm was extended and positioned at an angle of ~80° from the torso. FMD was assessed with the subject in the same position used for the LBNP protocol, so that no change in posture or movement between the interventions and testing procedures was necessary. A rapid inflation and deflation pneumatic cuff (D. E. Hokanson, Bellevue, WA) was positioned on the forearm, immediately distal to the olecranon process, to provide a stimulus to forearm ischemia. A 10-MHz multifrequency linear array probes, attached to a high-resolution ultrasound machine (T3000; Terason, Burlington, MA) was then used to image the brachial artery in the distal one-third of the upper arm on both sides. When an optimal image was obtained, the probe was held stable, and the ultrasound parameters were set to optimize the longitudinal, B-mode image of the lumen-arterial wall interface. Settings were identical between all assessments of FMD. Continuous Doppler velocity assessments were also obtained using the ultrasound and were collected using the lowest possible isonation angle (always

Table 1. *MAP, SV, HR, CO, brachial artery diameter, FMD, and SR_{AUC} before and after 10-min LBNP+heat and LBNP+cuff in healthy volunteers*

	LBNP		LBNP+Heat		One- and Two-Way ANOVA		
	Pre	Post	Pre	Post	Time	Arm	Time × Arm
MAP, mmHg	86 ± 4	87 ± 8			0.75		
SV, ml	71 ± 8	63 ± 9			**<0.01**		
HR, beats/min	58 ± 6	62 ± 6			**<0.01**		
CO, liters	4.1 ± 0.7	3.9 ± 0.7			0.14		
Diameter, mm	4.1 ± 0.7	4.1 ± 0.7	4.1 ± 0.7	4.0 ± 0.7	0.40	0.79	0.15
FMD, mm	0.22 ± 0.07	0.16 ± 0.11	0.22 ± 0.09	0.20 ± 0.10	**0.047**	0.63	0.12
SR_{AUC}, 10^3/s	17.6 ± 10.7	14.5 ± 5.2	18.4 ± 10.2	18.0 ± 7.1	0.36	0.41	0.11
			LBNP+Cuff				
			Pre	Post			
MAP, mmHg	86 ± 5	86 ± 9			0.85		
SV, ml	66 ± 13	62 ± 7			0.09		
HR, beats/min	58 ± 7	65 ± 7			**<0.01**		
CO, liters	3.8 ± 0.9	4.0 ± 0.5			0.36		
Diameter, mm	4.0 ± 0.7	3.9 ± 0.7	4.0 ± 0.6	4.0 ± 0.6	0.41	0.85	0.94
FMD, mm	0.24 ± 0.08	0.16 ± 0.09*	0.21 ± 0.09	0.15 ± 0.08*	**0.01**	0.41	0.31
SR_{AUC}, 10^3/s	15.2 ± 5.9	13.4 ± 4.4	16.7 ± 8.2	12.3 ± 3.1	0.16	0.90	0.20

Values are means ± SD; $n = 9$ subjects. MAP, mean arterial pressure; SV, stroke volume; HR, heart rate; CO, cardiac output; FMD, flow-mediated dilation; SR_{AUC}, area under the shear rate curve, LBNP, lower body negative pressure; LBNP+heat, LBNP combined with forearm heating; LBNP+cuff, LBNP combined with forearm cuff inflation to 75 mmHg; Pre, before LBNP; Post, after LBNP. *P* value represents a two-way ANOVA examining the impact of LBNP (Pre vs. Post; "time") and whether the impact of LBNP differed between both arms. *Post hoc significantly different from Pre at $P < 0.05$. *P* values in bold are significantly different.

<60°). We first performed a 1-min baseline assessment of brachial artery diameter and velocity. Simultaneously, heart rate (HR) and blood pressure were measured continuously and beat to beat throughout the protocol (Finometer, Finapres Medical Systems, Amsterdam, the Netherlands). Subsequently, the forearm cuffs were inflated (>200 mmHg) for 5 min. Diameter and flow recordings resumed 30 s before cuff deflation and continued for 3 min thereafter, in accordance with recent technical specifications (1, 31).

Blood flow and shear rate. Using the methods described above, brachial artery diameter and red blood cell velocity were recorded at the end of the 10- or 30-min intervention across a 1-min period, during which we continuously recorded diameter and red blood cell velocity (30 Hz) to provide beat-to-beat information. Using semiautomated software (see below) and the synchronized diameter and velocity data, we calculated the mean, antegrade, and retrograde blood flow and shear rate. Antegrade and retrograde mean blood velocities per cardiac cycle were calculated using the average of only positive or negative data points, respectively, yielding within-cardiac cycle values for these parameters.

Central hemodynamics. A Finometer PRO (Finapres Medical Systems) was used to measure changes in mean arterial pressure (MAP), HR, cardiac output (CO), and stroke volume (SV) via photoplethysmography. These data were exported to a data acquisition system PowerLab (LabChart 7, ADInstruments, Sydney, Australia) in real time. The finger cuff was placed around the middle phalanx of the index or middle finger of the hand of the noncuffed arm. The subject was instructed not to move their arm or finger during recording. Total peripheral resistance was calculated in real time in LabChart, while the cyclical measurement feature used systolic peaks to count and average HR.

Brachial Artery Diameter, Blood Flow and Shear Rate Analysis

Analysis of brachial artery diameter was performed using custom-designed edge-detection and wall-tracking software, which is independent of investigator bias. Recent papers contain detailed descriptions of our analysis approach (1, 31). From synchronized diameter and velocity data, blood flow (the product of lumen cross-sectional area and Doppler velocity) was calculated at 30 Hz. Shear rate (an estimate of shear stress without viscosity) was calculated as four times mean blood velocity/vessel diameter. Reproducibility of diameter measurements using this semiautomated software is significantly better than manual methods, reduces observer error significantly, and possesses an intraobserver coefficient of variation of 6.7% (31). For the FMD, we identified the postocclusion peak diameter using an algorithm, described in detail elsewhere (1).

Statistics

Statistical analyses were performed using SPSS 20.0 (SPSS, Chicago, IL) software. Following log transformation, blood levels of E and NE were compared using a paired Student's *t*-test. Our primary outcome was brachial artery FMD. A two-factor general linear model (time × arm) was used to analyze percent FMD (FMD%), and mean, antegrade, and retrograde shear rate after the 10- or 30-min LBNP intervention ("time"; pre- vs. post-LBNP), and whether these changes across time differed between both arms ("arm": cuffed arm vs.

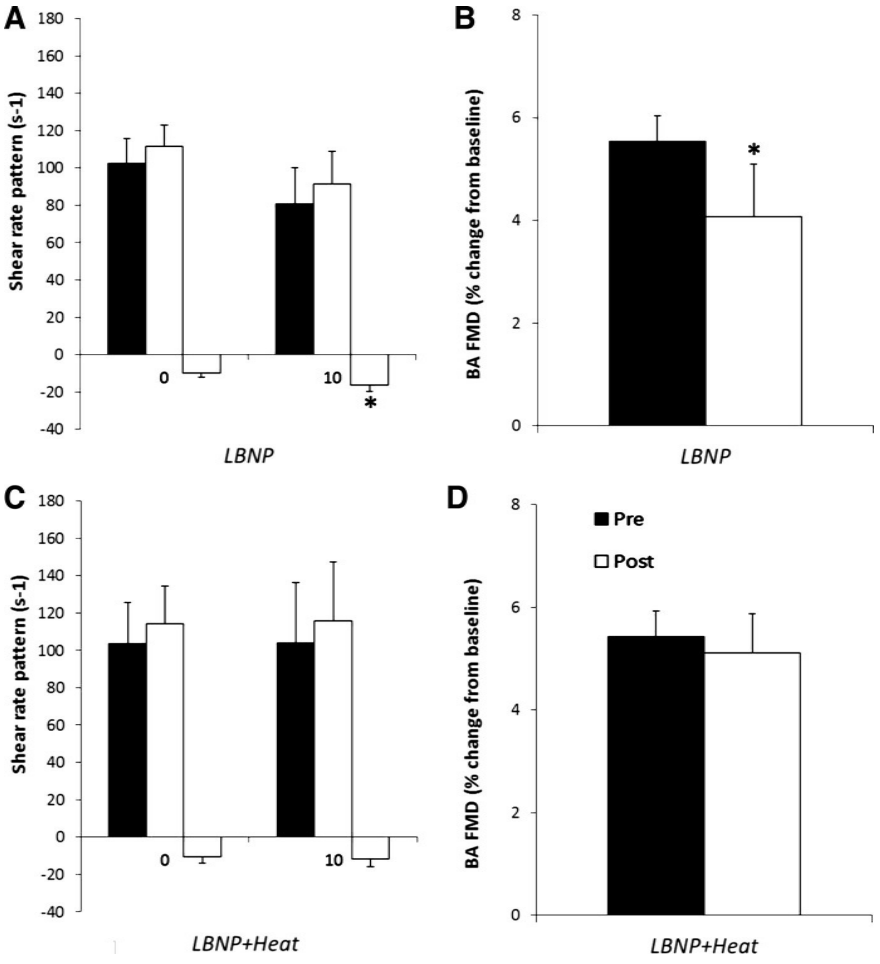

Fig. 1. Brachial artery (BA) mean (solid bars) and antegrade/retrograde shear rate (open bars) before (0) and following 10-min lower body negative pressure (LBNP) (10) in healthy volunteers (n = 9). Results are shown for LBNP alone (LBNP; *A*) and LBNP combined with forearm heating (LBNP+heat; *C*), which was simultaneously measured in the contralateral limb. BA flow-mediated dilation (FMD) is presented before (solid bars; Pre) and after (open bars; Post) the 10-min interventions in the LBNP (*B*) and LBNP+heat intervention (*D*). Values are means ± SE. *Post hoc significantly different from baseline at $P < 0.05$.

noncuffed arm, or heated arm vs. nonheated arm). All data are reported as means ± SD, unless stated otherwise, and statistical significance was assumed at $P < 0.05$. Across all analyses, post hoc analysis was performed using the least significant difference method for pairwise multiple comparisons (23, 24).

RESULTS

No differences in resting brachial artery diameter, blood flow or shear rate pattern, FMD%, FMD presented as absolute change in millimeter from baseline (FMDmm) or area under the shear rate curve (SR_{AUC}) were reported between the various days (all $P > 0.05$) or between arms (all $P > 0.05$). Because of technical problems, we were unable to examine the data from one subject during the 10-min LBNP+heat trial and two subjects for the 30-min LBNP+heat trial.

10-min LBNP: Role of Shear

$LBNP_{10}$+heat. LBNP protocol significantly increased HR and reduced SV, but did not change MAP or CO (Table 1). The 10-min LBNP protocol did not change NE plasma levels (622 ± 148 vs. 756 ± 299 pmol/l, $P = 0.33$), but significantly increased levels of E (52 ± 9 vs. 71 ± 25 pmol/l, $P = 0.02$). LBNP did not significantly change mean or antegrade brachial artery SR in either arm (Fig. 1*A*, $P = 0.23$ and $P = 0.24$, respectively), whereas retrograde SR was significantly increased (Fig. 1*A*, $P = 0.003$). In the contralateral arm, heat application prevented this increase in retrograde SR (Fig. 1*C*, $P = 0.47$). LBNP induced a significant decrease in brachial artery FMD%, which was not present in the contralateral arm (interaction effect, $P = 0.045$, Fig. 1, *B* and *D*, respectively). When presented as FMDmm, we found a comparable decrease after 10-min LBNP in both arms (Table 1).

$LBNP_{10}$+cuff. LBNP did not change MAP, SV, or CO, whereas a significant increase in HR was found (Table 1). LBNP did not change mean or antegrade brachial artery SR during the 10-min LBNP protocol (Fig. 2, $P = 0.12$ and $P = 0.31$, respectively). Retrograde SR was significantly increased (Fig. 2*A*, $P = 0.02$), whereas cuff inflation resulted in a significantly larger increase in retrograde SR (Fig. 2*C*, $P < 0.001$). We found a significant decrease in brachial artery FMD% and FMDmm following 10-min LBNP, which was similarly present in both arms (Fig. 2, *B* and *D*, and Table 1, respectively). No changes in brachial artery diameter and SR_{AUC} were found across the protocol (Table 1).

30-min LBNP: Role of Shear

$LBNP_{30}$+heat. The 30-min LBNP protocol significantly increased HR and lowered SV, whereas no change in MAP and CO was observed (Table 2). LBNP did not change plasma levels of NE (630 ± 296 vs. 840 ± 570 pmol/l, $P = 0.10$) or E (66 ± 30 vs. 63 ± 32 pmol/l, $P = 0.55$). Following LBNP, we found no change for mean, antegrade, or retrograde SR in

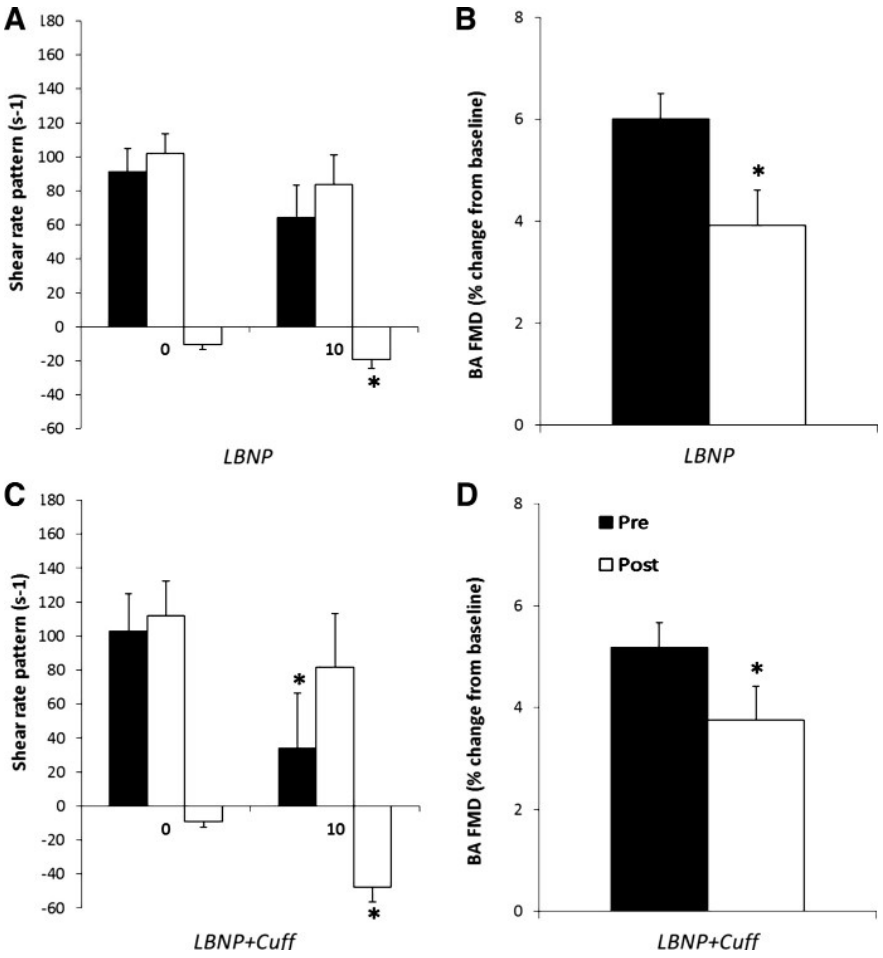

Fig. 2. BA mean (solid bars) and antegrade/retrograde shear rate (open bars) before (0) and following 10-min LBNP (10) in healthy volunteers ($n = 9$). Results are shown for LBNP alone (LBNP; *A*) and LBNP combined with forearm cuff inflation to 75 mmHg (LBNP+cuff; *C*), which was simultaneously measured in the contralateral limb. BA FMD is presented Pre (solid bars) and Post (open bars) the 10-min interventions in the LBNP (*B*) and LBNP+cuff intervention (*D*). Values are means ± SE. *Post hoc significantly different from baseline at $P < 0.05$.

Table 2. *MAP, SV, HR, CO, brachial artery diameter, FMD, and SR_{AUC} before and after 30-min LBNP+heat and LBNP+cuff in healthy volunteers*

	LBNP		LBNP+Heat		One- and Two-Way ANOVA		
	Pre	Post	Pre	Post	Time	Arm	Time × Arm
MAP, mmHg	84 ± 9	86 ± 8			0.22		
SV, ml	76 ± 6	68 ± 7			**0.01**		
HR, beats/min	59 ± 7	65 ± 10			**0.01**		
CO, liters	4.5 ± 0.8	4.4 ± 0.7			0.40		
Diameter, mm	4.2 ± 0.8	4.2 ± 0.7	4.1 ± 0.47	4.1 ± 0.4	0.70	0.62	0.70
FMD, mm	0.21 ± 0.05	0.19 ± 0.07	0.20 ± 0.06	0.26 ± 0.06*	**0.016**	0.36	**0.047**
SR_{AUC}, 10^3/s	17.1 ± 8.7	14.0 ± 6.8	18.9 ± 5.9	26.4 ± 9.0*	0.32	**0.043**	**0.003**
			LBNP+cuff				
			Pre	Post			
MAP, mmHg	84 ± 10	86 ± 9			0.58		
SV, ml	86 ± 5	72 ± 6			**<0.01**		
HR, beats/min	59 ± 8	63 ± 11			**0.04**		
CO, liters	5.1 ± 1.4	4.5 ± 1.1			**<0.01**		
Diameter, mm	4.0 ± 0.6	4.0 ± 0.6	4.0 ± 0.6	4.0 ± 0.7	0.63	0.94	0.69
FMD, mm	0.21 ± 0.11	0.20 ± 0.09	0.21 ± 0.11	0.14 ± 0.07*	0.08	0.38	**0.02**
SR_{AUC}, 10^3/s	16.5 ± 6.6	13.2 ± 6.3	16.9 ± 6.4	15.4 ± 5.3	0.12	0.60	0.40

Values are means ± SD; $n = 8$ subjects. P value represents a two-way ANOVA examining the impact of LBNP (Pre vs. Post; "time") and whether the impact of LBNP differed between both arms. *Post hoc significantly different from Pre at $P < 0.05$. P values in bold are significantly different.

the control arm ($P = 0.25$, 0.32, and 0.32, respectively, Fig. 3*A*). The heated arm revealed no change in antegrade SR ($P = 0.19$; Fig. 3*C*), but showed an increase in mean SR ($P = 0.05$; Fig. 3*C*) and lower retrograde SR ($P = 0.04$; Fig. 3*C*). LBNP did not change FMD% or FMDmm, whereas an increase in FMD% and FMDmm was observed in the heated arm (interaction effects, $P < 0.05$, Fig. 3, *B* and *D*, and Table 2, respectively).

$LBNP_{30}$+cuff. LBNP significantly increased HR and lowered SV and CO (Table 2), whereas MAP did not change (Table 2). At 30 min of LBNP, we found no difference in mean, antegrade, or retrograde SR compared with baseline ($P = 0.10$, $P = 0.23$, and $P = 0.13$, respectively, Fig. 4*A*). Cuff inflation significantly increased retrograde SR compared with the contralateral arm (interaction effect; $P < 0.01$, Fig. 4*C*). We found no change in brachial artery FMDmm or FMD% after LBNP, whereas a decrease was observed in the cuffed arm (interaction effects, $P < 0.05$, Table 2 and Fig. 4, *B* and *C*, respectively). In both arms, no change in diameter and SR_{AUC} was found (Table 2).

DISCUSSION

The purpose of this study was to examine the role of shear patterns in mediating changes in endothelial function during brief and more prolonged periods of SNS stimulation in healthy humans. Our findings confirm some previous studies (7, 10, 17), in that SNS stimulation using 10 min of LBNP increased E levels and retrograde SR and decreased brachial artery FMD. Within-subject manipulation of retrograde SR by local heating (which abolished the increase in retrograde SR), but not by subdiastolic cuff inflation (which increased retrograde SR), altered the magnitude of change in FMD. This suggests that changes in retrograde shear contribute, at least partly, to changes in FMD after short-term SNS stimulation. In contrast to these results after short-term stimulation, 30 min of SNS stimulation revealed no change in retrograde shear, NE/E levels, or FMD. Within-subject attenuation in retrograde SR by local heating increased FMD, whereas exaggeration of retrograde shear via cuff inflation during the 30-min period of LBNP resulted in a decrease in brachial artery FMD. These findings concur with those above, in that changes in retrograde shear rate play a role in changes in FMD during LBNP.

We adopted a 10-min protocol of −35-mmHg LBNP to increase sympathetic nerve activity. The significant increases in E levels and HR suggest that our protocol induced a significant increase in SNS activity. Furthermore, we found that the increase in SNS activity in our 10-min protocol was associated with a significant increase in retrograde shear and decrease in brachial artery FMD in healthy volunteers. This observation of an immediate decrease in FMD is in line with several other studies performed in conduit (7, 10, 17) and resistance vessels (25). However, the decline in FMD is not a universal finding and may depend on the method used to increase SNS activity (7).

Our primary aim was to understand the role of shear rate in mediating changes in FMD during LBNP. We found that LBNP resulted in an immediate increase in retrograde shear rate. This finding supports recent work from Padilla et al. (22), who reported that acute elevations in muscle sympathetic nerve activity, adopting three different methods, are associated with elevations in retrograde shear. To examine the link between LBNP-mediated increases in retrograde shear and FMD, we effectively manipulated retrograde shear by unilateral local heat application (to abolish the increase in retrograde shear) and inflation of a cuff around the forearm (to further increase retrograde shear) during LBNP (Figs. 1 and 2). As we performed bilateral simultaneous assessment of brachial artery FMD, both arteries were exposed to the same systemic LBNP stimulus. Abolishing the LBNP-mediated increase in retrograde shear rate effectively prevented the decrease in FMD, supporting our hypothesis that changes in FMD after SNS stimulation are, at least partly, mediated by changes in retrograde shear. Importantly, heating selectively prevented the change in retrograde shear, without affecting the antegrade

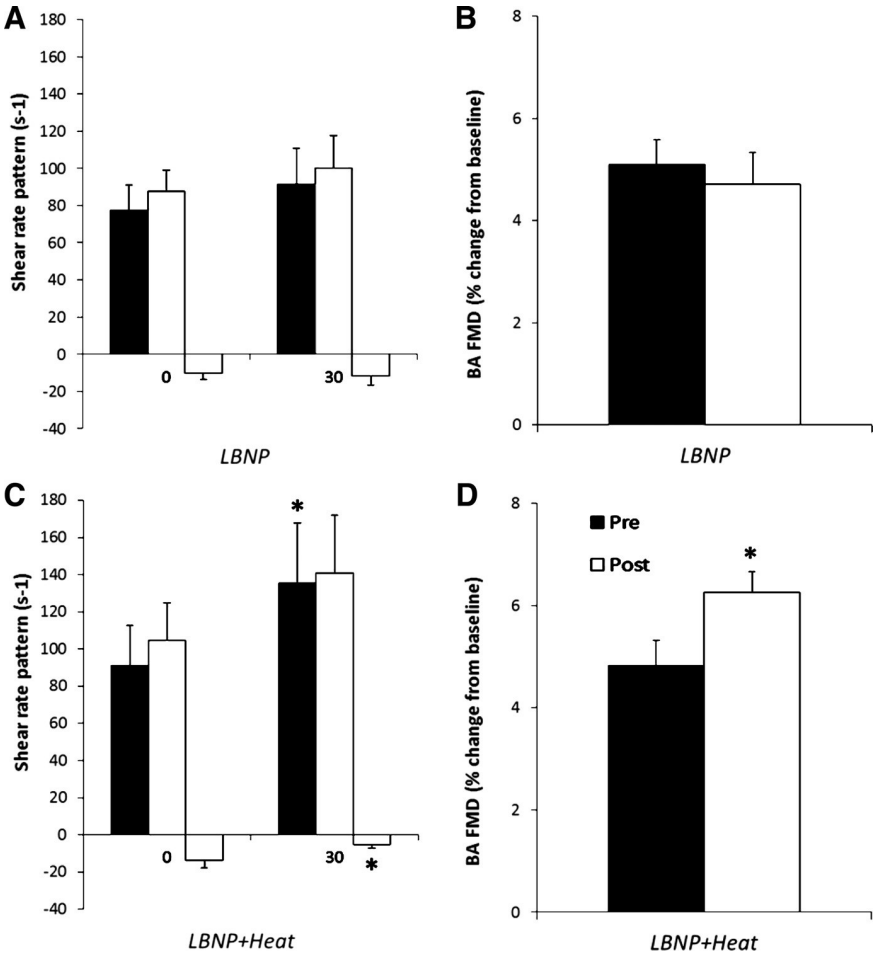

Fig. 3. BA mean (solid bars) and antegrade/retrograde shear rate (open bars) before (0) and following 30-min LBNP (30) in healthy volunteers ($n = 8$). Results are shown for LBNP alone (LBNP; *A*) and LBNP+heat (*C*), which was simultaneously measured in the contralateral limb. BA FMD is presented Pre (solid bars) and Post (open bars) the 30-min intervention in the LBNP (*B*) and LBNP+heat intervention (*D*). Values are means ± SE. *Post hoc significantly different from baseline at $P < 0.05$.

shear rate. This is relevant because previous findings have suggested an important role for antegrade shear in acutely changing diameter (3) and FMD (30). In contrast to the findings observed during local heating, exaggerating retrograde shear using cuff inflation did not further decrease FMD following 10 min of LBNP. Earlier findings suggest that the change in FMD after short-term change in retrograde shear is dose dependent (29). This suggests that the relatively brief period of retrograde shear associated with our 10-min stimulus was insufficient to induce a further diminution in FMD. In any event, our study provides evidence that SNS activity, elicited by 10 min of LBNP, leads to an immediate decrease in brachial artery FMD in healthy subjects, which is, at least partly, linked to changes in retrograde shear rate.

When we repeated the LBNP experiment using a 30-min stimulus, we unexpectedly found distinct responses. Although the significant increase in HR and drop in SV and CO indicate that 30 min of LBNP altered central hemodynamics, we found no change in NE/E levels, or SR patterns, relative to pre-LBNP measures. More importantly, we observed no change in brachial artery FMD response after 30-min LBNP. Interestingly, our data represent the first observation of a time-dependent effect of LBNP on FMD in humans and support the idea that prolonged exposure to LBNP may not inhibit endothelial function in humans. Although our study design was originally not set up to examine this, the contrast with the briefer period of 10-min LBNP suggests that the vasculature may accommodate to prolonged LBNP in a manner that normalizes retrograde shear and hence limits impacts on endothelial function. Despite the accommodation of the shear pattern, changes in central hemodynamics to 30-min LBNP were comparable to those observed after 10 min. At least this suggests that the regulation of retrograde shear rate is not simply explained through changes in peripheral vascular tone alone, but may also relate to other mechanisms, such as pressure from wave reflections (9). An effect of shear, as against direct effects of SNS activation per se, is supported by our observation that heating attenuated retrograde shear and increased FMD, whereas cuff inflation increased retrograde shear and decreased FMD. These effects occurred, despite simultaneous LBNP exposure. Our results, therefore, reinforce the 10-min data in that they also suggest that changes in FMD during prolonged SNS activation are, at least partly, mediated by changes in retrograde shear rate.

Several previous studies in animals have examined the impact of short-term increases in retrograde shear rate and have found that such increases lead to the upregulation of pro-atherogenic genes (e.g., vascular cell adhesion molecule 1, intercellular adhesion molecule 1, endothelin-1) and down-regulation of anti-atherogenic genes (e.g., endothelial nitric oxide synthase) (15, 21). Such changes in gene expression may impact endothelial function. Whether activation of the SNS

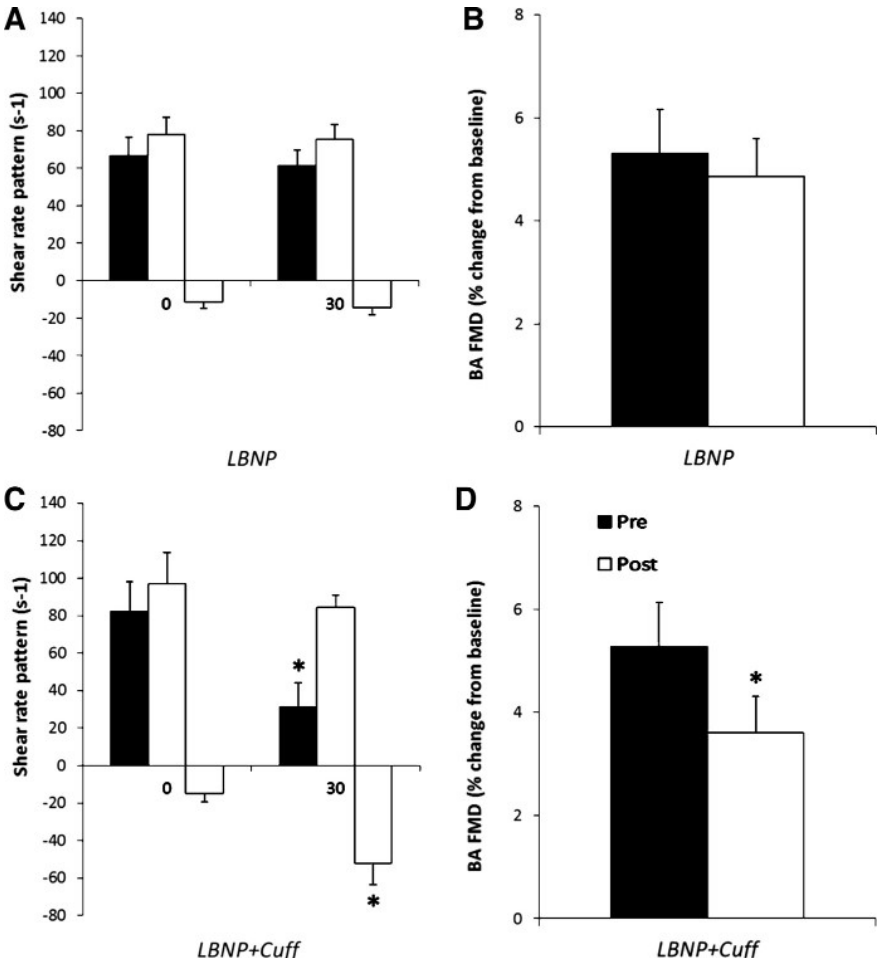

Fig. 4. BA mean (solid bars) and antegrade/retrograde shear rate (open bars) before (0) and following 30-min LBNP (30) in healthy volunteers (n = 8). Results are shown for LBNP alone (LBNP; *A*) and LBNP+cuff (*C*), which was simultaneously measured in the contralateral limb. BA FMD is presented Pre (solid bars) and Post (open bars) the 30-min intervention in the LBNP (*B*) and LBNP+cuff intervention (*D*). Values are means ± SE. *Post hoc significantly different from baseline at $P < 0.05$.

indeed mediates changes in endothelial function through these mechanisms needs to be assessed in future studies.

Our findings may shed some light on previous disparity in the literature regarding the impact of the SNS on FMD. A previous study highlighted that different SNS activation protocols may induce distinct FMD responses (e.g., LBNP, cold pressor test, mental arithmetic, muscle chemoreflex) (7). Different LBNP protocols (continuous vs. incremental pressure) and level of LBNP suction (−20 mmHg vs. −30 mmHg) have also been adopted. It is possible that each of these distinct protocols has different impacts on shear patterns, which in turn influences the magnitude of impact on FMD. These issues emphasize the importance of adopting within-subject experimental designs to understand the impact of SNS activity on the FMD.

Our observation of the distinct change in FMD during 10- vs. 30-min LBNP raises questions regarding the relationship between resting FMD and resting levels of SNS. Previous studies have suggested that resting levels of SNS activity [via HR variability (13) or catecholamine-levels (11, 12)] inversely relate to endothelial function in healthy subjects and clinical groups. Our results, however, suggest that FMD normalizes when exposed to a prolonged stimulus of LBNP that is likely associated with elevated levels of SNS activity. It is important to emphasize that we specifically focused on the acute impact of a substantial increase in SNS activity through LBNP, while these previous studies have focused on chronic resting levels of SNS activity. Therefore, the mechanisms relating the SNS to endothelial function in vivo may differ between acute or chronic activation.

Limitations

A potential limitation of our study is that our results are difficult to extrapolate to other populations, especially groups characterized by an elevation in SNS activity. Understanding the impact of the sympathetic nerve system on the vasculature in these populations will require disease-specific studies. Another potential limitation of our study is that we did not examine the impact of LBNP and/or cuff inflation on brachial artery endothelium-independent dilation. Although others found no effect of LBNP stimulation (10) or mental stress (8) on endothelium-independent dilation, we cannot exclude that the effects of SNS stimulation in our study are specific for endothelial function. Another potential limitation is that we did not randomize the order of the 10- and 30-min experiments. While this may have introduced an order effect, randomization was applied to *1*) the order of heat and cuff experiments (for the 10- and 30-min experiments); and *2*) the arm undergoing the heat/cuff experiment.

Previous studies have demonstrated a strong link between activation of the SNS and the development of cardiovascular

disease. However, the precise mechanisms underlying the relationship between SNS activation and endothelial function have not been fully characterized. We found that short-term exposure to LBNP leads to an immediate increase in retrograde shear and decrease in brachial artery FMD. Abolishing retrograde shear during LBNP attenuated the decrease in FMD, which supports a role for shear stress in the immediate change in FMD observed during SNS stimulation. While longer term LBNP did not change retrograde shear or FMD, unilateral shear rate manipulation resulted in significant and directional changes in FMD. These findings support a role for shear rate in the changes in FMD observed during SNS stimulation.

GRANTS

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: D.H.T. and D.J.G. conception and design of research; D.H.T., C.L.A., K.O., V.S.S., A.L.S., and C.J.P. performed experiments; D.H.T., C.L.A., and C.J.P. analyzed data; D.H.T., C.L.A., K.O., V.S.S., C.J.P., and D.J.G. interpreted results of experiments; D.H.T., C.L.A., C.J.P., and D.J.G. prepared figures; D.H.T., C.L.A., K.O., V.S.S., A.L.S., C.J.P., and D.J.G. drafted manuscript; D.H.T., C.L.A., K.O., V.S.S., A.L.S., C.J.P., and D.J.G. edited and revised manuscript; D.H.T., C.L.A., K.O., V.S.S., A.L.S., C.J.P., and D.J.G. approved final version of manuscript.

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