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Am J Physiol Heart Circ Physiol 303:H533-H538, 2012. First published 22 June 2012;
doi: 10.1152/ajpheart.00272.2012

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Remote ischemic preconditioning prevents reduction in brachial artery flow-mediated dilation after strenuous exercise

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Submitted 4 April 2012; accepted in final form 19 June 2012

Bailey TG, Birk GK, Cable NT, Atkinson G, Green DJ, Jones H, Thijssen DH. Remote ischemic preconditioning prevents reduction in brachial artery flow-mediated dilation after strenuous exercise. *Am J Physiol Heart Circ Physiol* 303: H533–H538, 2012. First published June 22, 2012; doi:10.1152/ajpheart.00272.2012.—Strenuous exercise is associated with an immediate decrease in endothelial function. Repeated bouts of ischemia followed by reperfusion, known as remote ischemic preconditioning (RIPC), is able to protect the endothelium against ischemia-induced injury beyond the ischemic area. We examined the hypothesis that RIPC prevents the decrease in endothelial function observed after strenuous exercise in healthy men. In a randomized, crossover study, 13 healthy men performed running exercise preceded by RIPC of the lower limbs (4 × 5-min 220-mmHg bilateral occlusion) or a sham intervention (sham; 4 × 5-min 20-mmHg bilateral occlusion). Participants performed a graded maximal treadmill running test, followed by a 5-km time trial (TT). Brachial artery endothelial function was examined before and after RIPC or sham, as well as after the 5-km TT. We measured flow-mediated dilation (FMD), an index of endothelium-dependent function, using high-resolution echo-Doppler. We also calculated the shear rate area-under-the-curve (from cuff deflation to peak dilatation; SR_{AUC}). Data are described as mean and 95% confidence intervals. FMD changed by <0.6% immediately after both ischemic preconditioning (IPC) and sham interventions ($P > 0.30$). In the sham trial, FMD changed from 5.1 (4.4–5.9) to 3.7% (2.6–4.8) following the 5-km TT ($P = 0.02$). In the RIPC trial, FMD changed negligibly from 5.4 (4.4–6.4) post-IPC and 5.7% (4.6–6.8) post 5-km TT ($P = 0.60$). Baseline diameter, SR_{AUC}, and time-to-peak diameter were all increased following the 5-km TT ($P < 0.05$), but these changes did not influence the IPC-mediated maintenance of FMD. In conclusion, these data indicate that strenuous lower-limb exercise results in an acute decrease in brachial artery FMD of ~1.4% in healthy men. However, we have shown for the first time that prior RIPC of the lower limbs maintains postexercise brachial artery endothelium-dependent function at preexercise levels.

exercise performance; endothelial function; cardiovascular risk; exercise training

RESEARCHERS EXAMINING the acute effect of exercise on endothelium-dependent dilation have reported conflicting results (10, 16, 34, 48, 52), which may be at least partly explained by differences in exercise intensity. Recently, we found that high-intensity exercise, but not low-intensity exercise, was associated with a significant reduction in upper-limb brachial artery endothelium-dependent function immediately after cycling exercise (9). This is in line with recent observations following running exercise (20). The impact of acute exercise on arterial function is important to examine as Goto et al. (15) found that training performed at different exercise intensities can induce distinct adaptation in vasodilator function, with high-intensity exercise training not leading to improvement in vasodilator function. Although speculative and others have suggested otherwise (35), the presence of a decline in endothelium-dependent function after exercise may relate to the absence of vascular adaptations after high-intensity exercise.

Repeated bouts of ischemia followed by reperfusion, commonly known as ischemic preconditioning (IPC), delays cardiac cell injury (33) and protects against myocardial damage (12). Studies have also reported that IPC of the upper limbs prevents damage to the endothelium after exposure of the same limb to prolonged ischemia (23). Moreover, even when IPC is performed on the contralateral upper limb [i.e., remote IPC (RIPC)], brachial artery endothelial dysfunction is prevented after prolonged ischemia (22, 28). Whether RIPC can also prevent the acute decrease in flow-mediated dilation (FMD) after strenuous, high-intensity exercise is currently unknown. This is of particular importance since previous studies have reported a beneficial effect of IPC on swimming (19) and cycling (7, 11) exercise performance. Therefore, the aim of the present study was to examine the effect of RIPC on brachial artery endothelial function (measured as FMD) after strenuous running in healthy volunteers. We hypothesized that preexercise RIPC would prevent the decrease in endothelium-dependent function typically observed in the brachial artery after strenuous lower-limb exercise.

METHODS

Participants

In a randomized, single-blind, crossover study, 13 healthy moderately trained men (25 ± 6 yr; 176 ± 4 cm; 77 ± 6.9 kg) volunteered to participate. Participants were recreationally active, measured via a self-report questionnaire, and typically engaged in low (e.g., walking) and moderate (e.g., running, stationary cycling) intensity aerobic activities (2 to 3 days/wk). We excluded subjects that performed >10 h exercise per wk. Based on their medical history, participants did not have any medical problems and were free of medication. Before testing, all participants were informed of the methods of the study, but remained naive to the study rationale. All participants provided providing written informed consent before participation. The study was approved by the Liverpool John Moores University Institutional Ethics Committee and adhered to the Declaration of Helsinki (2000).

Experimental Design

All participants refrained from exercise, alcohol, caffeine, and additional nutritional training supplements 24 h before all exercise

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testing. Participants reported twice to our laboratory to perform the same testing procedure, which was preceded by four cycles of 5-min bilateral cuff inflation of the lower limbs to 220 mmHg (i.e., RIPC) or to 20 mmHg (i.e., sham). Participants performed a graded maximal running test on a treadmill until voluntary exhaustion. A 45-min supine rest period then ensued. Participants then performed a 5-km running time trial (TT) on a treadmill (after being familiarized with this TT 3 to 4 times before performance of this test). Based on a possible relationship between the magnitude of exercise intensity and decline in brachial artery endothelial function (9, 20), we included two exercise sessions to provide a strenuous exercise stimulus to result in an acute decline in brachial artery endothelial function. Brachial artery endothelial function was examined before and after the RIPC- or sham-intervention as well as immediately after the 5-km TT (Fig. 1). The order of testing (IPC vs. sham) was randomized and counterbalanced between participants. Both tests were performed separated by 5–7 days to prevent a possible carryover effect of the acute exercise bout (39) or IPC (28).

Experimental Measures

Remote ischemic preconditioning. RIPC was performed in the supine position using bilateral arterial occlusion, in line with the methods of de Groot et al. (11). The automated occlusion cuffs were placed proximally around the upper thigh and inflated to 220 mmHg to block the arterial inflow for 5 min. This ischemic procedure was repeated four times bilaterally, with each ischemic episode separated by 5 min of rest. The sham intervention followed an identical protocol except the cuffs were inflated to 20 mmHg.

Strenuous running exercise. First, participants performed a maximal running test, starting with five stages of 3 min at 10–14 km/h running speed. These submaximal stages were followed by 2-min stages until volitional exhaustion (increased by 1 $km \cdot h^{-1} \cdot 2\ min^{-1}$ to a maximal running speed of 16 km/h, followed by an incline of the treadmill of 2% per 2 min). Breath-by-breath expired gases were continuously monitored and averaged over the last 15 s upon maximal exhaustion (Oxycon IV, Jaeger, Germany). Heart rate was also continuously measured with a chest strap and monitor (RS800i, Polar, Finland), whereas ratings of perceived exertion (RPE) were measured using Borg's 6–20 scale. After a 45-min rest in the supine position after completion of the incremental maximal running test (standardized for all participants), a 5-km running TT was performed on a motorized treadmill (Pulsar 4.0, H/P Cosmos, Germany). Participants were instructed to run 5 km as quickly as possible, with running time and speed blinded from the participant. Throughout the TT, participants were allowed to alter the speed of the treadmill. The only information available to the participants during each TT was total distance covered (in m). No further information or encouragement was provided. Heart rate was monitored continuously, with RPE measured at the end of each 1000-m stage. Before being tested, participants received at least three supervised familiarization tests on the same treadmill under comparable conditions as during the test. Data from the 5-km TT are reported elsewhere (1). These data were added in this paper as supportive data to provide detailed insight of the exercise bout.

Brachial artery endothelial function. Brachial artery endothelium-dependent function was measured using the FMD technique (44). For this purpose, participants were instructed to abstain from strenuous exercise, caffeine, and alcohol ingestion for 24 h before attending the laboratory. They were also asked to fast for 4 h before each visit. Measurements were performed in the supine position. Baseline assessment was performed after resting for 20 min, followed by assessment of heart rate and blood pressure using an automated sphygmomanometer (GE Pro 300V2, Dinamap, Tampa, FL). This was followed by assessment of brachial artery diameter and velocity. This procedure was repeated immediately after the RIPC or sham intervention as well as immediately after completion of the 5-km TT. The postexercise assessment of the brachial artery FMD was consistently collected within 5-min after cessation of the 5-km TT.

To examine brachial artery FMD, the arm was extended and positioned at an angle of ~80° from the torso. A rapid inflation and deflation pneumatic cuff (D. E. Hokanson, Bellevue, WA) was positioned on the forearm, immediately distal to the olecranon process to provide a stimulus to forearm ischemia. A 10-MHz multi-frequency 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. 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 the FMD. Continuous Doppler velocity assessments were also obtained using the ultrasound and were collected using the lowest possible isonation angle (always <60°). Following baseline assessments, the forearm cuff was 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 (3, 49).

Brachial Artery Diameter and Blood Flow Analysis

Analysis of brachial artery diameter was performed using custom-designed edge-detection and wall-tracking software, which is largely independent of investigator bias. Recent articles contain detailed descriptions of our analytical approach (3, 49). From synchronized diameter and velocity data, blood flow (the product of lumen cross-sectional area and Doppler velocity) were 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, significantly reduces observer error, and possesses an intraobserver coefficient of variation of 6.7% (49).

Statistics

Statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, IL) software. Our primary outcome was FMD. We deemed it important to control for the influence of moderators of FMD (shear rate and baseline diameter) in the event of these moderators being also influenced by exercise. We therefore analyzed the effects of trial and time on logarithmically transformed diameter changes using a generalized estimating equation that incorporated baseline diameter and shear rate as covariates. As variability in baseline diameter (46) and shear rate (45) (although to a lesser extent) may influence the magnitude of the FMD response, these parameters were included in the statistical analysis as covariates to correct for potential impact following exercise. The resulting mean differences between conditions and time (pre-/postintervention and post-5-km TT) were back transformed to provide an estimate of covariate-controlled FMD (in %).

Fig. 1. Protocol of the study. Rest, supine position; RIPC, remote ischemic preconditioning (220 mmHg) 4 × 5-min bilateral cuff occlusion; sham, reduced cuff pressure of 20 mmHg; FMD, flow-mediated dilation of the brachial artery; running test, incremental maximal running test; 5-km TT, running treadmill time trial (blinded for speed and duration).

Mean and 95% confidence intervals for the effect magnitudes of brachial artery FMD are cited.

A Students' paired *t*-test was used to analyze any differences in maximal values at the cessation of the incremental exercise test and 5-km TT performance (time to completion). Additionally, a two-factor general linear method (trial × time) was used to analyze any differences in running speed, heart rate, and RPE throughout the 5-km TT. We examined the potential presence of a trial order influence by examining the interaction effect between the order of testing (as between subjects factor) and the condition effect (as within subjects factor). All exercise data are reported as mean ± 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 (37, 41).

RESULTS

Exercise Protocol

All participants completed both exercise protocols. We found negligible and nonsignificant differences between both days in maximal running exercise performance, maximal heart rate, and maximal oxygen consumption during the graded running test (Table 1). The 5-km TT was performed in 1,355 ± 146 and 1,321 ± 114 s following the sham intervention and RIPC, respectively ($P = 0.027$, Table 1). This corresponds with a 2.3 ± 3.6% increase in 5-km TT performance. We found no significant interaction effect between the order of testing and the 5-km TT performance ($P = 0.46$).

Brachial Artery Endothelial Function

Baseline diameter, shear rate area-under-the-curve (from cuff deflation to peak dilatation; SR_{AUC}), and time-to-peak diameter were all increased following the TT ($P < 0.05$). At preintervention, differences in FMD were negligible and nonsignificant between the sham [5.3% (4.5–6)] and RIPC [4.8 (3.6–5.9)] trials ($P > 0.05$). FMD changed by <0.6% immediately after both the RIPC and sham interventions ($P > 0.30$, Table 2). In the sham trial, FMD changed from 5.1% (4.4–5.9) post-sham to 3.7% (2.6–4.8) following the 5-km TT ($P = 0.02$), as expected. However, in the RIPC trial, FMD was similar post-IPC and post-5-km TT, with an FMD of 5.4 (4.4–6.4) and 5.7% (4.6–6.8) ($P = 0.60$; Fig. 2), respectively. This is reflected in a significant interaction ($P = 0.010$) between the RIPC and sham trials, whereas IPC had no effect on the change in baseline or peak diameter, time-to-peak diameter, and SR_{AUC} (Table 2).

DISCUSSION

This study has resulted in several important findings. We found that strenuous running exercise is associated with a decrease in brachial artery endothelial function (FMD) by ~1.4%, confirming most of the recent observational data in this field (9, 16, 21, 27). Therefore, using an acute bout of strenuous exercise is a real-life model, we found that IPC of the lower limbs before strenuous running is practically important, since it prevents the exercise-induced decrease in FMD. These findings reveal, for the first time, that the reduction in endothelial function after strenuous exercise can be prevented by a noninvasive and easy applicable intervention in humans.

An important finding of this study is that brachial artery endothelium-dependent function is reduced immediately after strenuous running exercise in healthy, moderately trained individuals. This finding is in agreement with recent data (9, 21) which found that the acute impairment in brachial artery endothelial function is dependent on exercise intensity. These observations suggest that systemic reductions in endothelial function are present immediately after strenuous exercise. A potential explanation for the decrease in endothelium-dependent function may relate to the large and/or sustained increases in shear during the exercise bout that have resulted in an impaired nitric oxide (NO) biosynthesis because of depletion of L-arginine (10). Also, high-intensity exercise may impair endothelium-dependent vasodilation because of an increase in reactive oxygen species, resulting in a reduction in NO bioavailability (2, 8). Finally, intense exercise may also activate inflammation markers that may contribute to changes in endothelial function and NO bioavailability (16). Alternatively, it may be possible that the decrease in FMD relates to repair and potentially training adaptation mechanisms. Recurring periods of exercise-induced endothelial dysfunction may represent a beneficial stimulus that contributes to longer-term improvement of the endothelial function, commonly referred to as "hormesis" (35). In contrast, a previous well-controlled study

Table 1. *Exercise performance parameters during the graded running test and the 5-km TT in healthy volunteers*

	Sham	IPC	*P* Value
Maximal running test			
Maximal running speed, km/h	16 ± 2	16 ± 2	0.957
Maximal heart rate, beats/min	195 ± 4	195 ± 6	0.742
Maximal O_2 uptake, ml $O_2 \cdot kg^{-1} \cdot min^{-1}$	51.5 ± 4.3	52.1 ± 4.5	0.258
RER	1.18 ± 0.03	1.19 ± 0.04	0.613
Postexercise RPE	19 ± 1	19 ± 1	1.000
5-km TT			
Time to completion, s	1,355 ± 146	1,321 ± 114	IPC: 0.027
Heart rate, beats/min	175 ± 6	177 ± 7	Time: <0.001 IPC: 0.125 Time·IPC: 0.276
RPE	16 ± 2	16 ± 2	Time: <0.001 IPC: 0.136 Time·IPC: 0.091

Values are means ± SD; $n = 13$ healthy volunteers. IPC, ischemic preconditioning; RER, respiratory exchange ratio; RPE, rate of perceived exhaustion. *P* value refers to a paired Student's *t*-test. 5-km time trial (TT) variable *P* values refer to 2-way repeated-measures general linear method (data collected every 1 km).

Table 2. *Brachial artery FMD before and after the RIPC or sham intervention as well as after the 5-km TT in healthy volunteers*

	Intervention			
	Pre	Post	Post-5-km TT	*P* values
D_{rest}, mm				
Sham	0.41 (0.38–0.44)	0.41 (0.39–0.43)	0.44 (0.41–0.47)*	Time: <0.001
IPC	0.41 (0.39–0.44)	0.41 (0.38–0.43)	0.44 (0.41–0.47)*	IPC: 0.688
				Time·RIPC: 0.607
FMD corrected, %				
Sham	5.2 (4.5–6.0)	5.1 (4.4–5.9)	3.7 (2.6–4.8)*	Time: 0.093
RIPC	4.8 (3.6–5.9)	5.3 (4.4–-6.)	5.7 (4.6–6.8)#	IPC: 0.094
				Time·RIPC: 0.010
FMD, %				
Sham	5.4 (3.7–6.2)	5.2 (4.5–5.9)	3.5 (2.3–4.7)	Time: 0.110
RIPC	5.0 (3.9–6.0)	5.4 (4.6–6.3)	5.5 (4.5–6.6)	RIPC: 0.018
				Time·RIPC: 0.001
SR_{AUC}				
Sham	15,725 (12,440–19,009)	16,260 (12,771–19,749)	32,756 (24,546–40,964)*	Time: <0.001
IPC	11,428 (7,778–15,078)	13,803 (11,533–16,072)	26,801 (20,517–33,084)#	IPC: 0.009
				Time·RIPC: 0.262

Values are presented as means (95% confidence intervals); $n = 11$ healthy volunteers; 1 subject was missing because of technical problems. Flow-mediated dilation (FMD) values are presented classically (FMD in %change from baseline) and as the corrected values (FMD corrected) for changes in diameter and shear rate area under the curve (SR_{AUC}). Pre, before intervention; Post, after intervention; D_{rest}, diameter at rest; RIPC, remote IPC. *P* values refer to a generalized estimating equation (effect of time, IPC, and time·IPC). Post hoc significantly at $P < 0.05$ different from *preintervention or #sham.

from Goto et al. (15) found that exercise training performed at moderate intensity, but not at high-intensity level, was associated with improvements in vasodilator function. Taken together, it is currently not known whether the acute decrease in endothelial function after strenuous exercise prevents a potentially harmful or beneficial stimulus and should require further research.

A unique and novel finding in our study is that we revealed that the decrease in endothelium-dependent function was abolished when exercise was preceded by IPC. Several previous studies have established that RIPC can prevent endothelial injury of the brachial artery after prolonged periods of ischemia (23, 28, 30) and cardiac damage in clinical groups, as evidenced by smaller increments in ischemic markers (e.g., troponins) and infarct size (4, 5). In line with these observations, we add the novel observation that IPC protects against the acute decrease in endothelium-dependent function typically observed after strenuous exercise in healthy volunteers.

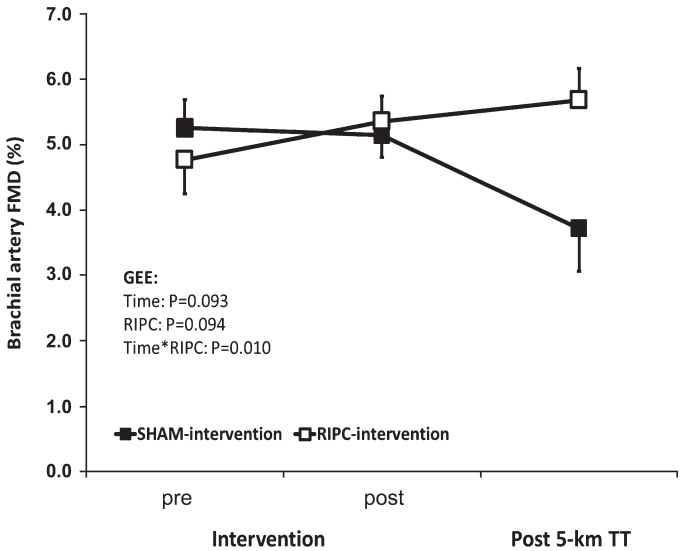

Fig. 2. Brachial artery FMD before (pre) and after (post) the intervention (RIPC or sham) as well as post-5-km TT in healthy volunteers ($n = 11$, 2 participants were not included because of technical problems) during the sham (black squares) and IPC (white squares) interventions. Error bars represent 95% confidence interval. Data from the generalized estimated equation (GEE) were included in the figure, meaning that FMD values are presented, which are statistically adjusted for changes in diameter and shear rate. *Post hoc pairwise comparison analysis represents a significant decrease in FMD between pre- and post-5-km TT in the sham trial ($P = 0.02$), whereas FMD was unchanged after RIPC between pre- and post-5-km TT ($P = 0.60$).

Our findings raise questions about the potential mechanisms that contribute to the effects of RIPC. While the mechanisms for RIPC are still not completely clear (12), our finding that IPC applied to the legs can prevent the decrease in brachial artery endothelial function indicates that the effects are systemic rather than localized. A potential explanation relates to the vascular effects of RIPC in the peripheral and coronary circulation. For example, studies in humans (51) and animals (42) demonstrated that IPC of the limbs increased coronary blood velocity. Also in peripheral arteries, it is demonstrated that IPC is associated with vasodilatation of the contralateral brachial artery (13). Therefore, these strong vascular effects of IPC on distant vessel beds may contribute to our findings. In addition, the protective effects of RIPC against endothelial ischemia-reperfusion injury were attenuated during autonomic ganglion blockade (14, 28), whereas RIPC is associated with enhancement of parasympathetic activity (13). The association between RIPC and the autonomic nerve system is of particular importance since exercise is associated with sympathetic activation and inhibition of parasympathetic activity, especially after strenuous exercise (25). Since activation of the sympathetic nervous system relates to a decrease in brachial artery endothelium-dependent function (18), the decrease in FMD after strenuous exercise may relate to activation of the sympathetic nerve system (47). Future studies should further examine whether our findings can be explained through an effect of RIPC on the sympathetic nervous system.

Another mechanism for the protective effects of RIPC in our study relates to the inflammatory response that may be responsible for the decline in FMD immediately after strenuous exercise (16). Previous studies in humans have demonstrated that an IPC stimulus on the forearm induces leukocyte inflammation gene expression (24), attenuates systemic neutrophil activation (23), and alters neutrophil function (43). These changes in inflammation induced by IPC may protect the endothelium against a decrease in vascular function after strenuous exercise. Alternatively, protection against endothelium-dependent dysfunction after exercise training (15) and acute exercise (20) via IPC may also be mediated through lower levels of oxidative stress during strenuous exercise. Previous studies in animals provided evidence that IPC is able to diminish increased levels of oxidative stress after a period of ischemia (6, 31). The protection against oxidative stress through IPC may be mediated through improved antioxidative defense mechanisms (e.g., increased superoxide dismutase activity and glutathione peroxidase) and/or lower generation of oxidative stress (e.g., decreased xanthine oxidase activity) (31, 50). Finally, (R)IPC has well-established effects on the local release of adenosine, which activates neurogenic pathways leading to systemic adenosine-receptor activation (26). The effect of RIPC on vasodilators, adenosine and bradykinin, may also contribute to the effects of IPC via protection against cellular damage (38). Despite the absence of a clear mechanistic explanation at this stage, our study is the first to demonstrate in vivo that exercise-associated reduction in endothelium-dependent dilation can be prevented by RIPC.

Another observation in our study was that RIPC before exercise was associated with a performance increase of 2.3% in 5-km run time. This finding is in line with recent studies that found improved cycling or swimming exercise performance when exercise was preceded by IPC (7, 11, 19). Endothelial function is believed to contribute to blood flow regulation, such as during exercise. Therefore, a decrease in endothelial function may impair blood supply during (strenuous) exercise. Although speculative, prevention of endothelial dysfunction by RIPC may contribute to the effect of IPC on exercise performance.

Limitations

A potential limitation of this study is that we performed a single postexercise FMD assessment only, especially since previous studies revealed a time-dependent decrease in FMD after exercise (9, 39). However, these studies revealed that the largest decrease in FMD was present immediately after exercise, followed by a rapid normalization of the FMD (9). As IPC prevented the decrease in FMD immediately after exercise, we believe it unlikely that repeated measurements after exercise would have changed the main findings of our study. Another limitation relates to the performance of an incremental running test preceding the 5-km TT. The reason for including this test is, based on the relation between exercise intensity and decline in FMD, to cause an exercise-induced decrease in FMD. The impact of this exercise bout on the 5-km performance is unclear and may have impacted changes in diameter and shear rate. Moreover, a recent study by Michelsen et al. (32) provided evidence that short bouts of high-intensity exercise (4 × 2 min) elicits a preconditioning effect through a humorally mediated pathway, similar to RIPC. Such a preconditioning effect of high-intensity exercise may have impacted our results. However, the type and duration of exercise performed in Michelsen's study is different to our study. Moreover, subjects in our study performed the same exercise protocol on both testing days and, therefore, received the same exercise (preconditioning) stimulus twice (including on the control day that was associated with a decrease in FMD after exercise). Taken together, the incremental exercise has unlikely impacted on the ability of IPC to prevent the decrease in brachial artery endothelial function.

In conclusion, we found that a strenuous running bout is associated with a decrease in brachial artery endothelium-dependent function, which is prevented by RIPC of the lower limbs before strenuous running exercise. These findings support the potential of IPC to prevent the decrease in endothelium-dependent function induced by strenuous exercise in humans in vivo.

GRANTS

D. H. J. Thijssen received funding for this study from UK SPORT (Ideas for Innovation Garage Innovators Award) and is recipient of the E. Dekker stipend (Netherlands Heart Foundation, 2009T064). D. J. Green received research funding support from the National Heart Foundation of Australia and the Australian Research Council.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

T.G.B. and G.K.B. performed experiments; T.G.B., G.A., and D.H.T. analyzed data; T.G.B., G.A., D.J.G., H.J., and D.H.T. interpreted results of experiments; T.G.B. prepared figures; T.G.B., H.J., and D.H.T. drafted manuscript; T.G.B., G.K.B., N.T.C., G.A., D.J.G., H.J., and D.H.T. edited and revised manuscript; T.G.B., G.K.B., N.T.C., G.A., D.J.G., H.J., and D.H.T. approved final version of manuscript; N.T.C., D.J.G., H.J., and D.H.T. conception and design of research.

REFERENCES

1. **Bailey T, Jones H, Gregson W, Atkinson G, Cable NT, Thijssen DH.** Effect of ischemic preconditioning on lactate accumulation and running performance. *Med Sci Sports Excer.* doi:10.1249/MSS.0b013e318262cb17.
2. **Bergholm R, Makimattila S, Valkonen M, Liu ML, Lahdenpera S, Taskinen MR, Sovijarvi A, Malmberg P, Yki-Jarvinen H.** Intense physical training decreases circulating antioxidants and endothelium-dependent vasodilatation in vivo. *Atherosclerosis* 145: 341–349, 1999.
3. **Black MA, Cable NT, Thijssen DH, Green DJ.** Importance of measuring the time course of flow-mediated dilatation in humans. *Hypertension* 51: 203–210, 2008.
4. **Botker HE, Kharbanda R, Schmidt MR, Bottcher M, Kaltoft AK, Terkelsen CJ, Munk K, Andersen NH, Hansen TM, Trautner S, Lassen JF, Christiansen EH, Krusell LR, Kristensen SD, Thuesen L, Nielsen SS, Rehling M, Sorensen HT, Redington AN, Nielsen TT.** Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial. *Lancet* 375: 727–734, 2010.
5. **Cheung MM, Kharbanda RK, Konstantinov IE, Shimizu M, Frndova H, Li J, Holtby HM, Cox PN, Smallhorn JF, Van Arsdell GS, Redington AN.** Randomized controlled trial of the effects of remote ischemic preconditioning on children undergoing cardiac surgery: first clinical application in humans. *J Am Coll Cardiol* 47: 2277–2282, 2006.
6. **Crestanello JA, Lingle DM, Kamelgard J, Millili J, Whitman GJ.** Ischemic preconditioning decreases oxidative stress during reperfusion: a chemiluminescence study. *J Surg Res* 65: 53–58, 1996.
7. **Crisafulli A, Tangianu F, Tocco F, Concu A, Mameli O, Mulliri G, Caria MA.** Ischemic preconditioning of the muscle improves maximal exercise performance but not maximal oxygen uptake in humans. *J Appl Physiol* 111: 530–536, 2011.
8. **Davies KJ, Quintanilha AT, Brooks GA, Packer L.** Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Commun* 107: 1198–1205, 1982.

9. **Dawson EA, Birk GK, Cable NT, Thijssen DH, Green DJ.** OP-PM04 Health: Effect of acute exercise intensity on brachial artery endothelial function in humans. *Oral Presentation*, 16th annual Congress of the ECSS, Liverpool, UK, 06 Jul 2011–09 Jul: 20, 2011.
10. **Dawson EA, Whyte GP, Black MA, Jones H, Hopkins ND, Oxborough D, Gaze D, Shave RE, Wilson M, George KP, Green DJ.** Changes in vascular and cardiac function after prolonged strenuous exercise in humans. *J Appl Physiol* 105: 1562–1568, 2008.
11. **de Groot PC, Thijssen DH, Sanchez M, Ellenkamp R, Hopman MT.** Ischemic preconditioning improves maximal performance in humans. *Eur J Appl Physiol* 108: 141–146, 2010.
12. **Eisen A, Fisman EZ, Rubenfire M, Freimark D, McKechnie R, Tenenbaum A, Motro M, Adler Y.** Ischemic preconditioning: nearly two decades of research. A comprehensive review. *Atherosclerosis* 172: 201–210, 2004.
13. **Enko K, Nakamura K, Yunoki K, Miyoshi T, Akagi S, Yoshida M, Toh N, Sangawa M, Nishii N, Nagase S, Kohno K, Morita H, Kusano KF, Ito H.** Intermittent arm ischemia induces vasodilatation of the contralateral upper limb. *J Physiol Sci* 61: 507–513, 2011.
14. **Gho CG, Schoemaker RG, van den Doel MA, Duncker DJ, Verdouw PD.** Myocardial protection by brief ischemia in noncardiac tissue. *Circulation* 94: 2193–2200, 1996.
15. **Goto C, Higashi Y, Kimura M, Noma K, Hara K, Nakagawa K, Kawamura M, Chayama K, Yoshizumi M, Nara I.** Effect of different intensities of exercise on endothelium-dependent vasodilation in humans: role of endothelium-dependent nitric oxide and oxidative stress. *Circulation* 108: 530–535, 2003.
16. **Harris RA, Padilla J, Hanlon KP, Rink LD, Wallace JP.** The flow-mediated dilation response to acute exercise in overweight active and inactive men. *Obesity (Silver Spring)* 16: 578–584, 2008.
18. **Hijmering ML, Stroes ES, Olijhoek J, Hutten BA, Blankestijn PJ, Rabelink TJ.** Sympathetic activation markedly reduces endothelium-dependent, flow-mediated vasodilation. *J Am Coll Cardiol* 39: 683–688, 2002.
19. **Jean-St-Michel E, Manlhiot C, Li J, Tropak M, Michelsen MM, Schmidt MR, McCrindle BW, Wells GD, Redington AN.** Remote preconditioning improves maximal performance in highly trained athletes. *Med Sci Sports Exerc* 43: 1280–1286, 2011.
20. **Johnson BD, Padilla J, Wallace JP.** The exercise dose affects oxidative stress and brachial artery flow-mediated dilation in trained men. *Eur J Appl Physiol* 122: 33–42, 2012.
21. **Johnson BD, Wallace JP.** A comparison of postexercise shear rate patterns following different intensities and durations of running in healthy men. *Clin Physiol Funct Imaging* 32: 234–240, 2012.
22. **Kharbanda RK, Mortensen UM, White PA, Kristiansen SB, Schmidt MR, Hoschtitzky JA, Vogel M, Sorensen K, Redington AN, MacAllister R.** Transient limb ischemia induces remote ischemic preconditioning in vivo. *Circulation* 106: 2881–2883, 2002.
23. **Kharbanda RK, Peters M, Walton B, Kattenhorn M, Mullen M, Klein N, Vallance P, Deanfield J, MacAllister R.** Ischemic preconditioning prevents endothelial injury and systemic neutrophil activation during ischemia-reperfusion in humans in vivo. *Circulation* 103: 1624–1630, 2001.
24. **Konstantinov IE, Arab S, Kharbanda RK, Li J, Cheung MM, Cherepanov V, Downey GP, Liu PP, Cukerman E, Coles JG, Redington AN.** The remote ischemic preconditioning stimulus modifies inflammatory gene expression in humans. *Physiol Genomics* 19: 143–150, 2004.
25. **Leuenberger U, Sinoway L, Gubin S, Gaul L, Davis D, Zelis R.** Effects of exercise intensity and duration on norepinephrine spillover and clearance in humans. *J Appl Physiol* 75: 668–674, 1993.
26. **Liem DA, Verdouw PD, Ploeg H, Kazim S, Duncker DJ.** Sites of action of adenosine in interorgan preconditioning of the heart. *Am J Physiol Heart Circ Physiol* 283: H29–H37, 2002.
27. **Llewellyn TL, Chaffin ME, Berg KE, Meendering JR.** The relationship between shear rate and flow-mediated dilation is altered by acute exercise. *Acta Physiol (Oxf)* 205: 394–402, 2012.
28. **Loukogeorgakis SP, Panagiotidou AT, Broadhead MW, Donald A, Deanfield JE, MacAllister RJ.** Remote ischemic preconditioning provides early and late protection against endothelial ischemia-reperfusion injury in humans: role of the autonomic nervous system. *J Am Coll Cardiol* 46: 450–456, 2005.
30. **Loukogeorgakis SP, Williams R, Panagiotidou AT, Kolvekar SK, Donald A, Cole TJ, Yellon DM, Deanfield JE, MacAllister RJ.** Transient limb ischemia induces remote preconditioning and remote postconditioning in humans by a K_{ATP}-channel dependent mechanism. *Circulation* 116: 1386–1395, 2007.
31. **Maczewski M, Duda M, Pawlak W, Beresewicz A.** Endothelial protection from reperfusion injury by ischemic preconditioning and diazoxide involves a SOD-like anti-O_2- mechanism. *J Physiol Pharmacol* 55: 537–550, 2004.
32. **Michelsen MM, Stottrup NB, Schmidt MR, Lofgren B, Jensen RV, Tropak M, St-Michel EJ, Redington AN, Botker HE.** Exercise-induced cardioprotection is mediated by a bloodborne, transferable factor. *Basic Res Cardiol* 107: 1–9, 2012.
33. **Murry CE, Jennings RB, Reimer KA.** Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 74: 1124–1136, 1986.
34. **Padilla J, Harris RA, Fly AD, Rink LD, Wallace JP.** The effect of acute exercise on endothelial function following a high-fat meal. *Eur J Appl Physiol* 98: 256–262, 2006.
35. **Padilla J, Simmons GH, Bender SB, Arce-Esquivel AA, Whyte JJ, Laughlin MH.** Vascular effects of exercise: endothelial adaptations beyond active muscle beds. *Physiology* 26: 132–145, 2011.
37. **Perneger TV.** What's wrong with Bonferroni adjustments? *Br Med J* 316: 1236–1238, 1998.
38. **Riksen NP, Smits P, Rongen GA.** Ischaemic preconditioning: from molecular characterisation to clinical application—part 1. *Neth J Med* 62: 353–363, 2004.
39. **Rognmo O, Bjornstad TH, Kahrs C, Tjonna AE, Bye A, Haram PM, Stolen T, Slordahl SA, Wisloff U.** Endothelial function in highly endurance-trained men: effects of acute exercise. *J Strength Cond Res* 22: 535–542, 2008.
41. **Rothman KJ.** No adjustments are needed for multiple comparisons. *Epidemiology* 1: 43–46, 1990.
42. **Shimizu M, Konstantinov IE, Kharbanda RK, Cheung MH, Redington AN.** Effects of intermittent lower limb ischaemia on coronary blood flow and coronary resistance in pigs. *Acta Physiol (Oxf)* 190: 103–109, 2007.
43. **Shimizu M, Saxena P, Konstantinov IE, Cherepanov V, Cheung MM, Wearden P, Zhangdong H, Schmidt M, Downey GP, Redington AN.** Remote ischemic preconditioning decreases adhesion and selectively modifies functional responses of human neutrophils. *J Surg Res* 158: 155–161, 2010.
44. **Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B, Widlansky ME, Tschakovsky ME, Green DJ.** Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *Am J Physiol Heart Circ Physiol* 300: H2–H12, 2011.
45. **Thijssen DH, Bullens LM, van Bemmel MM, Dawson EA, Hopkins N, Tinken TM, Black MA, Hopman MT, Cable NT, Green DJ.** Does arterial shear explain the magnitude of flow-mediated dilation?: a comparison between young and older humans. *Am J Physiol Heart Circ Physiol* 296: H57–H64, 2009.
46. **Thijssen DH, Dawson EA, Black MA, Hopman MT, Cable NT, Green DJ.** Heterogeneity in conduit artery function in humans: impact of arterial size. *Am J Physiol Heart Circ Physiol* 295: H1927–H1934, 2008.
47. **Thijssen DH, de Groot P, Kooijman M, Smits P, Hopman MT.** Sympathetic nervous system contributes to the age-related impairment of flow-mediated dilation of the superficial femoral artery. *Am J Physiol Heart Circ Physiol* 291: H3122–H3129, 2006.
48. **Tinken TM, Thijssen DH, Hopkins N, Black MA, Dawson EA, Minson CT, Newcomer SC, Laughlin MH, Cable NT, Green DJ.** Impact of shear rate modulation on vascular function in humans. *Hypertension* 54: 278–285, 2009.
49. **Woodman RJ, Playford DA, Watts GF, Cheetham C, Reed C, Taylor RR, Puddey IB, Beilin LJ, Burke V, Mori TA, Green D.** Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *J Appl Physiol* 91: 929–937, 2001.
50. **Wu YN, Yu H, Zhu XH, Yuan HJ, Kang Y, Jiao JJ, Gao WZ, Liu YX, Lou JS.** Noninvasive delayed limb ischemic preconditioning attenuates myocardial ischemia-reperfusion injury in rats by a mitochondrial K_{ATP} channel-dependent mechanism. *Physiol Res* 60: 271–279, 2011.
51. **Zhou K, Yang B, Zhou XM, Tan CM, Zhao Y, Huang C, Liao XB, Xiao HB.** Effects of remote ischemic preconditioning on the flow pattern of the left anterior descending coronary artery in normal subjects. *Int J Cardiol* 122: 250–251, 2007.
52. **Zhu W, Zeng J, Yin J, Zhang F, Wu H, Yan S, Wang S.** Both flow-mediated vasodilation procedures and acute exercise improve endothelial function in obese young men. *Eur J Appl Physiol* 108: 727–732, 2010.