Effect of acute sympathetic activation on leg vasodilation before and after endurance exercise

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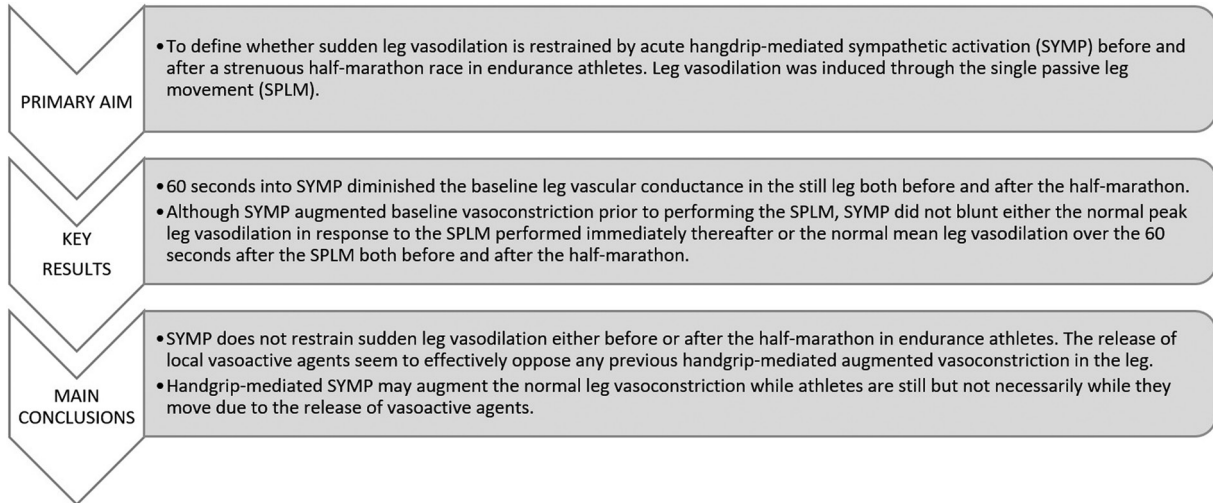[2]Department of Clinical and Biological Sciences, University of Turin, Turin, Italy
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Abstract

Vascular conductance (VC) regulation involves a continuous balance between metabolic vasodilation and sympathetic vasoconstriction. Endurance exercise challenges the sympathetic control on VC due to attenuated sympathetic receptor responsiveness and persistence of muscle vasodilation, especially in endurance athletes, predisposing them to blood pressure control dysfunctions. This study assessed whether acute handgrip-mediated sympathetic activation (SYMP) restrains sudden leg vasodilation before and after a half-marathon. Prior to, and within the 20 min following the race, 11 well-trained runners underwent two single passive leg movement (SPLM) tests to suddenly induce leg vasodilation, one without and the other during SYMP. Leg blood flow and mean arterial pressure were measured to assess changes in leg VC. Undertaking 60 sec of SYMP reduced the baseline leg VC both before (4.0 ± 1.0 vs. 3.3 ± 0.7 ml/min/mmHg; P=0.01; NO SYMP vs. SYMP, respectively) and after the race (4.6 ± 0.8 vs. 3.9 ± 0.8 ml/min/mmHg; P=0.01). However, SYMP did not reduce leg peak vasodilation immediately after the SPLM either before (11.5 ± 4.0 vs. 12.2 ± 3.8 ml/min/mmHg; P=0.35) or after the race (7.2 ± 2.0 vs. 7.3 ± 2.6 ml/min/mmHg; P=0.96). Furthermore, SYMP did not blunt the mean leg vasodilation over the 60 sec after the SPLM before (5.1 ± 1.7 vs. 5.9 ± 2.5 ml/min/mmHg; P=0.14) or after the race (4.8 ± 1.3 vs. 4.2 ± 1.5 ml/min/mmHg; P=0.26). This data suggest that the release of local vasoactive agents effectively opposes any preceding handgrip-mediated augmented vasoconstriction in endurance athletes before and after a half-marathon. Handgrip-mediated SYMP might improve normal vasoconstriction while athletes are still, but not necessarily while they move, as movements can induce a release of vasoactive molecules.

Key words: acute sympathetic activation, passive leg movement, endurance exercise, leg vasodilation, handgrip exercise

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- PRIMARY AIM
  - To define whether sudden leg vasodilation is restrained by acute hangdrip-mediated sympathetic activation (SYMP) before and after a strenuous half-marathon race in endurance athletes. Leg vasodilation was induced through the single passive leg movement (SPLM).

- KEY RESULTS
  - 60 seconds into SYMP diminished the baseline leg vascular conductance in the still leg both before and after the half-marathon.
  - Although SYMP augmented baseline vasoconstriction prior to performing the SPLM, SYMP did not blunt either the normal peak leg vasodilation in response to the SPLM performed immediately thereafter or the normal mean leg vasodilation over the 60 seconds after the SPLM both before and after the half-marathon.

- MAIN CONCLUSIONS
  - SYMP does not restrain sudden leg vasodilation either before or after the half-marathon in endurance athletes. The release of local vasoactive agents seem to effectively oppose any previous handgrip-mediated augmented vasoconstriction in the leg.
  - Handgrip-mediated SYMP may augment the normal leg vasoconstriction while athletes are still but not necessarily while they move due to the release of vasoactive agents.

Highlights of the study.

Introduction

The *in-vivo* regulation of vascular conductance is a complex process involving a continuous balance between metabolic vasodilation and sympathetic-mediated vasoconstriction (1). While nitric oxide inhibits Ca^{2+} influx and decreases free $[Ca^{2+}]_i$, therefore relaxing vascular smooth muscle (2), acute sympathetic activation (SYMP) generally induces a release of norepinephrine from sympathetic nerve endings and adrenal glands, which binds to post-synaptic α-adrenergic receptors on vascular smooth muscle to increase vasoconstriction by promoting Ca^{2+} influx (3). Consistent with this notion, SYMP has been shown to blunt the normal nitric oxide-mediated vasodilation of the upper limb after a brief period of ischemia to a different extent according to the sympathetic stressor employed (4–6).

Sympathetic vasoconstriction of the lower limb is particularly relevant among endurance athletes but can be countered by acute physical exercise. During endurance exercise, the vascular conductance of exercising limbs increases to augment blood flow in order to meet muscle oxygen demands (7, 8). This vascular conductance increase can be considerably large during large muscle mass exercise in athletes. Indeed, skeletal muscle vasculature dilation may exceed the ability of the heart to increase the cardiac output to maintain arterial blood pressure in athletes (9). Although its effect is weakened due to sympatholysis, sympathetic vasoconstriction occurs in exercising muscles (1, 10, 11) to limit the extent of local vasodilation. Sympathetic vasoconstriction also occurs in non-exercising tissues to maintain systemic arterial blood pressure and shunt blood towards working muscles (1). There are numerous factors that blunt sympathetic vasoconstriction during exercise (11). However, the high bioavailability of metabolites such as potassium, hydrogen ions, and adenosine resulting from high intensity exercises has been proposed to locally blunt post-synaptic sympathetic receptor responsiveness (12, 13). It has been shown that this functional reduction of sympathetic vasoconstriction may persist for some time after exercise (14). It has also been shown that dynamic exercise impairs the normal transduction of sympathetic activity into vasoconstriction leading to post-exercise hypotension (15). These effects seem to be greater in endurance athletes since they show greater vasodilation capacity (9, 16) along with lower central circulatory response and decreased effects on peripheral resistance in response to sympathetic stressors (17). Such outcomes predispose endurance athletes to dysfunction in blood pressure control. Indeed, it has been shown that the cardiac stroke volume is diminished post-exercise compared to pre-exercise in response to head-up tilt test

in endurance athletes, but not in sedentary subjects (18), suggesting an impaired venous return to the heart. This is likely due to weakened lower limb vasoconstriction after exercise.

To date, the effect of concurrent SYMP on sudden leg vasodilation before or after endurance exercise has not been investigated. If acute SYMP is successful in restraining leg vasodilation in endurance athletes, this strategy may improve the ordinary blood pressure control in a population particularly in need of it. The first objective of this study was to assess whether leg vasodilation is blunted during concurrent SYMP before and after a strenuous half-marathon in well-trained endurance runners. This type of race was chosen because it combines high intensity and long-lasting endurance exercise that may induce post-exercise hypotension (19). Additionally, within the last few years the half-marathon has gained the most popularity, in terms of number of participants, out of all long distance races (20). This has led to a large scientific interest in investigating the effect of half-marathons on health and on the physiological factors affecting athletic performance. The second objective of this study was to assess whether any effect of SYMP on leg vasodilation is more blunted after the race than before.

Methods

Participants

We tested 11 experienced half-marathon runners (9 males and 2 females; age: 43.3 ± 7.4 years old) both prior to and within the 20 min following a half-marathon race. Runners met inclusion (absence of any muscle-skeletal, metabolic, cardiovascular, and respiratory disease; between 18 and 55 years of age; took part in at least 240 min of running activity weekly for more than 1 year) and exclusion (BMI ≥ 28 kg/m^2; diabetes mellitus; hypertensive disorders; use of any drug that alters the cardiovascular response to exercise) criteria. An additional inclusion criterion was to have completed at least 3 half-marathon races under competitive conditions to ensure that the race was run at maximum effort. In addition, 7 sedentary individuals (4 males and 3 females; age: 36.8 ± 5.1 years old) were also tested before the race only. They met the previous inclusion and exclusion criteria except that they were physically inactive. The main judgement criterion was the sympathetic blunting of vasodilation among endurance runners. The number of subjects was calculated with an a priori power analysis (GPower 3.1.9.7; Universität Düsseldorf, Germany) for an F test (ANOVA, repeated measures, within-factors), partial eta squared of 0.20, statistical power $(1-\beta)$ of 0.80, level of significance of 0.05, which suggested a total of 11 runners to assess the sympathetic-mediated blunting of vasodilation. The effect size was calculated by assessing the magnitude of sympathetic blunting of the brachial artery vasodilation (4) since no data are available for the lower limb. Subjects were asked to abstain from alcoholic- and caffeine-rich beverages in the 48 h before the tests. This study was directed under the rules outlined in the Declaration of Helsinki. The Ethics Board of the Department of Neurosciences, Biomedicine, and Movement Sciences of the University of Verona approved all procedures involving human subjects (165038). Each participant provided written, informed consent before being involved in any test.

Experimental protocol

Runners completed a progressive incremental test (0.5 m/sec·min) to volitional exhaustion on a treadmill (Reax Run Reaxing, Milan, Italy) for the assessment of the maximal aerobic capacity one week before the race. They had to reach a plateau in their oxygen consumption kinetics, despite further workload increment, a respiratory quotient higher than 1.08, and a maximal theoretical heart rate (220–age) of higher than 90% to satisfy the assessment criteria. The heart rate, mechanical workload, and respiratory gas kinetics were collected with a metabolic cart (Quark CPET - Cosmed, Rome, Italy).

On race day, subjects were sitting with their right leg straight horizontally resting on a support. They were

fitted with an automatic blood pressure monitor (Tango+, SunTech Medical, Morrisville, NC, USA) at the level of their heart on their left arm as well as with a beat-by-beat finger blood pressure and heart rate monitoring system (Finapres Medical System BV, The Netherlands) on the third medial phalanx of their right hand. The beat-by-beat finger blood pressure system was calibrated with the automatic sphygmomanometer recording their brachial blood pressure. Data were synchronized throughout the experiment by the use of markers. While runners were asked to stay relaxed as much as they could and breathe normally, an expert operator began scanning the right common femoral artery using pulsed Doppler ultrasonography (LOGIQ S7 pro, GE, Milwaukee, WI, USA) to simultaneously detect mean blood velocity and measure the femoral artery diameter. The probe location was marked to assess the same section of the artery pre- and post-half-marathon. Data were acquired using a 4.4 MHz probe with a 60° angle of insonation, with the ultrasound gate adjusted to cover the width of the artery, and the sample volume aligned and adjusted according to vessel size as indicated by the recommendations for the assessment of flow-mediated dilation in humans (21).

After 5 min of baseline recording, an operator completed a single passive leg movement (SPLM) maneuver on the right leg as previously described (22). Briefly, an operator bent the leg 90 degrees to the knee joint and relocated it straight back on the support in approximately 1 sec. All data were collected for 60 sec after this maneuver. After 5 min of resting recovery with the leg straight horizontally on the support, participants started a static overhead handgrip exercise with their left hand at 30% of maximum voluntary contraction (MVC) (23) to acutely activate the sympathetic nervous system (SYMP), until conclusion of the experiment. Subjects were asked to keep their leg still and to raise their forearm about 50 cm above the heart to induce augmented SYMP as previously described (23). One minute into the handgrip exercise, another SPLM was performed. All data during concomitant SYMP were collected for the subsequent 60 sec. When all measurements were completed, the subjects released the handgrip knob.

This full procedure was repeated prior to and within 5 min after a competitive half-marathon race in endurance athletes and before the race only in sedentary individuals. Runners were instructed to attempt the race with maximum commitment. The rate of perceived exertion was assessed with the Borg CR100 scale upon reaching the finish line. Runners were allowed to drink water throughout the experiment. Athletes were weighed before and immediately after the race.

Data analysis

We calculated the leg vascular conductance by measuring the mean arterial pressure and femoral blood flow through the common femoral artery, since it branches off to feed the whole lower limb. Ultrasound (common femoral artery diameter, mean blood velocity) and Finapres (beat-by-beat mean arterial pressure and heart rate) data were analyzed beat-by-beat with a 3-beat rolling average and then fitted to a polynomial curve to prevent any artifacts from causing erroneous peaks in leg blood flow and vascular conductance kinetics, as well as to extrapolate second-by-second data. Ultrasound data were analyzed through the factory software installed within the Doppler ultrasound machine, which required the continuous detection of the artery edges along with the mean blood velocity calculation. Finapres data were exported through its proprietary software (BeatScope 1.1; Finapres Medical System BV, The Netherlands). Then, second-by-second leg blood flow was calculated as mean blood velocity $(cm \cdot s^{-1}) \cdot \pi \cdot r^2 \cdot 60$ $(ml \cdot min^{-1})$, where r is the radius of the common femoral artery. Finally, second-by-second leg vascular conductance was calculated as leg blood flow divided by mean arterial pressure.

We identified values of leg vascular conductance, leg blood flow, mean arterial pressure, and heart rate immediately before the SPLM, their peak values after the SPLM, and their mean values over the 60 sec after the SPLM. These were identified with and without acute SYMP, both before and after the race. Statistical

comparison was performed on the data collected in the absence and presence of SYMP as well as on the data collected before and after the half marathon race.

Statistics

Data normality was tested with the Shapiro-Wilk normality test. Two-way repeated-measure ANOVA with a Sidak post-hoc test was used to assess any effects of SYMP and the half-marathon race on the leg vascular conductance, leg blood flow, mean arterial pressure, and heart rate before and after the SPLM. Statistical significance was set at the 0.05 level of confidence. Two-way repeated-measure ANOVA with a Sidak post-hoc test was also used to assess any effects on vascular conductance in athletes compared to sedentary individuals pre-race only. The effect size to assess the magnitude of the effect of each condition was indicated with an F-index. GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) was used for statistical analysis and graphs. Results are expressed as the mean ± S.D.

Results

The preliminary aerobic power assessment revealed a V'O$_2$max of 58.65 ± 7.33 mlO$_2$ min^{-1} kg^{-1}. All participants (n=11) completed the race. Conclusion time was 100.55 ± 14.67 min. Runners' body weights were lower immediately after the race compared to before (69.3 ± 4.1 vs. 68.1 ± 5.9, P<0.01). The race day was cloudy with an average temperature of 15.1 °C and humidity between 65 and 83%. BORG CR100 score was 91.98 ± 8.22 arbitrary units, indicating the effort as 'extremely strong'. All data passed the normality test. Figure 1 shows the leg vascular conductance, leg blood flow and mean arterial pressure immediately after the SPLM, before and after the half-marathon race, with and without SYMP.

Baseline values in runners

As reported in Table 1 and Fig. 2, there was a reduction of the baseline leg vascular conductance after 1 min into SYMP while the leg was still, both before and after the race.

When comparing data after the race to the ones before, the baseline leg vascular conductance was higher, the leg blood flow and mean arterial pressure were similar, and the heart rate was increased. SYMP increased the mean arterial pressure and heart rate but did not change the leg blood flow. ANOVA revealed an effect of the race and SYMP on the leg vascular conductance (SYMP: P=0.01, F=9.7; race: P=0.0003, F=29.8), mean arterial pressure (SYMP: P<0.0001, F=109.2; race: P=0.02, F=6.4), and heart rate (SYMP: P<0.0001, F=101.1; race: P<0.0001, F=132.5). The race only affected the leg blood flow (SYMP: P=0.33, F=1.1; race: P=0.01, F=5.6). There was no effect of the half-marathon (P=0.75) or SYMP (P=0.87) on the common femoral artery diameter, which was similar at baseline before the race as afterward (0.907 ± 0.073 vs. 0.913 ± 0.069 cm; P=0.83; before vs. after the race) and did not vary over time after the SPLM (P=0.96).

Peak values after SPLM in runners

As shown in Fig. 1, the peak leg vascular conductance was reached approximately 5 sec after the SPLM. As reported in Table 1 and Fig. 3, the peak leg vascular conductance did not change during SYMP compared to without SYMP either before or after the race, but was blunted after the race compared to before, regardless of SYMP.

The peak leg blood flow was also blunted after the race compared to values prior to the race and augmented during SYMP regardless of the race. The mean arterial pressure at peak leg vasodilation did not change after
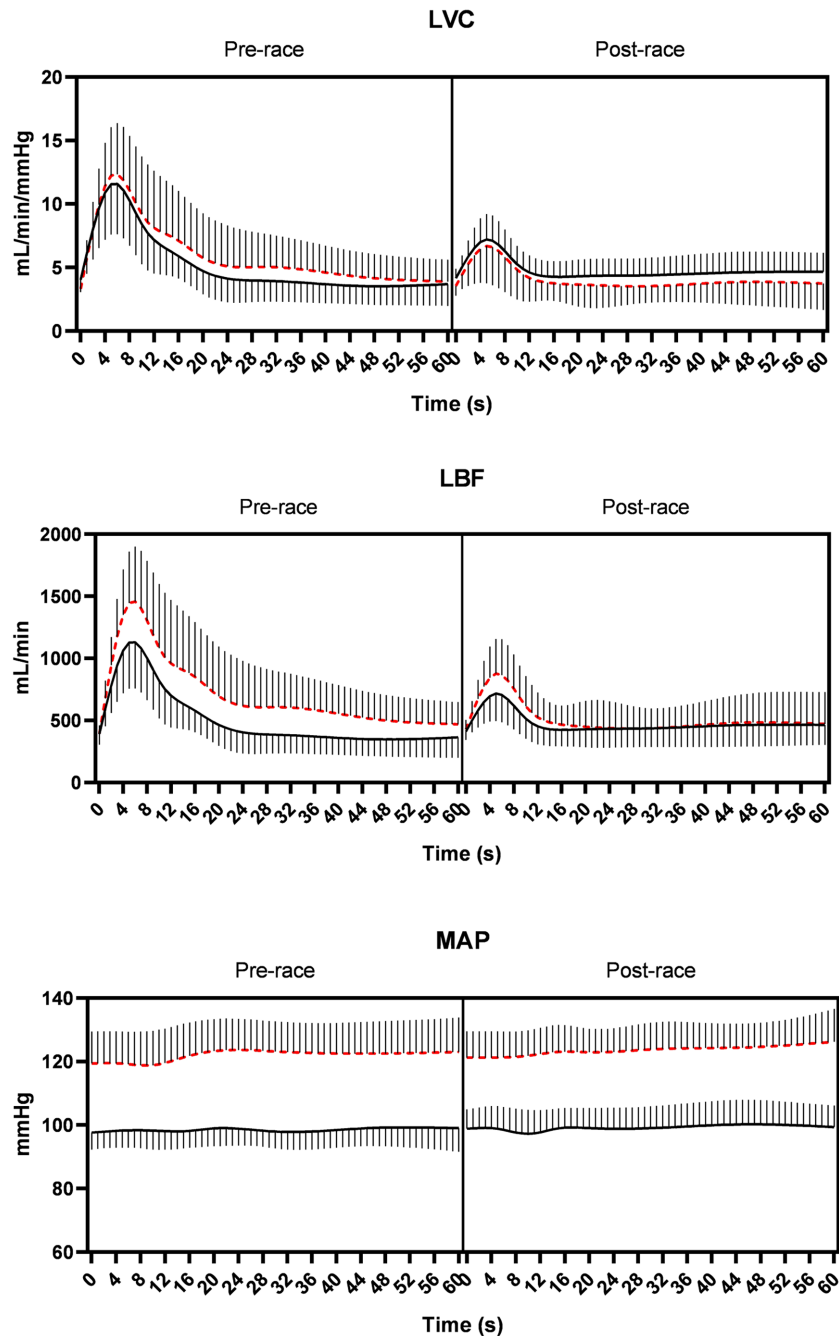
Fig. 1. Changes plotted as (mean ± S.D.) for the leg vascular conductance (LVC), leg blood flow (LBF) and mean arterial pressure (MAP) without (black solid line) and with (red dashed line) SYMP immediately after the SPLM, pre- and post-race in half-marathon runners (n=11).

the race in comparison to pre-race value but increased during SYMP regardless of the effects of the race. Heart rates were increased after the race and increased during SYMP regardless of the effects of the race. ANOVA indicated that the peak leg vascular conductance was only affected by the race (SYMP: P=0.45, F=0.6; race: P=0.0007, F=23.5), whereas peak leg blood flow (SYMP: P=0.0003, F=29.1; race: P=0.0004, F=26.8) and heart rate (SYMP: $P<0.0001$, F=100.4; race: $P<0.0001$, F=133.0) were affected by both SYMP and the race. The mean arterial pressure was only affected by SYMP (SYMP: P=0<0.0001, F=99.8; race: P=0.52, F=0.5).

Table 1. The table reports the data as (mean ± S.D.) for leg vascular conductance (LVC; ml/min/mmHg), leg blood flow (LBF; ml/min) and mean arterial pressure (MAP; mmHg), with and without sympathetic activation (SYMP), pre- and post-race. Data were analyzed via two-way repeated-measure ANOVA with a Sidak post-hoc test. P-values on the right hand side indicate SYMP vs. no SYMP, whereas those below indicate post- vs. pre-race.

|	Baseline values			Peak values after SPLM			Mean values after SPLM		
	No SYMP	SYMP		No SYMP	SYMP		No SYMP	SYMP	
LVCpre	4.0 ± 1.0	3.3 ± 0.7	$P=0.01$	11.5 ± 4.0	12.2 ± 3.8	$P=0.35$	5.1 ± 1.7	5.9 ± 2.5	$P=0.14$
LVCpost	4.6 ± 0.8	3.9 ± 0.8	$P=0.01$	7.2 ± 2.0	7.3 ± 2.6	$P=0.96$	4.8 ± 1.3	4.2 ± 1.5	$P=0.26$
	$P=0.02$	$P=0.03$		$P<0.001$	$P<0.001$		$P=0.62$	$P=0.002$	
LBFpre	391 ± 85	398 ± 68	$P=0.95$	1128 ± 372	1446 ± 412	$P<0.001$	501 ± 157	703 ± 270	$P=0.002$
LBFpost	430 ± 65	447 ± 76	$P=0.70$	716 ± 220	877 ± 278	$P=0.02$	475 ± 148	511 ± 186	$P=0.69$
	$P=0.22$	$P=0.08$		$P<0.001$	$P<0.001$		$P=0.82$	$P=0.003$	
MAPpre	97.6 ± 5.4	119.4 ± 10.1	$P<0.001$	98.3 ± 5.4	119.3 ± 10.4	$P<0.001$	98.5 ± 5.4	122.0 ± 9.9	$P<0.001$
MAPpost	93.8 ± 6.0	116.2 ± 8.2	$P<0.001$	98.8 ± 7.1	121.2 ± 8.1	$P<0.001$	99.2 ± 6.9	123.6 ± 7.8	$P<0.001$
	$P=0.31$	$P=0.43$		$P=0.97$	$P=0.73$		$P=0.95$	$P=0.76$	
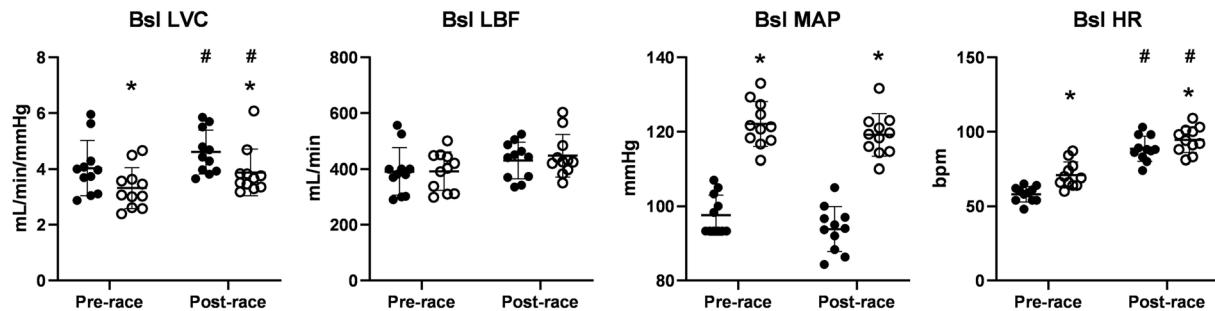
Fig. 2. Baseline values plotted as (mean ± S.D.; n=11) for leg vascular conductance (LVC), leg blood flow (LBF), mean arterial pressure (MAP), and heart rate (HR) without (black dots) and with (white dots) SYMP immediately before performing the SPLM, pre- and post-race in half-marathon runners (*$P<0.05$ SYMP vs. no SYMP; #$P<0.05$ post- vs. pre-race). Data were analyzed via two-way repeated-measure ANOVA with a Sidak post-hoc test.
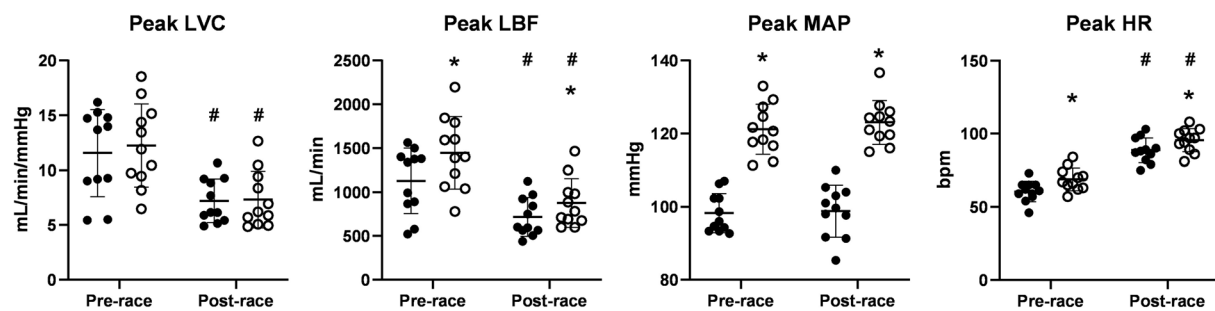

Fig. 3. Peak values plotted as (mean ± S.D.; n=11) for leg vascular conductance (LVC) with relative leg blood flow (LBF), mean arterial pressure (MAP), and heart rate (HR) without (black dots) and with (white dots) SYMP immediately after the SPLM, pre- and post-race in half-marathon runners (*$P<0.05$ SYMP vs. no SYMP; #$P<0.05$ post- vs. pre-race). Data were analyzed via two-way repeated-measure ANOVA with a Sidak post-hoc test.

Mean values after SPLM in runners

As reported in Table 1 and Fig. 4, the mean leg vascular conductance over the 60 sec after the SPLM was similar during SYMP compared to without SYMP, both before and after the race. However, there was an interaction between the effects of the race and SYMP. Indeed, the mean leg vascular conductance was similar
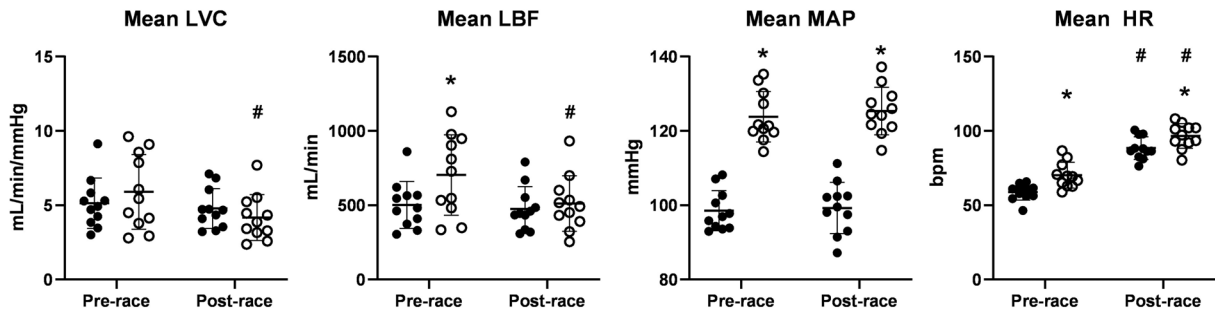
Fig. 4. Plots of the mean values (mean ± S.D.; *n*=11) for leg vascular conductance (LVC) with relative leg blood flow (LBF), mean arterial pressure (MAP), and heart rate (HR) without (black dots) and with (white dots) SYMP over the 60s after performing the SPLM, pre- and post-race in half-marathon runners (*$P<0.05$ SYMP vs. no SYMP; #$P<0.05$ post- vs. pre-race). Data were analyzed via two-way repeated-measure ANOVA with a Sidak post-hoc test.
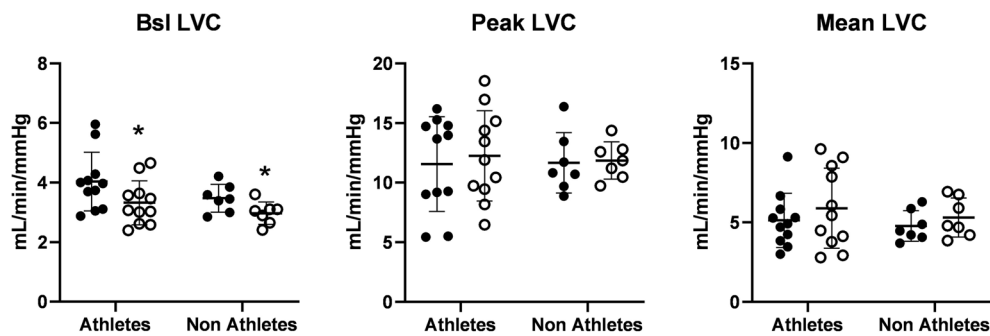
Fig. 5. Peak and mean values (mean ± S.D.; *n*=11 vs. *n*=7) for leg vascular conductance (LVC) without (black dots) and with (white dots) SYMP after the SPLM, pre-race in runners compared to sedentary individuals (*$P<0.05$ SYMP vs. no SYMP). Data were analyzed via two-way repeated-measure ANOVA with a Sidak post-hoc test.

without SYMP following the race compared to prior, but was lower during SYMP.

SYMP only elevated the mean leg blood flow before the race. In the condition without SYMP, the mean leg blood flow did not change after the race compared to prior to performing the race. However, it diminished in the condition with SYMP. The mean arterial pressure was similar after the race compared to before the race, but increased during SYMP regardless of the effects of the race. The mean heart rate was higher after the race compared to before the race and higher during SYMP regardless of the effects of the race. ANOVA revealed that the mean leg vascular conductance was not affected by SYMP and the race (SYMP: $P=0.83$, $F=0.2$; race: $P=0.09$, $F=3.5$), but that there was an interaction effect between these two effects ($P=0.03$, $F=6.4$). The mean leg blood flow was only affected by SYMP (SYMP: $P=0.01$, $F=7.8$; race: $P=0.09$, $F=3.6$) and there was an interaction effect between SYMP and the race ($P=0.02$, $F=6.9$). The heart rate was affected by both SYMP and the race (SYMP: $P<0.0001$, $F=105.8$; race: $P<0.0001$, $F=122.2$). The mean arterial pressure was affected by SYMP only (SYMP: $P<0.0001$, $F=111.3$; race: $P=0.53$, $F=0.5$).

Vascular conductance in sedentary individuals

As shown in Fig. 5, baseline vascular conductance (3.5 ± 0.5 vs. 2.9 ± 0.4 ml/min/mmHg; $P=0.03$) diminished after 1 min of SYMP in sedentary individuals but was similar compared to endurance runners before the race regardless of SYMP ($P>0.24$). Peak and mean leg vascular conductance after SPLM were not different in sedentary individuals compared to endurance runners before the race ($P>0.45$) regardless of SYMP ($P>0.80$).

Discussion

The first objective of this study was to investigate whether acute handgrip-mediated SYMP restrains the normal SPLM-induced leg vasodilation before and after a half-marathon in endurance runners. The second objective was to assess whether any effect of SYMP on leg vasodilation is more blunted after the race compared to before due to post-exercise vasodilation and high bioavailability of metabolites that may challenge the sympathetic vasoconstriction (12, 13). This study was focused on endurance athletes since this population is prone to attenuation in sympathetic vasoconstriction, which can predispose them to post-exercise hypotension (15, 17). Our data show that 1 min of SYMP decreased the leg vascular conductance while the leg was still, both before and after the race. However, SYMP did not blunt either the normal peak leg vasodilation or mean leg vasodilation in response to the SPLM performed immediately afterward, both before and after the race. While the mean leg vasodilation was similar after the race compared to before without SYMP, it was restrained during SYMP.

Leg vasodilation

We employed the SPLM technique to induce sudden vasodilation of the lower limb (22). This technique consists of a single passive 90-degree flexion of a horizontally straight leg. At rest, this maneuver has been shown to induce rapid leg vasodilation and increase the leg vascular conductance up to approximately 2.5-fold within approximately 5–10 sec to then return towards baseline within approximately 30 seconds. This trend was confirmed by our data (Fig. 1). The SPLM technique has more clinical potential than the traditional flow-mediated dilation (FMD) of an artery (22). It has the advantage of being minorly influenced by central hemodynamic changes and being mainly nitric oxide-dependent (24). Moreover, this maneuver can limit any artifacts during ultrasound measurements because the leg is still. By focusing on the entire leg vascular conductance, we performed a global assessment of leg vasodilation at the microvascular level as propounded by Venturelli et al. (22). Indeed, the analysis of a single arterial segment provides a measurement of conduit function, which is a local outcome that may be independent of the entire leg vasodilation. For instance, the common femoral artery may not dilate even during active exercise despite global leg vasodilation (25). As shown by our data, the common femoral artery did not dilate even after SPLM.

Handgrip-mediated sympathetic activation

The handgrip exercise was preferred among several non-invasive sympathetic stimulants for its direct applicability into daily or clinical practice, especially for athletes suffering from post-exercise hypotension. A similar handgrip exercise can be easily achieved by squeezing a tennis ball. Handgrip exercise at 30% MVC has been shown to increase the vascular resistance of the lower limb when it is kept still (26). Additionally, the large availability of microneurography recordings performed during this stimulus at rest (23, 26, 27) shows this stressor to reliably increase the muscle sympathetic nerve activity (MSNA). In our study, we specifically employed the overhead version of the static handgrip exercise at 30% MVC to augment the MSNA to a greater extent compared to when this exercise is performed at heart level (23). Indeed, 2 min of overhead handgrip exercise at 30% MVC increased the total MSNA up to 5 fold compared to baseline and up to 2 fold compared to the same exercise performed at heart level. In agreement with previous studies (26–29), SYMP increased the heart rate and mean arterial pressure. Moreover, SYMP decreased the leg vascular conductance after 60 sec of stimulation while the leg was still, both before and after the race. Therefore, 60 sec of SYMP augmented sympathetic vasoconstriction immediately before performing the SPLM maneuver both before and after the race.

Effect of SYMP on vascular conductance

We focused on the sympathetic capacity to restrain leg vasodilation. Since the *in-vivo* vascular conductance regulation is a continuous balance between metabolic vasodilation and sympathetic vasoconstriction, assessing the vasoconstriction effects elicited by a sympathetic stressor without considering the contribution of vasoactive agents may deliver misleading findings.

In agreement with previous studies (26, 27), our findings show that 60 sec of acute handgrip-mediated SYMP does decrease the preceding baseline vascular conductance while the leg is kept still not only at rest, but also after a strenuous endurance exercise. However, SYMP did not blunt the normal peak leg vasodilation or mean leg vasodilation in response to the SPLM performed immediately afterward, both before and after the race. To our knowledge, no study has investigated whether handgrip-mediated SYMP blunts limb vasodilation or artery FMD. However, the unchanged leg vasodilation during SYMP compared to without SYMP would suggest that the sudden release of local vasodilatory agents effectively opposes any augmented sympathetic vasoconstriction in endurance athletes both before and after endurance exercise.

Our data do not outline any differences in baseline, peak and mean leg vascular conductance in endurance runners compared to sedentary subjects before the race. This might suggest that the integrated control of vascular conductance at rest is unaffected by endurance exercise training. Chronic endurance training induces arterial remodeling, increasing arterial diameter and vasodilator function (9, 16, 30, 31). Indeed, the muscle vasodilation function in endurance athletes can occur to such an extent that the heart is unable to maintain blood pressure by increasing cardiac output after exercise (9). On the other side, handgrip-mediated SYMP (50% MVC to fatigue) changes the vascular conductance, arterial pressure, and heart rate to a lesser extent in fit than unfit subjects (17). However, these changes are accompanied by an increase in baseline sympathetic activity at rest (32). It has been proposed that this increase in sympathetic activity may be functional to restrain any enhanced vasodilation function at rest (32). An increased baseline sympathetic tone may offset any attenuated sympathetic receptor responsiveness or enhanced metabolic vasodilation in endurance athletes at rest. Since physiological adaptations to endurance training are functional to improve performance during exercise, it is possible that any change in the integrated control of vascular conductance is noticeable immediately after exercise. This would be consistent with the notion that endurance training augments the incidence of hypotension especially after exercise but not necessarily at rest (15, 33).

The preponderance of previous studies suggests that the transduction of sympathetic activity into vasoconstriction may be blunted in young females compared to males (34). Indeed, sympathetic activation increased vascular resistance to a lesser extent in young women than men with similar increment in MSNA (35). Although our study was open to both sexes, there were more male than female participants. This does not allow for sex differences to be identified. Since sex differences in neurovascular modulation may change according to changes in body dimension, hormonal status and menstrual phase (36), an independent study with targeted measurements is required to properly elucidate sex differences.

Effect of the half-marathon race on the leg vasodilation

The half-marathon blunted the normal peak leg vasodilation that took place approximately 5 sec after the SPLM. However, the half-marathon did not change the leg mean vasodilation over the 60 sec after the SPLM. This suggests that endurance exercise impairs the capacity to massively and quickly dilate in response to a mechanical stimulus. However, this effect is short-lasting and does not affect the vascular conductance in a large time frame. Rapid vasodilation is due to the sudden release of local vasodilatory agents produced by the vascular endothelium or the muscle itself, which quickly (<2 sec) relax the vascular smooth muscle to allow

sudden reactive hyperemia (37). Acute bouts of endurance exercise can decrease the vasodilator function due to several factors, including an increase in baseline diameter of arterial vessels, increased oxidative stress, shear-induced substrate depletion, enhanced retrograde shear, and decreased sensitivity or reduced shear stimulus (38). An increase in the baseline vascular conductance post-race compared to pre-race is confirmed by our data (Fig. 2) and may limit further vasodilation since the tissue is already dilated. Among the possible contributors to the post-race increase in baseline vascular conductance, adenosine may play a role by binding to A_{2A} receptors on arterial smooth muscle (39). Indeed, adenosine has been suggested to be responsible for 20 to 40% of the maintained phase of muscle vasodilatation following muscle contraction. A lesser contribution of ATP in vessel dilation may also take place during and after exercise by binding to P2Y receptors (39). Increased levels of extracellular potassium after exercise have also been suggested to induce hyperpolarization and consequent relaxation of vascular smooth muscle via Na^+/K^+-ATPase pump stimulation (40, 41). Indeed, the membrane potential of vascular smooth muscles regulates the open-state probability of voltage-gated Ca^{2+} channels and consequently Ca^{2+} influx. Hydrogen ions can further relax vascular smooth muscle by diminishing the intracellular Ca^{2+} concentration via an increase in pH (41). Nitric oxide release during exercise could also induce vasodilation via activation of guanylate cyclase in smooth muscle cells and probably by stimulating the release of vasodilator prostaglandins from endothelial cells (41).

The mean leg vasodilation over the 60 sec after the SPLM was similar post-race as pre-race without SYMP. However, it was reduced during SYMP. This reveals an interaction between the effects of the half-marathon and SYMP on the leg neurovascular control. Previous studies documented a preeminent role for the sympathetic nervous system in constricting the vascular tissue immediately after endurance exercise compared to that at rest via an α1-adrenergic mechanism. Indeed, the brachial artery FMD is normally reduced immediately after endurance exercise compared to at rest (42, 43). While α1-adrenoreceptor blocking does increase the brachial artery FMD immediately after endurance exercise, it does not change the brachial artery FMD at rest (42, 43). In the parallel investigation that we conducted in similar runners who ran the same half-marathon race, under similar conditions, we also showed that circulating blood catecholamines were consistently augmented after this race (44). Although epinephrine has a higher affinity for β2- than postjunctional α1- or α2-adrenoceptors, high epinephrine concentrations after exercise can also bind to α1- and α2-adrenoceptors and overpower the vasodilatory effects of β2-adrenoceptor stimulation that take place with low epinephrine concentrations at rest. However, defining the underlying mechanisms is beyond the scope of this project and requires further investigation. Although the interaction between the half-marathon and SYMP may suggest an apparent stronger sympathetic vasoconstriction post-race than pre-race, SYMP was, likewise, ineffective in restraining the normal mean leg vasodilation post-race as it was in pre-race. This suggests no evident stronger sympathetic vasoconstriction post-race than pre-race.

Practical applications

Our data show that the acute handgrip-mediated SYMP at 30% MVC increases leg vasoconstriction while the leg is kept still in endurance athletes both before and after exercise. However, the release of vasoactive agents induced by a mechanical stimulus appears to effectively oppose such vasoconstriction, providing no evident constraint of vasodilation. Therefore, a potential for this tool in improving sympathetic vasoconstriction of exercised muscles may be justified when endurance athletes are still, but not necessarily while active, given that movements induce the release of vasoactive molecules.

Limitations

Firstly, several non-invasive techniques can be used to stimulate the sympathetic nervous system, each with different effects on vascular control (4–6, 45, 46). Thus, the vascular effects we found may differ by using different sympathetic stressors. Secondly, this study aimed to define any possible difference of the effects elicited by the same sympathetic stressor on leg vascular conductance before and after a half-marathon. The investigation of the underlying mechanisms of action as well as sex differences requires additional investigation. The assessment of epinephrine, norepinephrine, cortisol and estrogen levels as well as body dimension and MSNA may provide further insight regarding the potential of the handgrip exercise to influence the vascular tissue before and after the race in both sexes.

Conclusions

One minute of handgrip-mediated SYMP decreased the leg vascular conductance while the leg was still, both before, as well as after the race. However, SYMP did not blunt the normal peak leg vasodilation or mean leg vasodilation after the SPLM performed immediately afterward, both before and after the race. This suggests that the release of local vasoactive agents effectively opposes any previous handgrip-mediated augmented vasoconstriction in endurance athletes. Thus, the handgrip-mediated SYMP may improve the normal vasoconstriction while athletes are still, but not necessarily while they move, as movements induce the release of vasoactive agents.

Author contributions

AG contributed to the research concept and study design, literature review, data collection, data analysis and interpretation, statistical analyses, manuscript writing, and review; CT and KS contributed to the half-marathon organization and manuscript review; AC and FS contributed to the data interpretation, data analysis, and manuscript review. All people designated as authors qualify for authorship, and all those who qualify for authorship are listed. All authors read and approved the final version of the manuscript.

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Conflicts of interests

None.

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