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Effects of Unilateral Arm Warming or Cooling on the Modulation of Brachial Artery Shear Stress and Endothelial Function during Leg Exercise in Humans

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Aim: We examined the effect of modulating the shear stress (SS) profile using forearm warming and cooling on subsequent endothelial function in the brachial artery (BA) during exercise.

Methods: Twelve healthy young subjects immersed their right forearm in water (15℃ or 42℃) during a leg cycling exercise at 120–130 bpm for 60 min. The same exercise without water immersion served as a control. The BA diameter and blood velocity were simultaneously recorded using Doppler ultrasonography to evaluate the antegrade, retrograde, and mean shear rates (SRs, an estimate of SS) before, during, and after exercise. The endothelial function in the right BA was evaluated using flow-mediated dilation (FMD) (%) using two-dimensional high-resolution ultrasonography before (baseline) and 15 and 60 min after exercise.

Results: During exercise, compared with the control trial, higher antegrade and mean SRs and lower retrograde SRs were observed in the warm trial; conversely, lower antegrade and mean SRs and higher retrograde SRs were observed in the cool trial. At 15 min postexercise, no significant change was observed in the FMD from baseline in the warm (Δ%FMD: +1.6%, tendency to increase; $p=0.08$) and control trials (Δ%FMD: +1.1%). However, in the cool trial, the postexercise FMD at 60 min decreased from baseline (Δ%FMD: −2.7%) and was lower than that of the warm (Δ%FMD: +1.5%) and control (Δ%FMD: +1.2%) trials. Accumulated changes in each SR during and after exercise were significantly correlated with postexercise FMD changes.

Conclusion: Modulation of shear profiles in the BA during exercise appears to be associated with subsequent endothelial function.

Key words: Endothelial function, Shear stress, Exercise, Thermal stimulation

Introduction

As demonstrated in many epidemiological studies, participants who possess risk factors for cardiovascular diseases (e.g., hypertension, type 2 diabetes mellitus, or obesity) often exhibit impaired endothelial function. However, interventional studies have revealed improvements in endothelial function in conduit arteries following exercise. In recent animal and human studies, it has been demonstrated that physical exercise can increase nitric oxide production and bioavailability, resulting in acutely and chronically improved endothelial function (e.g., see Green *et al.*'s review[1]). It is postulated that this beneficial effect on the endothelium may be partly derived from the exercise-induced elevation of shear stress (SS), which is the frictional force of blood flow to the wall of conduit arteries.

In addition to the elevation of SS itself, its profile may also have important implications in the adapta-

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tion of arterial vascular function[2, 3]. Antegrade SS, which is directed toward the periphery mainly during systole, provides a key stimulus to the arterial wall to improve endothelial function. In this line, several studies have reported positive effects of repeated exposure to elevations in antegrade shear rate (SR) (an estimate of SS without viscosity), using the manipulation of warming to the entire body or a peripheral limb[4-6]. By contrast, retrograde SS, which is directed backward to the heart mainly during diastole, seems to adversely influence endothelial cells in both *in vivo* and *in vitro* studies[1, 7]. For example, an inverse and dose-dependent relationship has been described between acute increases in retrograde SR and brachial artery (BA) endothelial function in young men[2, 8]. Therefore, it has been suggested that the profiles of SS that improve endothelial function may be the elevation of antegrade SS and the reduction and/or abolishment of retrograde SS.

Forearm warming is a simple manipulation that can locally modulate the shear profile of a conduit artery (i.e., BA)[3, 8, 9]. Specifically, forearm warming can elicit the elevation of antegrade SR and the reduction and/or abolishment of retrograde SR[3, 8, 9]. It has been demonstrated in recent studies that acute and chronic arm warming improves flow-mediated dilation (FMD), the most common measure of endothelial function, in the BA *via* a shear-dependent mechanism[3-6]. If the exercise-induced adaptation of arterial endothelial function is associated with the magnitude of the SR profile, a simultaneous arm warming maneuver during leg cycling exercise would provide an additional positive effect on the endothelial function of the BA. To our knowledge, this hypothesis has not yet been elucidated. Contrary to warming maneuver, although forearm cooling stimulation using a cold water-perfused suit during exercise has been shown to lead to reduced antegrade and increased retrograde SRs[10], there is no existing evidence on the effect of arm cooling during exercise on the modulation of the SR profile and the endothelial function of the BA. Cooling is well known to potentially suppress the elevation of core temperature during exercise and to accelerate recovery (so-called "icing"), that is, to smoothly remove postexercise heat accumulation[11, 12].

Aim

We, therefore, investigated the effects of simultaneous forearm warming and cooling during leg cycling exercise on the modulation of SR and endothelial function. We hypothesize that arm warming might lead to improved endothelial function in the BA, whereas arm cooling might attenuate this function.

Methods

Participants

Twelve healthy subjects (9 females, 3 males; age: 21 ± 3 years; height: 162 ± 8 cm; weight: 56 ± 5 kg; body mass index: 22 ± 2 kg/m^2 [mean ± standard deviation]) participated in this study. The sample size was determined according to a previous study[13]. The participants were young, healthy, and normotensive. No participants smoked or took any medication, and none had any history of autonomic dysfunction, cardiovascular/cerebrovascular disorder, and/or dyslipidemia. The study protocol was performed in compliance with the Declaration of Helsinki, and the study was approved by the Ethics Committee of the Prefectural University of Hiroshima (approval number: 18HH001). Before the commencement of the study, each participant provided written informed consent for participation.

Preliminary Test Session

In order to determine individual target work rates, a ramp incremental exercise test using a semi-recumbent cycle ergometer (Angio; Lode, Groningen, the Netherlands) was performed at least one week prior to the experiment. This test included 4 min of baseline rest in a semisupine position, followed by 4 min of baseline exercise at 20 W and an incremental ramp exercise at 13–15 W/min until reaching the individual's tolerance limit. The subjects were instructed to maintain a pedal frequency of 60 rpm. The test was terminated when the subjects could not maintain 50 rpm despite maximal exertion. Individual target work rates at 120–130 bpm were then determined (71 ± 19 W), and exercises with this intensity and same duration (i.e., 60 min) were adopted for three trails, as explained in the next section.

Main Trial Session

The subjects arrived at the laboratory at around 3:00 p.m. on the trial days after abstaining from strenuous exercise and alcohol/caffeine for at least one day. All subjects were randomly assigned to participate in three experimental protocols on separate days that were at least one week apart and set in the early follicular phase for female subjects. In order to measure the baseline variables, the subjects laid in a supine position in a quiet room for 30 min, and the temperature and humidity were maintained at 23℃ ± 1℃ and 40% ± 5%, respectively. After 5 min of sitting on the cycle ergometer, the subjects immersed their right forearm into either a cool or a warm water bath (15℃ or 42℃, respectively, maintained using an automatic circulatory controller with a thermal feedback system)

10 s before beginning a single bout of leg cycling exercise for 60 min. In the control trial, the subjects performed the same exercise with a bath of no water immersion. After the cycling exercise, the subjects laid in a supine position for 60 min.

Measurements

The heart rate (HR) was continuously monitored using an electrocardiogram (ECG) (DYNASCOPE DS-8100; Fukuda Denshi Co., Tokyo, Japan) throughout the protocol. Skin blood flow (SBF) was monitored in the center of the right forearm and in the ball of the thumb region (i.e., palm) of the right hand using a laser Doppler flowmeter (ALF21; Advance Co., Tokyo, Japan) to measure the red blood cell flux. The circulatory variables described above were converted to digital data using an AD conversion device and software (PowerLab 8/30; ADInstruments, Colorado Springs, CO, USA) at 1 kHz. Beat-by-beat BV through the right BA to the distal third of the right inactive upper limb and the vessel diameter were measured using a pulse-echo Doppler ultrasound (Aplio 300; Toshiba Medical Systems Co., Ltd., Tochigi, Japan) with a linear 11.0 MHz probe with an insonation angle below 60°. The sample volume was positioned at the center of the vessel and adjusted to cover the full diameter of the BA. For every cardiac cycle, Doppler tracing was analyzed using integral software to obtain the antegrade and retrograde velocities (mean velocity = antegrade velocity + retrograde velocity) in the BA. BF was calculated from the blood velocity and the cross-sectional area of the vessel, as previously described[14-16]. Briefly, audio-range signals for the antegrade and retrograde velocities reflected from the moving blood cells and the ECG signal were digitally sampled using a 20 kHz AD conversion device (PowerLab 8/30; ADInstruments). Audio-range signal spectra were processed offline with Doppler signal processing software (using a fast Fourier transfer analysis and a 256-point Hamming window) to yield instantaneous antegrade and retrograde velocities. Velocity signals were recorded at 100 Hz on a computer system, in addition to the ECG, so that the beat-by-beat data could be analyzed. Finally, second-by-second time courses of antegrade, retrograde, and mean net velocities were calculated by interpolation of the beat-by-beat data. B-mode echo images of the right BA were recorded simultaneously using a hard disk recorder, and the diameter of the vessel was measured with on-screen calipers using the ImageJ software. The vessel diameters were summarized at rest and every 10 min during and after exercise. In this study, the SR was calculated as 4 × each profile of blood velocity (i.e., antegrade, retrograde, and mean)/vessel diameter, according to a previous study[17]. The oscillatory shear index (OSI) was also calculated according to a previous study[18]. This index represents a measure of the magnitude of shear oscillation and is known to be associated with endothelial dysfunction[19]. For the latter correlation analysis, the shear profiles and OSI responses were further calculated as the incremental area under the curve (iAUC) above baseline during and until 5 min after exercise (from $t=0$ to $t=65$ min) and during exercise and recovery (from $t=0$ to $t=105$ min; see the transverse axis in **Fig. 2**).

A high-resolution ultrasonography equipment (UNEXEF18G; UNEX Co., Nagoya, Japan) was used to evaluate FMD[20-23] by an expert examiner who was trained for 2 months prior to the experiment. This novel device is manufactured for wide-ranging clinical purposes with a semiautomatic procedure. The superior reproducibility (between the two visits) of the FMD measurement using this system was reported, as a coefficient of variance, to be 11.2%[24], 10.1%[25], and 4.5%[26]. In addition, we evaluated the repeatability of the FMD measurements using UNEXEF18G preliminarily (Pearson's correlation coefficient of the FMD between the two visits was 0.896, $P<0.01$, and the coefficient of variation was 9.38%, $n=22$). At baseline ($t=-15$ min) and at 15 min ($t=75$ min) and 60 min ($t=120$ min) after exercise, the subjects laid in a supine position with their right arm extended horizontally 80°–90° from the trunk at the heart level. A blood pressure cuff was placed around the forearm. The details of the semiautomatic procedures with the validity have been described in previous studies[24-26]; therefore, we have briefly summarized them. The BA was scanned at the same position to measure the SR (approximately 5–10 cm above the elbow). When the clearest two-dimensional gray-scale B-mode image of the anterior and posterior intimal interfaces between the lumen and the vessel wall was obtained, the transducer was held at the same position throughout the scan by an automatic tracking probe holder and not by the hand of the examiner manually to ensure image consistency. The depth and gain were set to optimize the images of the arterial lumen wall interface. When the tracking gate was placed on the intima, the artery diameter was automatically tracked, and the waveform of diameter changes over the cardiac cycle was displayed in real time using the FMD mode of the tracking system. This allowed the ultrasound images to be optimized at the start of the scan and the transducer position to be automatically adjusted immediately for optimal tracking performance throughout the scan. Pulsed-wave Doppler flow was assessed at baseline and during postocclusive hyperemic flow, which was con-

firmed to occur within 15 s after cuff deflation. In order to measure the baseline BA diameter (D-base), the baseline longitudinal image of the artery was acquired for 30 s, and then the blood pressure cuff was inflated to 50 mmHg above systolic pressure for 5 min. The longitudinal image of the artery was recorded continuously until 2 min after cuff deflation, and then the peak diameter of the BA (D-peak) was obtained. Changes from D-base to D-peak in the BA diameter were immediately expressed as a percentage change relative to the vessel diameter before cuff inflation. The FMD was calculated as follows: %FMD ([peak diameter − baseline diameter] / baseline diameter) × 100. Furthermore, to adjust for differences in the baseline diameter among subjects, allometric scaling of %FMD (i.e., adjusted %FMD) was conducted in our analysis according to a method of the previous study[27].

Statistical Analysis

Data are expressed as means and standard deviations (SDs) of the means. The effects of time and treatment on all measurements were tested using two-way repeated measures analysis of variance. When a significant effect was detected, Dunnett's and Tukey's *post hoc* tests were conducted to reveal the effects of time (change from baseline) and trials, respectively. The relationships between the change in %FMD from baseline (Δ%FMD) and iAUCs for shear profiles (i.e., antegrade, retrograde, and mean SRs) and for the OSI by exercise (i.e., until 5 min after the cessation of exercise) and exercise and recovery (i.e., until the end of the protocol) were analyzed using Pearson's correlation coefficient. The two-sided statistical significance level was set at $P \leq 0.05$. All statistical analyses were conducted using the SPSS PASW 18 statistical software (SPSS Inc., Chicago, IL, USA).

Results

HR, Blood Pressure, and SBF

The baseline measurements (i.e., before the onset of exercise) of HR and mean arterial blood pressure did not differ significantly among the three trials. In all trials, the HR values increased significantly from baseline both during and 5 min (t=65 min) after exercise **(Fig. 1A)**. The HR values were lower in the cool trial than in the warm trial during and after exercise. Conversely, the HR values were higher in the warm trial than in the control trial from 20 to 60 min during exercise. In all trials, the MAP increased from baseline to 5 min (t=65 min) after exercise (data not shown). However, the MAP did not differ significantly among the three trials after exercise.

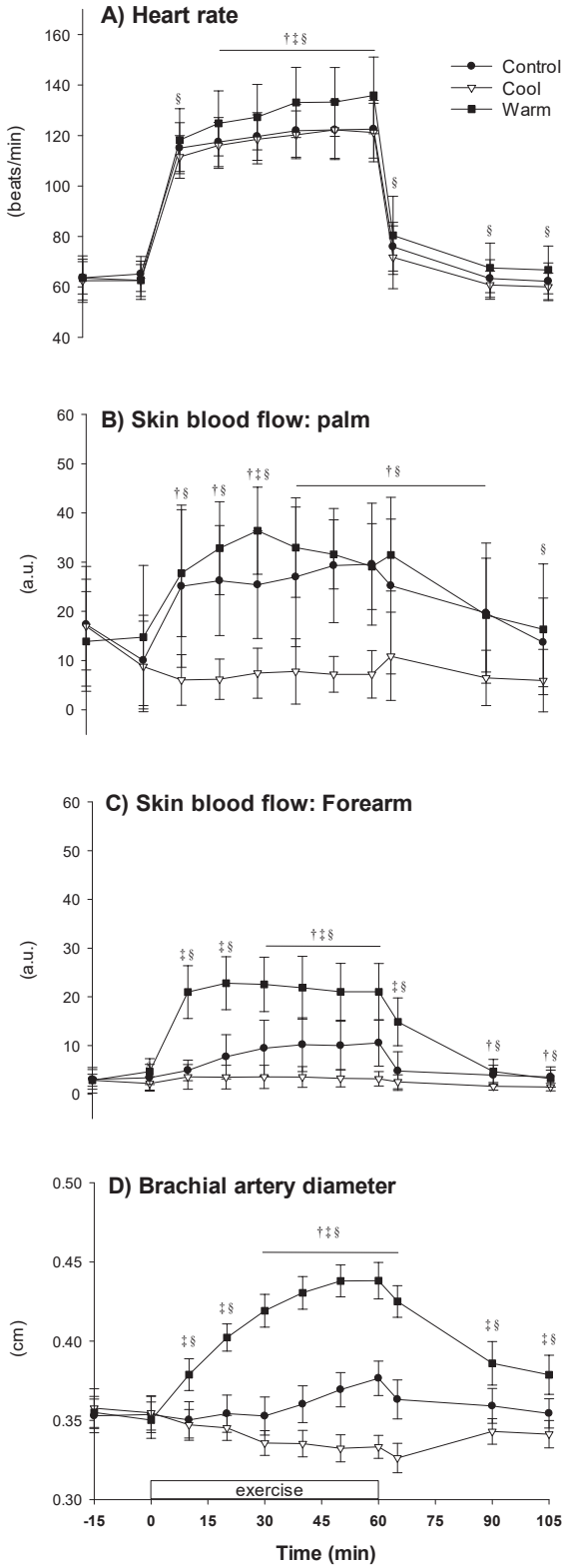

Fig. 1. HR (A); SBF in the palm (B) and forearm (C); BA diameter (D) before, during, and after exercise

Exercise was performed from 0 to 60 min as shown in the transverse axis. Mean ± SD. †Cool versus control. ‡Warm versus control. §Cool versus warm. $P<0.05$.

The baseline measurements of SBF in the palm and forearm did not differ significantly among the three trials. In the control trial, the palm SBF responses increased significantly from baseline to between 50 and 60 min during exercise **(Fig. 1B)**. In the cool trial, the palm SBF responses significantly decreased from baseline to 60 min during and 30–45 min (t=90–105 min) after exercise. In the warm trial, the palm SBF responses increased significantly from baseline to between 10 and 60 min during and 5 and 30 min (t=65 and 90 min, respectively) after exercise. The palm SBF responses were lower in the cool trial than in the control trial from 10 to 60 min during and 5 min (t=65 min) after exercise. The palm SBF responses were lower in the cool trial than in the warm trial both during and after exercise. The palm SBF responses were higher in the warm trial than in the control trial at 30 min during exercise.

In the control trial, the forearm SBF responses increased significantly from baseline to between 20 and 60 min during exercise **(Fig. 1C)**. In the cool trial, the forearm SBF responses during and after exercise did not change from the baseline values. In the warm trial, the forearm SBF responses increased significantly from baseline to between 10 and 60 min during and 5 and 30 min (t=65 and 90 min, respectively) after exercise. The forearm SBF responses were lower in the cool trial than in the control trial from 30 to 60 min during and 30 and 45 min (t=90 and 105 min, respectively) after exercise. Both during and after exercise, the forearm SBF responses were lower in the cool trial than in the warm trial, and from 30 to 60 min during and 30 and 45 min (t=90 and 105 min, respectively) after exercise. The forearm SBF responses were higher in the warm trial than in the control trial from 30 to 60 min during and 30 and 45 min (t=90 and 105 min, respectively) after exercise.

Diameter, Shear Profiles, and OSI of the BA

The baseline measurements of each shear profile, OSI, and diameter in the BA did not differ significantly among the three trials. In the control trial, the BA diameter was significantly dilated from baseline to between 50 and 60 min during exercise **(Fig. 1D).** In the cool trial, the BA diameter was significantly constricted from baseline to between 30 and 60 min during and 5 and 45 min (t=65 and 105 min, respectively) after exercise. In the warm trial, the BA diameter was significantly dilated from baseline both during and after exercise. The BA diameter was smaller in the cool trial than in the control trial from 30 to 60 min during and 5 min (t=65 min) after exercise. Both during and after exercise, the BA diameter was smaller in the cool trial than in the warm trial. Conversely, the BA diameter was larger in the warm trial than in the control trial both during and after exercise.

In the control trial, the antegrade SR responses of the BA increased significantly from baseline to between 10 and 60 min during and 5 min (t=65 min) after exercise **(Fig. 2A)**. In the cool trial, the antegrade SR responses decreased significantly from baseline just before (t=0 min) to 30 and 45 min (t=90 and 105 min, respectively) after exercise. In the warm trial, the antegrade SR responses increased significantly from baseline to between 10 and 60 min during and 5 min (t=65 min) after exercise. The antegrade SR responses were lower in the cool trial than in the control trial from 20 to 60 min during and 5 min (t=65 min) after exercise. The antegrade SR responses were lower in the cool trial than in the warm trial both during and after exercise. The antegrade SR responses were higher in the warm trial than in the control trial from 20 to 65 min during and 45 min (t=105 min) after exercise.

In the control trial, the retrograde SR responses of the BA increased significantly (i.e., in a negative direction) from baseline to between 10 and 30 min and 60 min during exercise **(Fig. 2B)**. In the cool trial, the retrograde SR responses increased significantly from baseline throughout the exercise period. In the warm trial, the retrograde SR responses did not change during or after exercise compared with the baseline values. The retrograde SR responses were higher (i.e., in a negative direction) in the cool trial than in the control trial from 30 to 60 min during exercise. The retrograde SR responses were higher in the cool trial than in the warm trial during exercise. The retrograde SR responses were lower in the warm trial than in the control trial at between 10, 50, and 60 min during exercise.

In the control trial, the mean SR responses of the BA increased significantly from baseline to between 20 and 60 min during and 5 min (t=65 min) after exercise **(Fig. 2C)**. In the cool trial, the mean SR responses decreased significantly from baseline to just (t=0 min) before exercise, during the entire exercise period, and at 30 and 45 min (t=90 and 105 min, respectively) after exercise. In the warm trial, the mean SR responses increased significantly from baseline to between 10 and 60 min during and 5 min (t=65 min) after exercise. The mean SR responses were lower in the cool trial than in the control trial during the entire exercise period and at 5 and 30 min (t=65 and 90 min, respectively) after exercise. The mean SR responses were lower in the cool trial than in the warm trial both during and after exercise. The mean SR responses were higher in the warm trial than in the control trial during the entire exercise period and at 5 and 60 min (t=65 and 105 min, respectively) after

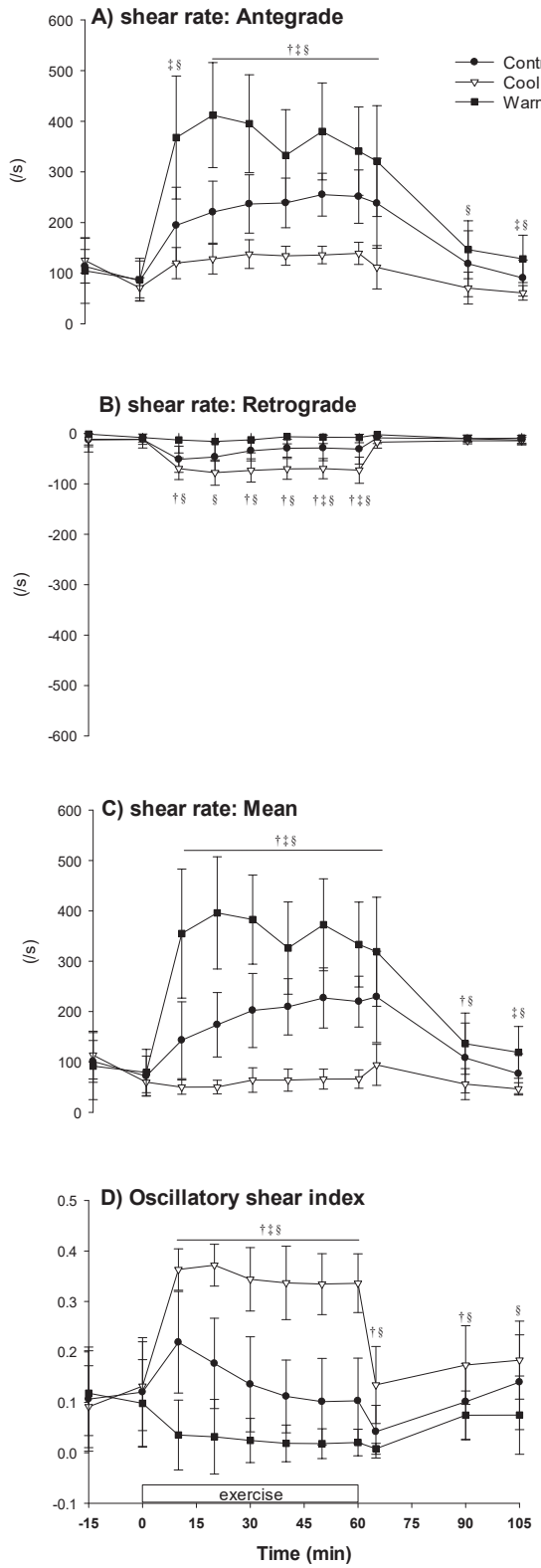

Fig. 2. SR profile (A: antegrade, B: retrograde, and C: mean) and OSI (D) before, during, and after exercise

Exercise was performed from 0 to 60 min and is shown in the transverse axis. Mean ± SD. †Cool versus control. ‡Warm versus control. §Cool versus warm. $P<0.05$.

exercise.

In the control trial, the OSI increased significantly from baseline to 10 min during exercise **(Fig. 2D)**. In the cool trial, the OSI increased significantly from baseline to between 10 and 60 min during and 30 and 45 min ($t=90$ and 105 min, respectively) after exercise. In the warm trial, the OSI decreased significantly from baseline to between 10 and 60 min during and 5 min ($t=65$ min) after exercise. The OSI was higher in the cool trial than in the control trial during the entire exercise period and at 5 and 30 min ($t=65$ and 90 min, respectively) after exercise. The OSI was higher in the cool trial than in the warm trial both during and after exercise. The OSI was lower in the warm trial than in the control trial during exercise.

The iAUCs for the antegrade, retrograde, and mean SRs during exercise were the lowest in the cool trial and higher in the warm trial compared with the control trial. In addition, the iAUCs for the OSI during exercise and recovery were the highest in the cool trial and lower in the warm trial compared with the control trial.

%FMD before and after Exercise

The baseline values of D-base, D-peak, and %FMD in the BA did not differ among the three trials **(Table 1, Fig. 3)**. In the control trial, D-base, D-peak, and %FMD after exercise did not change from baseline to the period after exercise. In the cool trial, D-base and D-peak decreased significantly from baseline to 15 min ($t=75$ min) after exercise (Δ D-base, ΔD-peak, and Δ%FMD: −0.021 cm, −0.025 cm, and −1.12%, respectively; $P<0.05$), and %FMD and adjusted %FMD decreased significantly from baseline to 60 min ($t=120$ min) after exercise (Δ %FMD and adjusted Δ%FMD: −2.68 and −2.34%, respectively; $P<0.05$). In the warm trial, D-base increased significantly from baseline to 15 min ($t=75$ min) after exercise (ΔD-base: +0.072 cm; $P<0.05$), and D-peak increased significantly at 15 and 60 min ($t=75$ and 120 min, respectively) after exercise (Δ D-peak at 15 and 60 min: +0.082 and +0.020 cm; $P<0.05$). %FMD showed a tendency to increase from baseline to 15 min ($t=75$ min) after exercise (Δ %FMD: +1.64%; $P=0.08$). The adjusted %FMD also showed a tendency to increase from baseline at 15 and 60 min ($t=75$ and 120 min) after exercise (adjusted Δ %FMD at 15 and 60 min: +1.91 and +1.42%, respectively; $P=0.09$ and $P=0.09$, respectively).

D-base was lower in the cool trial than in the warm trial and higher in the warm trial than in the control trial at 15 min ($t=75$ min) after exercise. D-peak was lower in the cool trial than in the control and warm trials and higher in the warm trial than in

Table 1. FMD variables before and after exercise

	Baseline	After exercise	
Time	−15 min	15 min	60 min
D-base (cm)			
Control	0.356 ± 0.03	0.358 ± 0.04	0.353 ± 0.03
Cool	0.359 ± 0.03	0.338 ± 0.03*, §	0.353 ± 0.02
Warm	0.353 ± 0.03	0.426 ± 0.04*, ‡	0.367 ± 0.03
D-peak (cm)			
Control	0.376 ± 0.03	0.382 ± 0.04	0.377 ± 0.03
Cool	0.380 ± 0.03	0.354 ± 0.04*, †, §	0.365 ± 0.03(*), §
Warm	0.374 ± 0.03	0.455 ± 0.04*, §	0.394 ± 0.03*, §
%FMD (%)			
Control	5.69 ± 1.35	6.74 ± 1.60	6.87 ± 2.49
Cool	5.87 ± 0.70	4.75 ± 1.89†, §	3.19 ± 1.82*, †, §
Warm	5.68 ± 1.86	7.33 ± 2.27(*)	7.19 ± 2.06
Adjusted %FMD (%)			
Control	5.67 ± 1.78	6.75 ± 1.78	6.82 ± 1.79
Cool	5.69 ± 1.70	4.39 ± 1.71(*)	3.35 ± 1.67*
Warm	5.47 ± 2.13	7.37 ± 2.45(*)	6.88 ± 1.93(*)

*Versus baseline. †Cool versus control. ‡Warm versus control. §Cool versus warm. $P<0.05$.
(*)Versus baseline. $0.05<P<0.1$.
Mean ± SD.

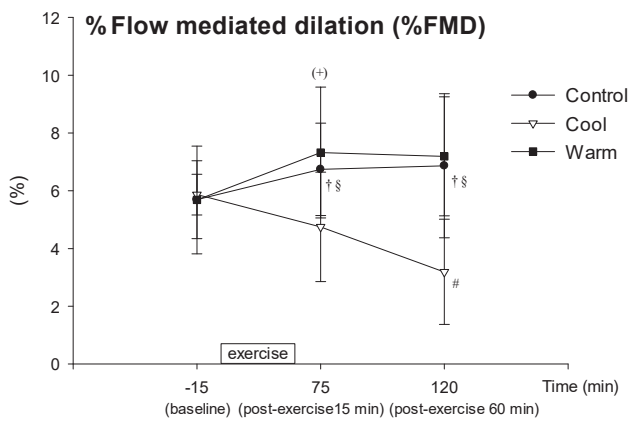

Fig. 3. %FMD in the BA before and after exercise

In the transverse axis, −15, 75, and 120 min indicate baseline (before exercise), 15 min and 60 min after the cessation of exercise during recovery, respectively. Mean ± SD. #Versus baseline (−15 min) in the cool trial. $P<0.05$. (+)Versus baseline (−15 min) in the warm trial. $P=0.08$. †Cool versus control. §Cool versus warm. $P<0.05$.

the control trial at 15 min ($t=75$ min) after exercise. D-peak was lower in the cool trial than in the warm trial and higher in the warm trial than in the control trial at 60 min ($t=120$ min) after exercise. %FMD was lower in the cool trial than in the control and warm trials at 15 and 60 min ($t=75$ and 120 min, respectively) after exercise.

Correlation between the iAUCs for the SR Profile and OSI and the Change in %FMD

The iAUCs for each SR and OSI during exercise were significantly correlated with Δ%FMD after exercise (antegrade, retrograde, mean SRs, and OSI: $r=0.54$, 0.54, 0.58, and −0.69, respectively; $P<0.05$) **(Fig. 4)**. The iAUCs for each SR and OSI during exercise and recovery were also significantly correlated with Δ%FMD from baseline to the end of protocol (antegrade, retrograde, mean SRs, and OSI: $r=0.41$, 0.47, 0.43, and −0.51, respectively, data not shown; $P<0.05$).

Discussion

In this study, we investigated the effects of the modulation of SR using unilateral arm warming and cooling on the %FMD value of the BA in healthy participants. As shown in **Fig. 2**, using arm warming and cooling during exercise, we succeeded in producing SR profiles distinctly different from the control trial, which highlights three key findings. First, arm warming during exercise exhibited a tendency to increase the %FMD value of the BA from baseline. Second, arm cooling during exercise significantly decreased the FMD of the BA from baseline and resulted in a lower %FMD of the BA compared with the warm and control trials. Third, the accumulated changes in each SR during and after exercise were significantly correlated with the change in postexercise FMD. These results

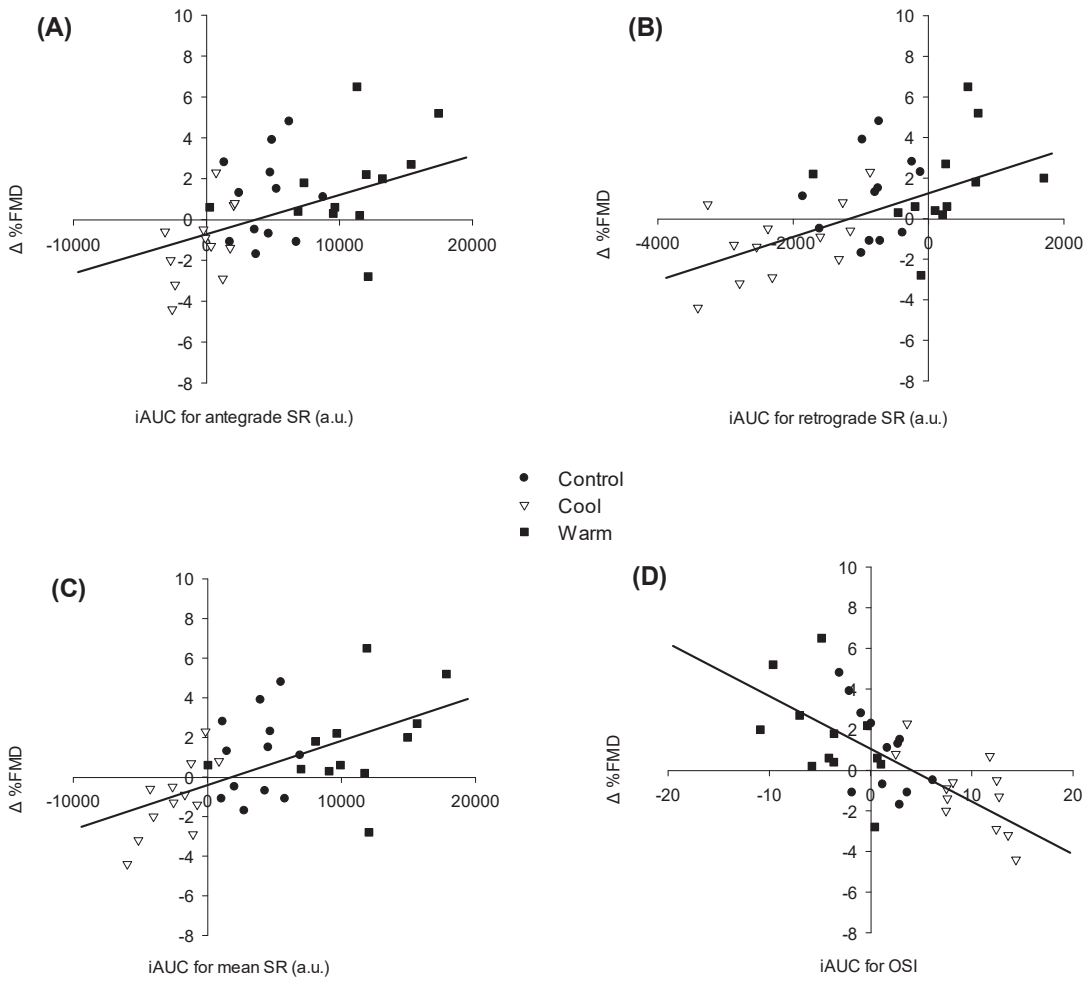

Fig. 4. Correlation between the iAUC for SR (A: antegrade, B: retrograde, and C: mean) and for the OSI (D) in the BA during exercise and the change of %FMD (Δ%FMD) from baseline to immediately after the cessation of exercise

See details in the Methods section.

suggest that, in healthy young participants, the modulation of shear profiles in the BA during exercise appears to be associated with subsequent changes in endothelial function.

SR and Diameter in the BA and SBF in the Palm and Forearm during Exercise

Each SR profile in the BA clearly differed among the three trials. In the control trial, at the onset of the cycling exercise, the retrograde SR was increased in accordance with an elevation in vascular resistance in downstream tissues (e.g., cutaneous and skeletal muscle) *via* sympathetic activation. Thereafter, the reduction of the retrograde SR and the elevation of antegrade SR gradually occurred *via* thermoregulatory mechanisms, such as the elevation of forearm SBF to facilitate heat exchange[10]. Presumably, the elevation of SBF in the palm also contributed to the reduction of retrograde SR and the elevation of antegrade SR in the BA. However, the study of Simmons *et al.*[10] did not elucidate the contribution of glabrous SBF to the SR profile due to the lack of measurements. As shown in **Fig. 1B**, the SBF in the palm gradually increased during exercise and also played a role in facilitating heat exchange. In the warm trial, a greater antegrade SR and lower retrograde SR were observed compared with the control trial during exercise. These results support the recent work of Thijssen *et al.*[8], in which unilateral arm heating can abolish the increase in retrograde SR during lower body negative pressure (i.e., a maneuver to increase muscle sympathetic nerve activity [MSNA]). Those SR profiles induced by arm warming could be explained as resulting from an exaggerated dilatory response in the resistance vasculature of arm tissues (skin and muscle) induced by local heating[28]. Although we did not measure the core body

temperature, because the warm trial resulted in the highest HR values during and after exercise among the three trials, the systemic circulatory effect (not only local effect) might also affect the SR response in the warm trial. In addition, the reduction of retrograde SR in the warm trial might be potentially induced by locally attenuated sympathetic activation in the forearm. Takahashi *et al.*[29] reported that when the anterior region of the leg was locally warmed at 41℃ using a hydrocollator pack, MSNA in the peroneal nerve was significantly decreased irrespective of systemic change.

Contrary to the control and warm trials, in the cool trial, the antegrade SR did not change, and greater retrograde SR and OSI remained the same as the baseline values throughout the exercise. As mentioned above, the reduction in the retrograde SR and the elevation in the antegrade SR during exercise occurred gradually *via* thermoregulatory mechanisms with elevated forearm SBF to facilitate heat exchange[10]. Thus, this finding seems to suggest that forearm cooling locally suppressed thermoregulatory responses in the right forearm. Consequently, in the cool trial, the modulation of shear profiles of the BA during exercise could be mainly reflected by exercise-induced sympathetic activation. It was shown in a previous study that increased retrograde SR was evoked by elevating sympathetic nervous system activity with graded lower body negative pressure[30]. In addition, Ishida e*t al.*[31] reported that when the anterior region of the leg was locally cooled at 15℃ using ice packs, MSNA in the peroneal nerve was significantly increased irrespective of systemic change. As immersing the hand in 15℃ cool water for 2 min did not increase MSNA in the peroneal nerve[32], the authors suggested that MSNA is activated by nonnoxious local cooling. Therefore, in this study, a sustainable increase in the retrograde SR might result from excited sympathetic activation *via* both an exercise-induced systemic response and localized cooling.

As shown in the time course of the BA diameter in each trial **(Fig. 1D)**, the diameter changes in the BA differed among the three trials and seemed to be affected by the SR profiles during exercise. In particular, the greater antegrade and smaller retrograde SRs in the warming trials led to stronger vasodilation of the BA (i.e., an enlarged diameter), and nearly opposite SR profiles in the cooling trial resulted in vasoconstriction of the BA (i.e., a smaller diameter). Until now, to enhance retrograde SR and OSI in the BA, unilateral arm cuff pressure has been commonly used because it can gradually increase the retrograde SR according to cuff pressure[2, 33, 34]. Previous studies that used the cuff pressure maneuver have helped us interpret our results (i.e., change in diameter and its consequent FMD in the BA). Our results were consistent with those of a previous study[35] demonstrating that the modulation of SR using unilateral cuff pressure during exercise and arm warming provides an important stimulus on the diameter change of the BA. Padilla *et al.*[36] revealed that NO synthase inhibition in the forearm circulation of young healthy participants increased retrograde and oscillatory shear. Taken together, the SR profiles play an important role in NO synthase, which contributes to the regulation of diameter change. Thus, arm warming/cooling during exercise can cause a significant diameter change, which could be associated with the modulation of SR responses (profiles).

Postexercise Endothelial Function in the BA

Modulation of SR profiles using forearm warming/cooling during exercise can potentially affect postexercise endothelial function in the BA. Our results are consistent with those of previous studies demonstrating that increases in antegrade SR are associated with antiatherogenic effects[37], and oscillatory and retrograde SRs lead to a proatherogenic state[38]. In the control trial, the %FMD value did not change even when it was statistically adjusted for individual changes in baseline diameter. There is no full agreement on the effects of low- to moderate-intensity exercise on postexercise %FMD. Low- or moderate-intensity cycling exercise exhibited improved[39] or unchanged[40-42] %FMD in young healthy humans. However, in the present study, even the adjusted %FMD increased by 1.4%–1.9% (mean increase, not significant) in the warm trial and decreased significantly by 2.3%–2.7% in the cool trial. Moreover, the iAUCs of each SR variable during and after exercise were significantly correlated with postexercise Δ %FMD. This is partly supported by the significant negative correlation between the cuff inflation-induced change in retrograde SR and the change in the %FMD value of the BA in healthy young individuals[2]. Hence, we suggest that the modulation of shear profiles in the BA during exercise is associated with postexercise endothelial function.

In order to explain the change in FMD after acute exercise in this study, we should consider the influences of not only the modulation of the SR (profile) but also other factors, such as oxidative stress, sympathetic activation, and systemic circulatory change[43]. It is postulated that an increase in oxidative stress can lead to a reduction in NO production and/or bioavailability and a subsequent reduction in the FMD[43]. Although we did not measure the reactive oxygen species (ROS) levels, they are unlikely to play

a key role because it has been reported in previous studies that ROS remain unchanged following mild or moderately intense aerobic exercise[40, 44-46]. Conversely, the attenuated endothelial function in the cool trial in the present study might be induced *via* a mechanism related to oxidative stress. When the retrograde SR in the BA was increased using the cuff pressure maneuver during leg cycling exercise or rest, the subsequent %FMD was attenuated[3]. This attenuation was abolished by supplementation with antioxidant vitamin C, which typically lowers oxidative stress[39, 47]. This suggests that the increased oxidative stress induced by the increases in retrograde shear was responsible for the attenuation in postexercise FMD. In addition, Jenkins *et al.*[48] provided the first direct evidence that local disturbed blood flow using the cuff pressure maneuver for 20 min acutely induces endothelial activation and apoptosis in humans, as reflected by the release of microparticles from activated [CD62E(+)] and apoptotic [CD31(+)/CD42b(−)] endothelial cells. Following this line, Storch *et al.*[49] demonstrated that the elevation of oscillatory shear using the cuff pressure maneuver for 30 min induced disturbances in platelet microparticle release, coagulation–fibrinolysis, and matrix metalloproteinase-9 activity in healthy individuals. The magnitudes of oscillatory shear values were 0.27 and 0.43 in the studies by Jenkins *et al.*[48] and Storch *et al.*[49], respectively. These values were nearly equivalent to those in the present cool trial. Therefore, we suspect that the molecular mechanisms underlying the elevation of oscillatory shear could also be associated with attenuated %FMD in the cool trial.

Limitations

In order to examine the effect of "local" modulating SR on endothelial function, we used the forearm cooling/warming maneuver. HR values were higher in the warm trial than in the other trials during and after exercise. This reflects the different physiological strain among the trials, which potentially affects the elevation in ROS production and sympathetic activation. Such factor(s) potentially attenuate the beneficial effects of exercise on the endothelium[42, 50]. Moreover, each heartbeat elicits a pulsatile shear force on the arterial wall, directly affecting endothelial function[51]. Thus, the different HRs among the three trials could result in accumulated changes in each SR profile, which might affect postexercise FMD. In order to understand the relationship between the modulation of SR profiles and endothelial function, further research is warranted to minimize the systemic circulatory change among trials. Finally, in the cool trial, postexercise FMD did not return to the baseline values. Thus, we merely recorded FMD data until 60 min after exercise. In future studies, the adaptive effect of immersing the forearm in cool water on endothelial function should also be investigated.

Conclusion

We revealed that forearm cooling during exercise decreases postexercise FMD by modulating SR profiles (i.e., reduced antegrade SR and elevated retrograde SR and OSI). Modulation of shear profiles in the BA during exercise appears to be associated with subsequent endothelial function. In conclusion, this study provides supportive evidence that SR profiles play a key role in determining the acute adaptation of endothelial function.

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