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Repeated increases in blood flow, independent of exercise, enhance conduit artery vasodilator function in humans

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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* 300: H664–H669, 2011. First published December 3, 2010; doi:10.1152/ajpheart.00985.2010.—This study aimed to determine the importance of repeated increases in blood flow to conduit artery adaptation, using an exercise-independent repeated episodic stimulus. Recent studies suggest that exercise training improves vasodilator function of conduit arteries via shear stress-mediated mechanisms. However, exercise is a complex stimulus that may induce shear-independent adaptations. Nine healthy men immersed their forearms in water at 42°C for three 30-min sessions/wk across 8 wk. During each session, a pneumatic pressure cuff was inflated around one forearm to unilaterally modulate heating-induced increases in shear. Forearm heating was associated with an increase in brachial artery blood flow ($P < 0.001$) and shear rate ($P < 0.001$) in the uncuffed forearm; this response was attenuated in the cuffed limb ($P < 0.005$). Repeated episodic exposure to bilateral heating induced an increase in endothelium-dependent vasodilation in response to 5-min ischemic ($P < 0.05$) and ischemic handgrip exercise ($P < 0.005$) stimuli in the uncuffed forearm, whereas the 8-wk heating intervention did not influence dilation to either stimulus in the cuffed limb. Endothelium-independent glyceryl trinitrate responses were not altered in either limb. Repeated heating increases blood flow to levels that enhance endothelium-mediated vasodilator function in humans. These findings reinforce the importance of the direct impacts of shear stress on the vascular endothelium in humans.

nitric oxide; heating

ENDOTHELIAL DYSFUNCTION is an early manifestation of atherosclerosis, which independently predicts future cardiovascular events (27, 28, 39), and improvement in endothelial function decreases cardiovascular risk (13, 19). Exercise training is a potent physiological stimulus that enhances endothelial function and induces endothelium-dependent arterial remodeling in humans (9, 17).

The increase in blood flow associated with repeated exercise bouts may be responsible for enhancing endothelial function and increasing artery size (i.e., outward remodeling). Hambrecht et al. (10) demonstrated increased endothelial nitric oxide (NO) synthase (eNOS) mRNA and protein content, phosphorylation of shear-sensitive eNOS moieties, and enhanced NO vasodilator function following exercise training in humans, linking shear stress and NO-mediated vasodilator function.

More recently, we used repeated forearm handgrip exercise performed simultaneously in both arms, with a cuff inflated on one forearm to unilaterally “clamp” exercise-induced hyperemia at near baseline levels (33). Despite similar increases in grip strength, the volume and girth in both limbs, vasodilator function, and indexes of arterial remodeling improved only in the arm that was exposed to the increased blood flow during exercise. These data, combined animal studies (17), strongly suggest that exercise induces an improvement in endothelial function via shear-dependent mechanisms.

Nevertheless, exercise is a complex stimulus, and it remains unclear whether the mechanisms responsible for training-induced changes in arterial function and remodeling are related to the modification of flow and shear. Therefore, the aim of the present study was to determine whether repeated episodic increases in blood flow, induced by forearm heating, can induce conduit artery adaptation in humans, independent of an exercise stimulus. Using a within-subjects, between-limb design, with simultaneous measures to minimize potential sources or error, we hypothesized that the limb exposed to larger changes in blood flow as a result of repeated heat exposure would exhibit enhanced vascular function following repeated forearm heating.

METHODS

Ethical Approval

All study procedures were approved by the Human Research Ethics Committee of the University of Western Australia. Written, informed consent was obtained from all subjects, and studies conformed to the Declaration of Helsinki.

Subject Characteristics

Ten young recreationally active men were recruited to undertake an 8-wk experimental protocol. Complete data were unavailable from one subject across the 8-wk period, so analysis was undertaken on the remaining 9 men (21.5 ± 1.4 yr, Table 1). The subjects were young and healthy and a preparticipation questionnaire confirmed the absence of known cardiovascular disease or risk factors and excluded subjects with unsuitable lifestyle traits, those on any medications or drugs. Because of possible antiatherogenic effects of estrogen, women were excluded from this study along with individuals taking medications or drugs of any kind.

Study Design

Subjects underwent an initial testing session, assessing endothelium-dependent and -independent brachial artery vasodilator function. These measures were then collected at weeks 2, 6, and 8 of the intervention at the same time of day (8:00 to 9:00 AM). During the

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Table 1. Baseline subject characteristics

Age, yr	21.5 ± 1.4
Height, cm	183.8 ± 0.1
Systolic blood pressure, mmHg	127 ± 8
Diastolic blood pressure, mmHg	58 ± 7
Mean arterial pressure, mmHg	83 ± 6
Heart rate, beats/min	61 ± 5

Values are means ± SD.

8-wk intervention period, the subjects reported to the laboratory 3 times/wk for a 30-min intervention consisting of bilateral forearm heating. In one arm, a pneumatic cuff was inflated (100 mmHg) to minimize increases in brachial artery shear. This enabled the comparison of heat exposure alone versus heat exposure combined with increases in shear. All study and training sessions were undertaken in a thermostatically controlled laboratory with an ambient room temperature between 22 and 24°C.

Experimental Procedures: Dependent Variables

Assessments were conducted in a quiet, temperature-controlled environment. All studies were conducted at the same time of day to eliminate the possible impact of circadian variation on vascular function, and the subjects fasted for 8 h and abstained from alcohol and/or caffeine and exercise for 24 h before testing.

Assessment of endothelium and flow-mediated responses to 5 min forearm ischemia. After a 20-min rest period, brachial artery diameter and velocity responses to flow-mediated dilation (FMD) were simultaneously assessed in both arms, using 10-MHz multifrequency linear array probes, attached to high-resolution ultrasound machines (T3000; Terason, Burlington, MA). Detailed descriptions of this technique are provided elsewhere (2, 3, 32). Briefly, a rapid inflation/deflation pneumatic cuff (AG 101, Hokanson) was placed around each arm immediately distal to the olecranon process. When an optimal B-mode image was obtained, images were collected using an insonation angle (always <60°), which did not vary during each study or within individuals across the intervention. Baseline images were recorded for 1 min, before the forearm cuff was inflated to 220 mmHg for 5 min. Recording resumed 30 s before cuff deflation, and continued for 5 min postdeflation. Heart rate and mean arterial pressure were determined from an automated sphygmomanometer (GE Pro 300V2, Dinamap; Tampa, FL). This measure provides an index of conduit artery dilation that is endothelium dependent and that most (6, 11, 15, 20), but not all (25), studies suggest is largely NO mediated.

Assessment of endothelium and flow-mediated responses to ischemic exercise. Following a >20-min rest period, we examined bilateral brachial artery dilation after 5 min of ischemic exercise, described in detail previously (22). While seated upright, subjects performed repeated handgrip exercise at a rate of 20 contractions/min for the middle 3 min of a 5-min ischemic period. This protocol results in dilation that is endothelium dependent but may not be highly NO dependent (20).

Assessment of endothelium-independent glyceryl trinitrate-mediated vasodilation. Following another 20-min rest period, a 1-min baseline recording was again taken simultaneously from both brachial arteries. Subsequently, brachial artery diameter and velocity were examined for 10 min after a sublingual administration of glyceryl trinitrate (GTN; 400 µg).

Brachial artery diameter and blood flow analysis. Analysis of diameter and velocity was performed using custom-designed edge-detection and wall-tracking software, which is independent of investigator bias (37) and previously described in detail (2). From synchronized diameter and peak velocity envelope data, blood flow and shear rate (an estimate of shear stress without viscosity) were calculated (24). The reproducibility of the FMD using this semiautomated software possesses a coefficient of variation of 6.7–10.5% (30, 37).

Repeated Water Bath Exposure Protocol

After the initial assessments, the subjects attended the laboratory 3 times/wk for 8 wk for a 30-min bilateral water bath exposure. A pneumatic cuff was placed on one forearm, below the elbow, and inflated to 100 mmHg using a pneumatic device (AG101, Hokanson). Previous research suggests that unilateral cuff inflation in this manner alters patterns of flow and shear (30, 32, 33). The cuff pressure used in this experiment was chosen following pilot trials. The contralateral arm remained uncuffed. Left or right forearm cuff placement was randomized but consistent for a given individual across the intervention period.

Both arms were then immersed in warm water (42°C) above the level of the elbow for 30 min. The water was maintained at a constant 42°C in a storage reservoir using a thermostatically controlled heating unit. This reservoir was connected via tubing to both forearm immersion tanks, and a submersible pump ensured that water of identical temperature was continuously circulated to both tanks.

We confirmed that the unilateral cuff placement induced differences in blood flow and shear patterns between the limbs in a substudy of five subjects who underwent direct assessments of blood flow and artery shear in both arms during bilateral heating with unilateral cuff placement. We have also previously demonstrated that a 30-min heating intervention induces improvement in FMD immediately after the heating period in the uncuffed arm, whereas the placement of an inflated cuff during the heating period abolishes this acute effect (32).

Data Analysis

Flow and GTN-mediated dilation are presented as the relative (%) dilation or rise from the preceding baseline diameter. See previous studies for further detail (2). In accordance with recent findings (2, 26), we calculated the shear rate stimulus responsible for endothelium-dependent FMD following cuff deflation. The area under the shear rate curve (AUC_{SR}), calculated as the sum of all shear rate data up to the point of maximal postdeflation diameter (2), was calculated for each individual.

Statistics

Statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, IL). All data are reported as means (SD) unless stated otherwise. Statistical significance was assumed at $P < 0.05$. All variables were analyzed using two-factor general linear models with repeated measures. The factors were time (week of training) and group (uncuffed vs. cuffed limb). Statistically significant interactions were followed up with post hoc t -tests.

We have previously published a detailed and comprehensive within- and between-subject assessment of variability of measurement of %dilation using our observer-independent and automatic wall-tracking system analysis system (37). This revealed that, assuming 80% power and an α of 0.05, eight subjects would be required in an intervention study to detect an absolute 1.5% change in FMD. We also performed a power analysis based on differences observed in a recent study of the impact of cuff placement on exercise training responses (33), which used an updated version of our analysis system. This indicates that, to detect the 1.5% difference in FMD during exercise training, a sample of six is required. This study was therefore adequately powered.

RESULTS

Impact of Cuff Placement During Bilateral Forearm Heating: Efficacy of Independent Variable Manipulation

Brachial artery measures immediately preceding water bath immersion demonstrated no significant difference in blood flow between the arms at rest (Fig. 1).

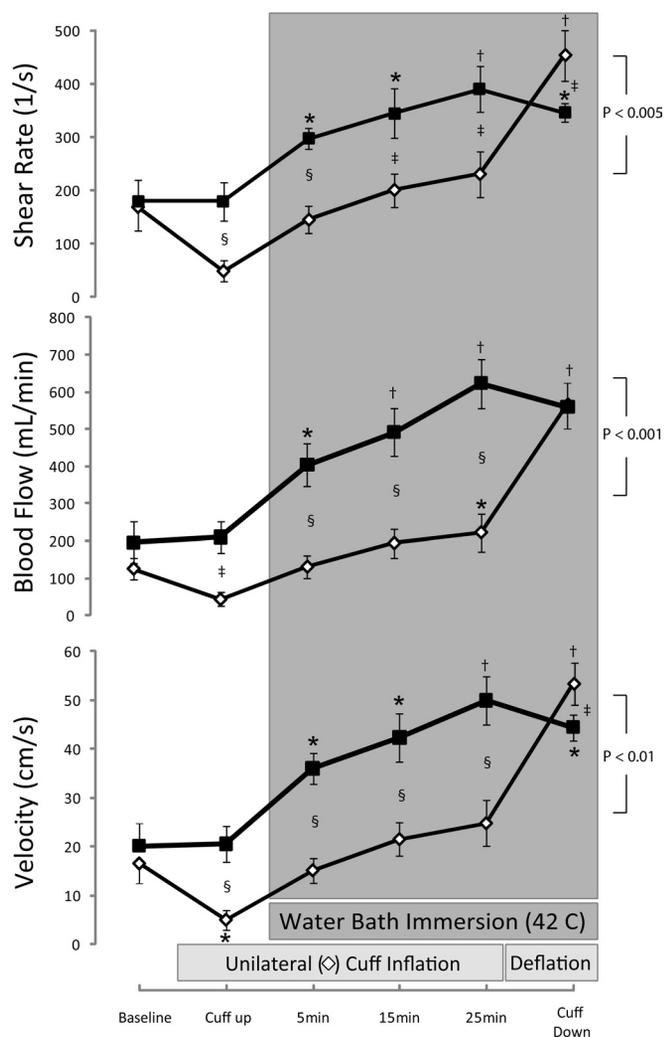


Fig. 1. Acute impact of cuff placement during bilateral forearm heating. Brachial artery shear rate (top), blood flow (middle), and velocity (bottom) at baseline and at 5-min intervals in a subgroup of 5 subjects who underwent bilateral assessments throughout an acute bout of 30 min bilateral forearm heating (42°C) in the uncuffed (■) and cuffed arm (◇) are shown. Error bars represent means \pm SE. * $P < 0.05$ or † $P < 0.01$, significantly different from baseline. ‡ $P < 0.05$ or § $P < 0.01$, significantly different between limbs at same time point.

The measures taken from each arm at 5-min intervals during the heating stimulus revealed a significant impact of cuff placement (Fig. 1). Velocity ($P > 0.001$, 1-way ANOVA), flow ($P > 0.001$), and shear ($P > 0.001$) were significantly

higher in the uncuffed arm relative to baseline as a result of heating (Fig. 1).

While there was also an increase in the cuffed arm (velocity, flow, and shear, all $P < 0.001$), the magnitude of change was greater in the uncuffed limb ($P < 0.005$, 2-way ANOVA, Fig. 1). These data confirm our recent findings that indicated forearm heating acutely increased FMD in the uncuffed, but not the cuffed, forearm (32).

Impact of 8 wk of Repetitive Heating on Brachial Artery Responses

Endothelium-dependent flow-mediated responses to 5 min ischemia. Subjects presented with no baseline differences in resting artery diameter (Table 2). Repeated localized heating resulted in a significant difference between cuffed and uncuffed dilation (%) responses (main limb effect at weeks 0, 2, and 8; $P = 0.017$, Fig. 2A). Post hoc analysis revealed that dilation (%) values at week 2 were significantly higher compared with baseline in the uncuffed arm ($P < 0.05$, Fig. 2A). In the cuffed arm, no changes in dilation (%) were evident at any time point relative to baseline (Fig. 2A). Comparisons between limbs revealed significant differences at week 2 ($P < 0.05$), but not baseline, weeks 6 or 8 (Fig. 2A). Shear rate following the 5-min ischemic stimulus decreased between weeks 0 and 2 in the uncuffed limb ($P < 0.05$, Table 2). No differences were evident in the cuffed limb.

Endothelium-dependent flow-mediated responses to ischemic handgrip exercise. In the uncuffed arm, the responses to ischemic exercise showed an increase across the intervention period, particularly at 8 wk (Fig. 2B) (1-way ANOVA, $P < 0.01$). In the cuffed arm, no changes in response to ischemic exercise were evident across the intervention (Fig. 2B). ANOVA (2-way) revealed a significant effect for the cuffed/uncuffed limb ($P < 0.01$) and also an interaction effect between the limb and time ($P < 0.01$). Post hoc *t*-tests revealed significant increases in the uncuffed limb between baseline and week 8 ($P < 0.005$), with no differences between baseline and weeks 6 or 8 in the cuffed limb. Comparisons between limbs revealed significant differences at week 2 ($P < 0.05$) and week 8 ($P < 0.05$) (Fig. 2B). No significant effect for time, limb, or interaction between these factors was evident for AUC_{SR} in response to ischemic handgrip exercise (Table 2).

Endothelium-independent GTN-mediated vasodilator responses. There were no significant difference in response to GTN (Table 2). Both cuffed and uncuffed responses remained unchanged from baseline across the intervention period (Table 2).

Table 2. Brachial artery characteristics throughout an 8-wk exercise intervention in the uncuffed and cuffed arms

	Week 0	Week 2	Week 6	Week 8
Uncuffed arm				
Resting diameter, mm	4.0 \pm 0.5	3.9 \pm 0.6	4.1 \pm 0.7	4.0 \pm 0.8
5-min ischemia AUC _{SR}	23,528 \pm 9,327	16,253 \pm 7,703*	20,246 \pm 16,252	20,368 \pm 9,060
Ischemic exercise dilation AUC _{SR}	45,251 \pm 22,492	35,682 \pm 16,338	39,002 \pm 13,967	36,292 \pm 16,471
GTN, %	19.9 \pm 5.1	17.4 \pm 9.0	22.5 \pm 5.6	21.6 \pm 6.4
Cuffed arm				
Resting diameter, mm	4.0 \pm 0.5	3.8 \pm 0.5	3.9 \pm 0.6	3.8 \pm 0.6
5-min ischemia AUC _{SR}	24,775 \pm 21,586	18,297 \pm 10,032	26,431 \pm 11,320	36,447 \pm 10,872
Ischemic exercise dilation AUC _{SR}	54,731 \pm 29,736	42,432 \pm 14,754	42,985 \pm 18,279	37,264 \pm 24,263
GTN, %	17.7 \pm 2.2	18.4 \pm 2.4	19.7 \pm 3.4	19.4 \pm 3.7

Values are means \pm SD. AUC_{SR}, shear rate area under the curve from the time of cuff deflation to peak diameter detection; GTN, glyceryl trinitrate. * $P = 0.05$, significant from value at week 0.

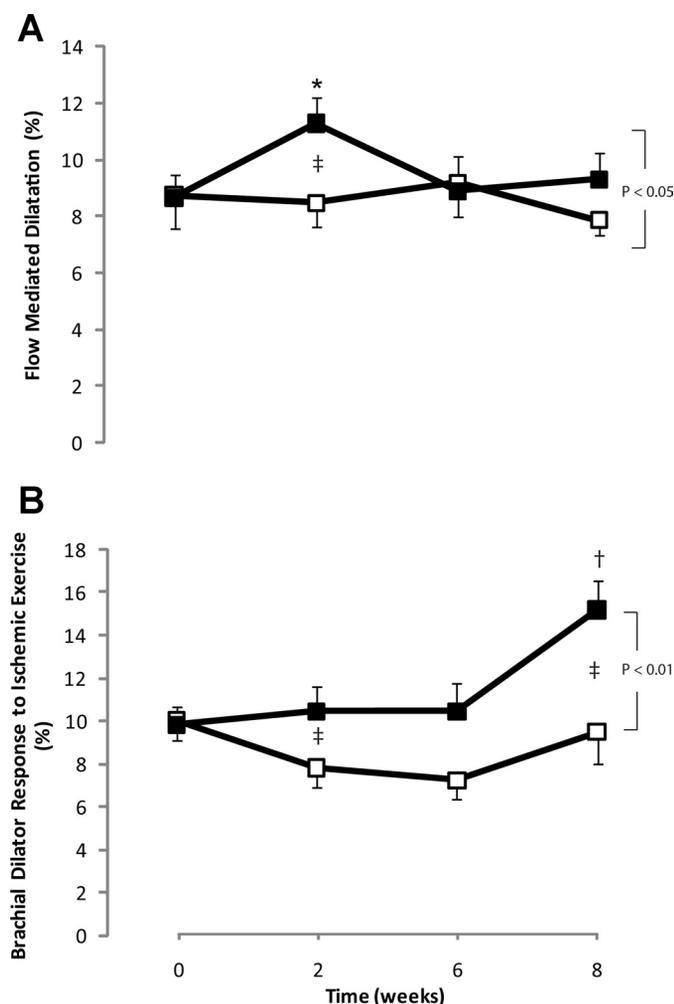


Fig. 2. Chronic impact of 8 weeks of repetitive heating on brachial artery response. Relative change in flow-mediated dilation from baseline (A) and in response to ischemic exercise (B) across the 8-wk heating intervention in healthy young men ($n = 9$) is shown. Data were presented for the uncuffed arm (■) as well as the cuffed arm (□). Error bars represent means \pm SE. * $P < 0.05$ or † $P < 0.005$, significantly different from baseline. ‡ $P < 0.05$, significantly different between limbs at same time point.

DISCUSSION

The aim of the present study was to determine whether episodic increases in blood flow, independent of an exercise stimulus, induce conduit artery adaptation in humans. Using a repeated simultaneous bilateral forearm heating stimulus, we induced increases in blood flow and shear in one forearm, while attenuating these responses using a cuff on the contralateral limb. This provided a within-subjects design to determine the effects of shear modulation on endothelium-dependent and -independent vasodilation. Outcome measures were simultaneously assessed in both limbs to minimize potential sources of error. Our principal findings include the following: 1) a repeated exercise-independent shear-mediated stimulus can induce adaptation in endothelial function, 2) cuff placement and shear modulation attenuates this response, 3) different endothelial mechanisms may be involved in the above findings, and 4) adaptation is limited to the endothelium and not smooth muscle layer. These findings indicate that repeated heating increases shear to levels capable of inducing adaptation in

endothelial function and that exercise-independent increases in shear can alter arterial function in vivo.

It is well established that exercise training improves endothelial function in conduit and resistance arteries (9). Animal studies suggest that this is related to the repeated increases in shear which occur during exercise (17, 23). Furthermore, adaptation in endothelial function occurs rapidly, within 1 or 2 wk following the onset of exercise training (4, 5, 14, 18, 29, 35). In humans, the link between exercise, repeated shear stimulation, and vascular function was first suggested by Hambrecht et al. (10) who observed improvement in NO-mediated vasodilator function alongside increases in eNOS expression and endothelial content of phospho-eNOS_{Ser1177}, Akt, and phospho-Akt. They concluded that the change in endothelium-dependent vasodilation was closely related to shear stress-induced Akt-dependent phosphorylation of eNOS. More recently, we adopted experimental approaches similar to those used in the present study to bilaterally manipulate the magnitude of increase in shear during dual handgrip exercise training (33). We observed enhanced endothelium-dependent vasodilation in the limb exposed to increased shear during exercise but not in the contralateral limb in which shear was clamped near baseline levels. We concluded that exercise-induced changes in shear provide a crucial physiological stimulus to adaptation in flow-mediated endothelial function in healthy humans. As in the present study, we observed transient changes in NO-mediated vasodilator function, which were superseded by increases in vasodilatation attributable to non-NO-dependent mechanisms or, perhaps, vascular structure, as previously discussed (9, 16, 31, 33). Nonetheless, exercise may conceivably induce adaptations via mechanisms other than shear modulation (17).

This is the first study in humans, to our knowledge, to indicate that endothelium-dependent vasodilator function improves in response to a repeated blood flow and shear stimulus that is independent of exercise. We observed changes in endothelial function in the uncuffed limb but no such changes in the contralateral cuffed limb that received a lower shear rate stimulus. An interesting observation is that, despite being diminished compared with the uncuffed arm, blood flow and shear rate nonetheless increased during heat exposure in the cuffed limb. The lack of change in endothelial function in the cuffed limb, despite some increase in flow and shear during heating, suggests that there may be a threshold below which endothelial adaptations are less apparent. Further studies will be required to fully address this issue.

We used two distinct vasodilator stimuli in the present experiment. The vasodilator response to a 5-min period of ischemia has been reported by most (6, 11, 15, 20), but not all (25), studies to be largely NO dependent, whereas dilation in response to ischemic handgrip exercise is likely less NO dependent (20). Whatever the precise mechanisms responsible for the dilation to each stimulus, the combination of both measures provides complementary information regarding the status of endothelium-dependent and shear stress-mediated conduit artery vasodilation in humans. Our findings of enhanced arterial dilation in response to these stimuli are therefore consistent with the upregulation of endothelium-dependent conduit artery function. The present study reinforces our recently observed changes in response to handgrip training (33)

and adds the novel finding that repeated shear stimulation, whether associated with exercise or not, may induce conduit artery endothelial adaptation. It appears that the brachial artery became somewhat hyperresponsive following heat training, as shear AUC decreased in the presence of maintained FMD and increased ischemic handgrip responses. These findings contrast somewhat with the impact of exercise training on shear-mediated dilator responses, and the explanation is not entirely clear, but it may be that heat training has less impact on resistance vessel remodeling than exercise training, even if it does modulate cutaneous microvascular function (8).

We observed no evidence for change in GTN responses in either limb of the subjects in the present study. This suggests that, despite evidence for a change in endothelium-dependent vasodilator responses to ischemic stimuli, repeated exercise-independent increases in shear did not alter NO-mediated smooth muscle function. Many, but not all, studies of exercise training have also reported improvement in endothelial, but not smooth muscle, vasodilator function in humans (9).

We could not find many examples of the impact of repeated passive heating on conduit artery adaptation in humans. One study reported the improved clinical status and brachial FMD, but not GTN, responses as a result of 2 wk of daily sauna exposure in patients with chronic heart failure (12). The authors suggested that the peripheral circulation improved, but they did not test this. Our data also suggest that the purported benefits of heat therapy may be related to a shear stress-mediated improvement in conduit endothelial function. However, the clinical implications of our findings in response to repeated heating are not clear. While a number of studies have established the independent prognostic relevance of the dilator response to 5 min ischemia (19, 27, 28, 39), possibly attributable to its dependence on antiatherogenic NO function, nothing is currently known regarding the prognostic relevance of the ischemic handgrip protocol, despite its endothelium dependence. We cannot, therefore, extend our findings to possible prognostic implications of the changes observed with heating in this study, and we prefer to limit our conclusions to the impact of shear manipulation on vascular adaptation.

There are several limitations to this study. Our sample size was small. However, significant differences were observed in our principal outcome variables, indicating that it was adequate. Moreover, previous studies using %dilation and interventions such as exercise have used similar sample sizes (7, 34, 36, 38). A post hoc power analysis revealed that we had 90% power to observe the difference in %dilation between baseline and week 2 for the ischemic dilator response. This indicates that we have a strong design to detect changes in vascular function across an 8-wk intervention in healthy men. We chose not to normalize %dilation for its eliciting shear because of the large variability and statistical issues associated with shear assessment and normalization (1). However, we believe it unlikely that our conduit dilator data can be explained by changes in the shear rate stimulus, since increases in dilation were associated with no change, or decreases, in shear rate. In any event, our study clearly indicates that cuff placement and shear manipulation have an impact on endothelial function in humans and that these effects are not evident in smooth muscle. Our results do not provide evidence relating to resistance vessels, and although we recently reported data on microvascular adaptations using the experimental approach

outlined in this study (8), larger studies, which are specifically designed to assess resistance vessel responses, would be a valuable addition to the present data set. Finally, we did not include a nonheating control group to assess the effects of time on vascular responses in the present study. This is because we have previously published data of this nature in a very similar group of subjects, and no differences across 8 wk were evident in either ischemic or ischemic exercise responses (31).

In summary, our findings indicate that repeated heating increases shear stress to levels that enhance endothelium-mediated conduit artery vasodilator function. These findings reinforce the importance of direct impacts of shear stress on the vascular endothelium in humans and provide further insight into the mechanisms responsible for exercise training-mediated vasodilator changes.

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GRANTS

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DISCLOSURES

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

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