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RESEARCH ARTICLE

Heart and musculoskeletal hemodynamic responses to repetitive bouts of quadriceps static stretching

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Venturelli M, Rampichini S, Coratella G, Limonta E, Bisconti AV, Cè E, Esposito F. Heart and musculoskeletal hemodynamic responses to repetitive bouts of quadriceps static stretching. *J Appl Physiol* 127: 376–384, 2019. First published May 30, 2019; doi: 10.1152/japplphysiol.00823.2018.—The role of sympathetic and parasympathetic activity in relation to the repetitive exposure to static stretching (SS) on heart and musculoskeletal hemodynamics in stretched and resting muscles is still a matter of debate. The aim of the study was to determine cardiac and musculoskeletal hemodynamics to repetitive bouts of unilateral SS. Sympathetic and parasympathetic activity contribution to the central hemodynamics and local difference in circulation of stretched and resting muscles were also investigated. In eight participants, heart rate (HR), cardiac output (CO), mean arterial pressure (MAP), HR variability (HRV), blood pressure variability (BPV), and blood flow in passively stretched limb (SL) and control (CL, resting limb) were measured during five bouts of unilateral SS (45 s of knee flexion and 15 s of knee extension). SS increased sympathetic (~20%) and decreased parasympathetic activity (~30%) with a prevalence of parasympathetic withdrawal. During SS, HR, CO, and MAP increased by ~18 beats/min, ~0.29 l/min, ~12 mmHg, respectively. Peak blood flow in response to the first stretching maneuver increased significantly ($+377 \pm 95$ ml/min) in the SL and reduced significantly (-57 ± 48 ml/min) in the CL. This between-limb difference in local circulation response to SS disappeared after the second SS bout. These results indicate that heart hemodynamic responses to SS are primarily influenced by the parasympathetic withdrawal rather than by the increase in sympathetic activity. The balance between neural and local factors contributing to blood flow regulation was affected by the level of SS exposure, likely associated with differences in the bioavailability of local vasoactive factors throughout the stretching bouts.

NEW & NOTEWORTHY Repetitive exposure to static stretching (SS) on heart and musculoskeletal hemodynamics in stretched and remote muscles may be influenced by neural and local factors. We documented that SS-induced heart hemodynamic responses are primarily influenced by parasympathetic withdrawal. The balance between neural and local factors contributing to the regulation of musculoskeletal hemodynamics is dependent on SS exposure possibly because of different local vasoactive factor bioavailability during the subsequent stretching bouts.

hemodynamics; parasympathetic activity; stretching; sympathetic activity

INTRODUCTION

The control of musculoskeletal blood flow is a complex integrative mechanism that equalizes the vasoconstrictive and vasodilatory triggers to distribute blood flow within and between skeletal muscles (6, 36). The homeostasis of systemic (neural) and local factors is crucial for the control of skeletal muscle blood flow, with an important factor being the balance between sympathetically mediated vasoconstriction and the vasodilation induced by local factors, such as nitric oxide (NO) (15). At rest, the equilibrium between vasoconstriction and vasodilation results in a smooth muscle tone near 60% of the total resistance vessel vasodilatory capacity (39). However, when this balance is altered by the increases in muscle sympathetic nerve activity, the vasculature of resting skeletal muscles can achieve elevated levels of vasoconstriction (39).

During physical exercise, general agreement exists on the key role of the autonomic nervous system in response to voluntary skeletal muscle activation (30). Indeed, sympathetic and parasympathetic activity are modulated, at least in part, by the parallel activation of the central motor pathways and the feedback that arises from mechano- and metaboreceptor activation in the skeletal muscle (1, 10, 16, 42). Different from physical exercise, static stretching (SS) is characterized by the absence of exercise-induced increase in muscle metabolism and the lack of central command, both of which would generate a sympathetic-mediated increase in heart rate (HR). Hence, the increase in HR observed during SS has been attributed to a decrease in vagal activity and the concomitant small rise in sympathetic discharge (9). However, whether or not cardiac output (CO), stroke volume (SV), and mean arterial pressure (MAP) response to passive SS are primarily influenced by the sympathetic-mediated activation or by the parasympathetic withdrawal is still poorly understood (30).

The acute physiological effects of passive SS on the above-mentioned heart and musculoskeletal hemodynamics have been recently debated (18, 19, 42), documenting either no detectable change in net blood flow, MAP, and popliteal artery vascular conductance (VC) (18, 19) or an increase in HR and CO, coupled with a hyperemia to the stretched skeletal muscles (42). The latter observation has been likely explained by the mechanoreflex activation (42) and local NO release (29, 37). Other seminal studies on this subject issue, however, reported that the HR and blood pressure responses to calf muscle stretch are independent of the metaboreflex activation (10) and can

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decrease spontaneous baroreflex sensitivity and other indexes of vagal tone (7). However, the mechanisms underpinning the contribution of local (vasodilatory) and systemic (vasoconstrictive) factors to these physiological changes and their transitory nature (~35 s) are not completely understood. Further insights on this phenomenon may come from a new approach involving repetitive exposures to SS. Given the generally low bioavailability of NO as a local vasodilator factor (22), several bouts of SS should deplete temporarily the NO reserve and blunt the SS-induced hyperemia. Moreover, whether or not the mechanoreflex-induced increases in sympathetic activity may differently affect the circulation of stretched and remote muscles not directly involved in SS is not clear.

Therefore, the aim of the present study was to compare the heart and musculoskeletal hemodynamic responses to repetitive bouts of unilateral quadriceps muscle SS. The contribution of sympathetic and parasympathetic activity to the heart hemodynamics was also investigated. Specifically, by studying HR variability (HRV), heart hemodynamics, blood pressure variability (BPV), and blood flow in passively stretched limb (SL) and control (CL, resting limb) during five bouts of unilateral SS (45 s of knee flexion and 15 s of knee extension), we tested the following hypotheses: *1*) the heart hemodynamic responses to SS might be primarily influenced by the parasympathetic withdrawal rather than by the increase in sympathetic activity; *2*) the blood flow response to SS in the passively stretched limb would be initially greater compared with the contralateral limb; and *3*) this regional difference in peripheral circulation response to SS would be dependent on the repetitive exposures to SS (number of bouts) that are administered to the investigated muscle.

METHODS

Participants. Eight young healthy men (age: 25 ± 2 yr; body mass: 71 ± 8 kg; stature: 1.80 ± 0.06 m; means ± SD) participated in this study. None of the participants were smokers, and all were moderately physically active. All procedures conformed to the standards set by the Declaration of Helsinki and were approved by the ethical committee of the University of Milan. The participants gave written, informed consent before their participation after full explanation of the purpose of the study and of the experimental procedures. The participants reported to the laboratory in the morning (9–10 AM) in a fasted state. They were asked to abstain from consuming caffeine 24 h before the test and to report to the laboratory without any form of physical exercise of heavy intensity in the previous 48 h.

Experimental design. After a first visit for familiarization purpose, the participants reported to the laboratory a second time, during which single-leg SS was performed. All the experimental procedures, from which experimental data were collected, were accomplished during the second visit.

SS. The participants rested in a supine position for 20 min before starting the data collection and remained in this position throughout the entire duration of the data collection (Fig. 1). As previously reported, SS protocol consisted of 5 min of resting baseline followed by passive static knee flexion for 45 s and passive knee extension for 15 s, repeated five times (5, 20, 21). During the entire SS protocol, knee extensors were stretched by the same operator up to a point of discomfort lower than 2. This cut-off of discomfort level was chosen in the present investigation to minimize the activation of peripheral pain pathways that might interact with group III and IV afference feedback and potentially accentuate the central hemodynamic response (1). The level of discomfort was assessed by a 0–10 visual analog scale; 0 = no discomfort at all and 10 = maximum discomfort

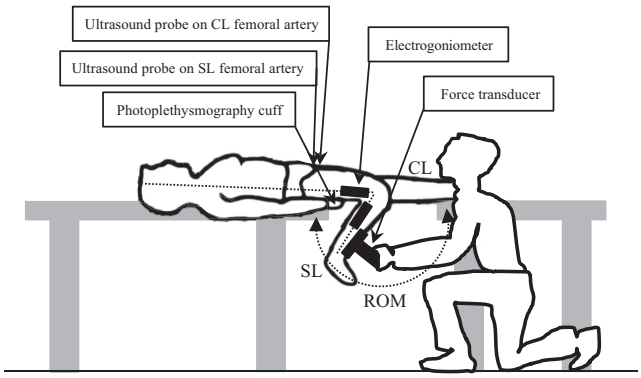

Fig. 1. Schematic figure showing the position of the subject during the static stretching procedure. SL, stretched leg; CL, control resting leg; ROM, range of motion.

(25). The knee joint angle was continuously recorded using a dual-axial goniometer (model no. TSD 130A; Biopac Systems, Goleta, CA). Force output (FO) between the passively stretched leg (SL) and the operator arms was recorded during the protocol by a load cell (model SM-2000 N; Interface, Crowthorne, UK). Specifically, the load cell was positioned 5 cm above the ankle of the passively SL, and a member of the research team pushed perpendicularly the load cell to stretch the leg extensor for 45 s (Fig. 1). The mean FO during the 45 s of the consecutive flexion of the SS protocol was then recorded.

Central hemodynamics. HR, SV, CO, and MAP were determined on a beat-by-beat basis using a finger photoplethysmography device (FinometerPro; Finapres Medical Systems, Amsterdam, The Netherlands). The photoplethysmographic cuff was placed on the third finger of the left hand. The height adjustment sensor and reference were positioned following the manufacturer's instructions. The blood pressure signal was calibrated in accordance to the procedure indicated by the manufacturer. SV was estimated using the Modelflow algorithm (Beatscope version 1.1a; Finapres Medical Systems) (4). CO was then calculated as the product of HR and SV. The same method has been documented to accurately track CO during exercise (2, 35), and, as reported in previous investigations, the absolute changes from rest values have been demonstrated to be accurate (38, 40, 41).

Femoral blood flow. The measurements of arterial blood velocity and vessel diameter were performed in the passively SL and control resting leg (CL), distal to the inguinal ligament and proximal to the deep, superficial femoral bifurcation with two Logiq S7pro ultrasound systems (General Electric Medical Systems, Milwaukee, WI). The systems were equipped with 12- to 14-MHz linear array transducers. The common femoral artery diameters were determined along the central axis of the scanned areas. The blood velocity (v) was measured using the same probe at a frequency of 5 MHz. The measurements of v were obtained second by second with the probe positioned to maintain an insonation angle of 60° or less, and the sample volumes were centered and maximized according to vessel size. After arterial diameter and mean v (v_{mean}) assessment, femoral blood flow (FBF) was automatically calculated using the Logiq S7pro software as

$$\text{FBF} = v_{mean} \times \pi \times \left(\frac{\text{vessel diameter}}{2}\right)^2 \times 60$$

where FBF is in milliliters per minute. All scanning and blinded analyses were performed by experienced and skilled sonographers. To account for potential differences in MAP, VC was calculated as FBF/MAP.

HRV. The computer analysis of spontaneous HR and interbeat interval oscillation in consecutive cardiac cycles has been recognized to be a credible quantitative marker to assess the activity of the

sympathetic and parasympathetic branches of the autonomic nervous system (35a). Several indexes have been developed, in both the time and frequency domain, to characterize the contribution of the vagal and the sympathetic efferent activity to the cardiovascular control. In the time domain, the root mean square of the squared differences of successive RR (RMSSD) estimates short-term variation of HR (35a), thus detecting high-frequency (HF) oscillations caused by parasympathetic activity. In the frequency domain analysis, the variance of the signal, namely the distribution of power as a function of frequency (power spectral density, PSD), is calculated by means of short fast Fourier transform, and, according to the frequency band classification proposed by the HRV Task Force (35a), it is divided in three components, very low frequency, low frequency (LF), and HF. The very-low-frequency component ($\leq$0.04 Hz) is not usually considered in short recordings (5 min). Power component of LF band (0.04–0.15 Hz) includes sympathetic and parasympathetic influences, whereas HF (0.15–0.4 Hz) band is mainly influenced by the efferent activity of the vagal tone (35a). Both markers could be measured in absolute units of power (ms^2) and in normalized units. Although the former provides information about the total power of the band, the latter allows one to assess the fractional contribution to HR oscillation given by the two bands (LF and HF), excluding the-very-low frequency component (43). Therefore, the ratio between normalized LF and HF (LF/HF) is computed as an index of the sympathovagal balance (3).

During this study HR was derived from the electrocardiographic signal (ECG) collected by the photoplethysmography device at 500 Hz. R-peaks of each QRS complex from the continuous ECG signals were detected by a derivative-threshold algorithm. The interbeat interval series (R-R interval tachogram) was obtained as the difference between the occurrence times of consecutive R-peaks. An expert operator checked the signal, and, in case of ectopic beats, the RR series were corrected through a cubic spline interpolation (28). The time domain and frequency domain analysis of the R-R series were conducted considering 5 min of signal for each condition (rest vs. stretching) (35a). In view of the frequency domain analysis, the unevenly time-sampled tachogram was interpolated at 4 Hz by a cubic spline function and successively downsampled at 1 Hz. The PSD was calculated by means of short fast Fourier transform, and the normalized LF and HF bands were subsequently obtained to compute LF/HF values. The very-low-frequency component requiring longer data series was not addressed in the present study.

The cardiac sympathovagal balance was obtained by the LF/HF index, whereas variation in parasympathetic activity was estimated, from the time domain analysis, by the RMSSD.

BPV. Blood pressure measurement, if collected concurrently with HR, is known to allow the simultaneous assessment of markers of efferent sympathetic vascular modulation (31). In light of this, the beat-by-beat systolic blood pressure (SBP) and diastolic blood pressure (DBP) series were obtained from the continuous blood pressure signal to characterize blood pressure oscillations (BPV). By the previously described photoplethysmography approach, SBP and DBP were measured for 5 min during baseline condition and 5 min during the SS procedure. SBP series was composed by the maximum of BP in each RR interval, whereas the DBP series was made by the minimum of BP following each SBP detection. Given that DBP changes negatively related to muscle sympathetic nerve activity burst incidence (23, 34), changes in the mean values of DBP series were computed and considered as an index of the (vessel) sympathetic activity. Moreover, like HRV analysis, SBP series can be evaluated in the frequency domain; therefore, as oscillation in the LF component of the SBP power spectral density (LF_{SBP}) is associated with an increase in the sympathetic drive (32), it was used as an additional BPV marker of sympathetic activity (11).

Data collection and analysis. SV, CO, MAP, ECG, and knee joint angle and FO underwent A/D conversion system (model no. UM150; Biopac Systems) and were simultaneously acquired (1,000 Hz) by commercially available data acquisition software (AcqKnowledge 4.2; Biopac Systems). The software allowed beat-by-beat analysis of HR, SV, CO, and MAP throughout the experimental protocols. v_{mean} was analyzed with 1-Hz resolution on the Doppler ultrasound systems (GE Logiq S7pro) for 30 s at rest and during the 5 min of repetitive single-leg SS. From the velocity and femoral artery diameter, net FBF was calculated on a second-by-second basis. Before analysis, all hemodynamic data were smoothed using a 3-s rolling average. As the response to passive stretching is transient and varies between individuals, a peak response was determined for all variables on an individual basis. Maximal absolute (peak), relative changes (Δpeak), and the area under the curve (AUC) were determined for each subject in all measured variables.

Statistical analysis. Raw data were analyzed using a statistical software package (SPSS Statistics v. 22; IBM, Armonk, NY). In light of a previous article of our group (42), in which a difference of ~15% in FBF (main outcome) was observed under SS, a sample size of eight participants was selected to ensure a statistical power higher than 0.80 with a type 1 error $<$0.05. To check the normal distribution of the parameters, a Shapiro-Wilk test was applied. Student's *t*-test was utilized to determine potential differences between baseline and passive SS measurements in the HRV and BPV normally distributed data. A two-way ANOVA for repeated measures [time (6 levels: baseline + 5 stretching bout) $\times$ limb (2 levels: SL and CL)] was used to establish differences among conditions for peripheral hemodynamic data. A two-way ANOVA for repeated measures [time (6 levels: baseline + 5 stretching bout) $\times$ knee joint position (2 levels: flexion and extension)] was used to establish differences among conditions for central hemodynamic data. A one-way ANOVA [time (5 stretching bouts)] was used to establish differences for range of motion (ROM) and FO. A Tukey's post hoc test was applied to define the location of the difference, when necessary. If Shapiro-Wilk test did not disclose a normal distribution, for central hemodynamic, knee joint angle of stretched limb, and the FO during the repetitive bouts of SS, then a repeated-measure ANOVA on ranks test was applied. A Wilcoxon signed-rank test was conversely applied whereby HRV and BPV variables failed the normality test. The level of significance was set at $\alpha < 0.05$. Unless otherwise stated, data are presented as means $\pm$ SE.

RESULTS

All the participants took part in this experimental protocol without reporting discomfort during the stretching procedures. On a scale from 0 to 10, the average discomfort across all five stretch cycles on the passively SL was 1.4 $\pm$ 1.1 and did not differ among the repetitive bouts of SS, 1.3 $\pm$ 0.9, 1.6 $\pm$ 0.9, 1.6 $\pm$ 1.3, 1.7 $\pm$ 1.1, and 1.5 $\pm$ 1.2 during first, second, third, fourth, and fifth flexion, respectively.

HRV response to passive SS. The effect of five consecutive bouts of one-leg SS on HRV indexes is summarized in Fig. 2, *A* and *B*. After the passive SS, RMSSD significantly dropped by ~20% ($P = 0.041$, Fig. 2*A*), whereas the LF/HF index significantly increased by ~63%, $P = 0.039$ (Fig. 2*B*).

Blood pressure and BPV response to passive SS. After five consecutive bouts of one-leg SS, the Wilcoxon signed-rank test found a significant increase in mean DBP values (~13%, $P = 0.008$; Fig. 2*C*), whereas no changes occurred in LF_{SBP} (Fig. 2*D*).

Central hemodynamics during flexion and extension phases of consecutive bouts of one-leg SS. All central hemodynamic values during five consecutive bouts of one-leg SS are summarized in Table 1 and Fig. 3. ANOVA disclosed significant main effects in MAP for time ($F = 42.7$; $P < 0.001$) and knee joint position ($F = 1702$; $P < 0.001$), as well as a time $\times$ knee joint position interaction ($F = 135$; $P < 0.001$). Similarly, main effects for time ($F = 59.1$; $P < 0.001$) and knee joint

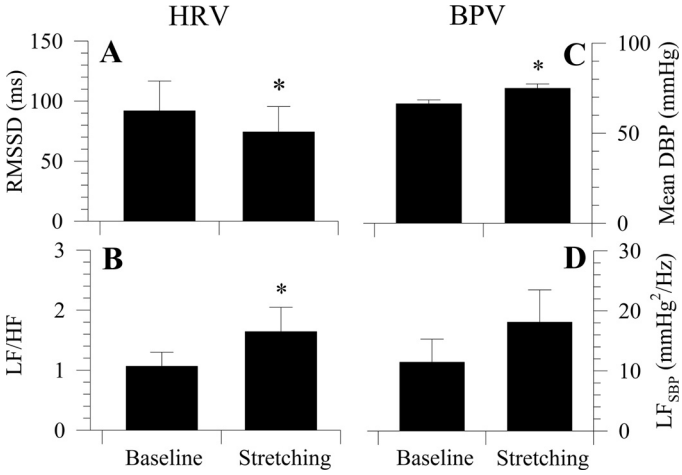

Fig. 2. Sympathetic and parasympathetic indexes during baseline and passive static stretching. *A–D*: root mean square of the squared differences of successive NN intervals (RMSSD), the ratio between low and high frequency (LF/HF) of the heart rate variability (HRV), mean diastolic blood pressure (DBP), and the low frequency component of systolic (LF_{SBP}) blood pressure variability (BPV). Data are means ± SE; *significantly different from baseline.

position ($F = 62.7$; $P < 0.001$) and time × knee joint position interaction ($F = 3.1$; $P = 0.012$) were retrieved in SV. In HR, no main effect for time was found ($F = 0.39$; $P = 0.85$), whereas there was a main effect for factor knee joint position ($F = 1266$; $P < 0.001$) and an interaction between time and knee joint position ($F = 44.4$; $P < 0.001$). Similarly, in CO, no main effect CO for time was retrieved ($F = 0.98$; $P = 0.43$), whereas a main effect for knee joint position ($F = 29.6$; $P < 0.001$) and a time × knee joint position ($F = 2.4$; $P = 0.046$) were found. During the first flexion procedure, MAP was initially increased by 5% ($P < 0.05$; Fig. 3*A*). This transitory MAP increase was followed by a significant drop 8% ($P < 0.05$) and a subsequent rise 11% ($P < 0.05$) of the MAP. This MAP sinusoidal response to SS was not present during the second, third, fourth, and fifth flexions, whereas there was a robust and sustained increase in MAP Δpeak and AUC (Table 1 and Fig. 3). During all the passive knee extension phases of SS, MAP rapidly dropped to values similar to baseline (Table 1; Fig. 3*A*). During the first flexion, SV increased by ~22 ml ($P < 0.05$; Fig. 3*B*) and remained significantly elevated from baseline for ~30 s. This SV Δpeak was blunted (~13 ml) during the second, third, fourth, and fifth flexions, whereas the response was longer than 42 s (Table 1 and Fig. 3). During all the passive extension phases of SS, SV increased by ~13 ml (Table 1; Fig. 3*B*). During all the flexion phases of SS, both HR and CO rapidly increased by ~18 beats/min and 0–31 l/min, respectively ($P < 0.05$; Fig. 3, *C* and *D*). HR and CO values remained significantly elevated from baseline for ~42 s. During all the extension phases of SS, HR and CO rapidly dropped to values similar to baseline (Table 1; Fig. 3, *C* and *D*).

Knee joint ROM and FO during consecutive bouts of SS. The ROMs and FO attained during the consecutive bouts of SS are reported in Table 2. Both ROM and FO did not increase from

Table 1. *Central hemodynamics during flexion and extension phases of consecutive bouts of 1-leg SS*

	1st	2nd	3rd	4th	5th
			MAP		
Δpeak, mmHg					
FL	−7.1 ± 1.2	14.2 ± 1.3†	11.7 ± 1.5†	10.8 ± 1.4†	11.8 ± 1.3†
EX	−15.0 ± 1.3*	−15.1 ± 1.4*	−18.7 ± 1.7*	−19.8 ± 1.6*	−19.0 ± 1.8*
AUC, mmHg·s					
FL	0.01 ± 0.01	7.5 ± 0.03†	6.6 ± 0.09†	6.9 ± 0.07†	7.6 ± 0.05†
EX	−2.08 ± 0.01*	−2.58 ± 0.02*	−2.25 ± 0.02*	−2.56 ± 0.02*	−2.88 ± 0.03*
			SV		
Δpeak, ml					
FL	21.8 ± 2.4	13.6 ± 2.5†	14.3 ± 1.9†	12.1 ± 1.9†	13.0 ± 2.2†
EX	16.7 ± 2.5	9.0 ± 2.2†	8.1 ± 2.9†	10.2 ± 1.6†	8.0 ± 3.2†
AUC, ml·s					
FL	10.6 ± 0.32	8.3 ± 0.28	8.9 ± 0.22	7.9 ± 0.22	7.1 ± 0.24
EX	1.2 ± 0.02*	0.5 ± 0.08*†	0.4 ± 0.02*†	0.6 ± 0.02*†	0.7 ± 0.04*†
			HR		
Δpeak, beats/min					
FL	18 ± 1.9	16 ± 1.9	18 ± 2.0	20 ± 1.8	20 ± 2.8
EX	−16 ± 2.1*	−16 ± 2.3*	−18 ± 2.9*	−18 ± 2.9*	−18 ± 3.8*
AUC, beats					
FL	11.5 ± 0.9	8.5 ± 0.8	11.3 ± 0.9	12.2 ± 0.82	12.9 ± 0.9
EX	−2.46 ± 0.11*	−2.91 ± 0.10*	−3.35 ± 0.10*	−3.36 ± 0.11*	−3.25 ± 0.09*
			CO		
Δpeak, l/min					
FL	0.32 ± 0.08	0.26 ± 0.07	0.25 ± 0.09	0.35 ± 0.09	0.27 ± 0.10
EX	−0.16 ± 0.07*	−0.30 ± 0.05*	−0.28 ± 0.07*	−0.26 ± 0.08*	−0.31 ± 0.09*
AUC, l					
FL	0.14 ± 0.02	0.16 ± 0.03	0.15 ± 0.03	0.21 ± 0.02	0.15 ± 0.02
EX	−0.03 ± 0.01*	−0.04 ± 0.01*	−0.04 ± 0.01*	−0.04 ± 0.01*	−0.05 ± 0.02*

Data are presented as means ± SE. Δpeak, absolute change; AUC, area under the curve; CO, cardiac output; EX, extension; FL, flexion; HR, heart rate; MAP, mean arterial pressure; SV, stroke volume; SS, static stretching. *$P < 0.05$ from FL; †$P < 0.05$ from 1st.

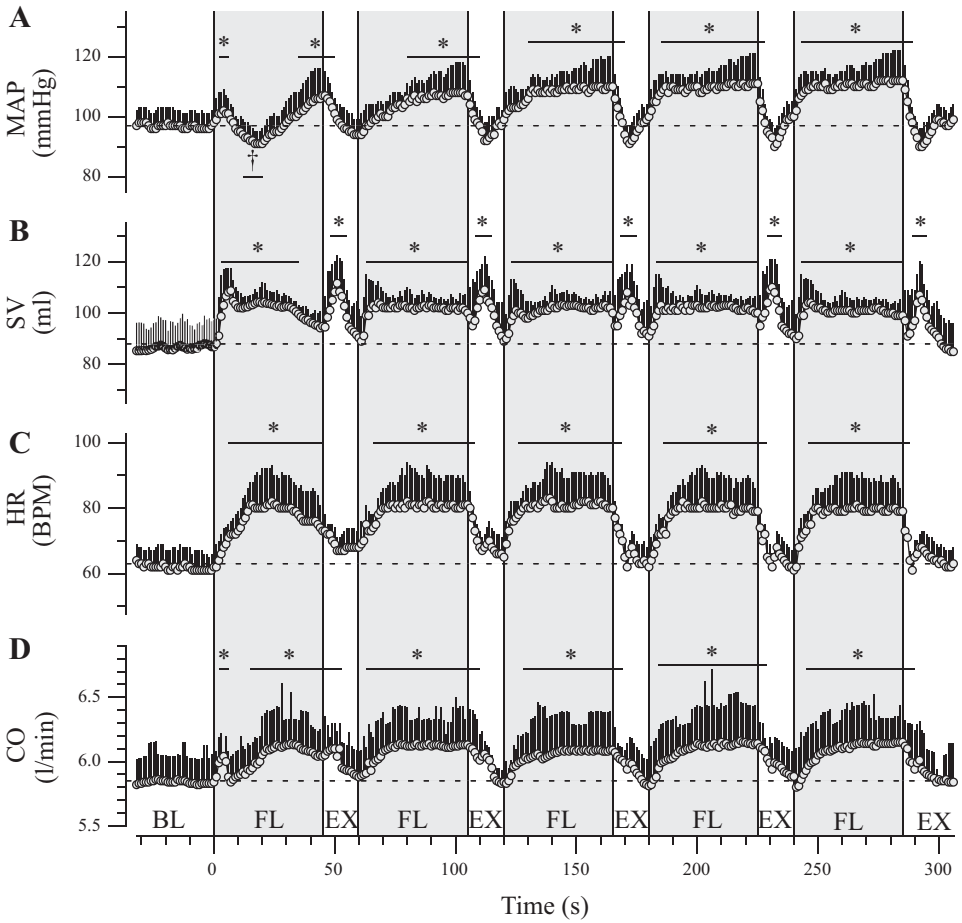

Fig. 3. Changes in central hemodynamic responses to repetitive bouts of 1-leg static stretching. *A–D*: mean arterial pressure (MAP), stroke volume (SV), heart rate (HR), and cardiac output (CO), at baseline (BL), during knee static passive flexion (FL), and static passive extension (EX), respectively. Data are means ± SE; *significantly increased from BL; †significantly reduced from BL. BPM, beats/min.

the first to the fifth SS bout, and no differences were found in any comparison ($P = 0.203$ and $P = 0.993$, respectively).

Peripheral hemodynamics during flexion and extension phases of consecutive bouts of unilateral SS. All peripheral hemodynamic outcomes recorded during five consecutive bouts of SL and resting CL are summarized in Table 2 and Fig. 4. ANOVA disclosed significant main effects in FBFpeak for time ($F = 207$; $P < 0.001$) and limb ($F = 2741$; $P < 0.001$) and a significant time × limb interaction ($F = 270$; $P < 0.001$). Similarly, main effect in VC peaks for factor time ($F = 190$; $P < 0.001$) and limb ($F = 2453$; $P < 0.001$) and a significant time × limb interaction ($F = 243$; $P < 0.001$) were found. In the SL, during the first flexion procedure, both FBF and VC were transiently increased from the 3rd to the 24th second (Fig. 4, *A* and *B*). This initial stretch-induced hyperemic response of SL, in terms of Δpeak and AUC, was greater in comparison to the second, third, fourth, and fifth flexions. Interestingly, a local reduction of FBF and VC was present in the SL from the third to the fifth flexion. Notably, during all the flexion phases of SS, FBF and VC of CL rapidly dropped below the baseline value (Table 2; Fig. 4, *A* and *B*). During the third, fourth, and fifth flexion procedures, FBF and VC of SL and CL were similar. During all the extension phases of SS, FBF and VC of SL rapidly increased by ~455 ml/min and ~72 $ml \cdot min^{-1} \cdot mmHg^{-1}$, respectively (Table 2; Fig. 4, *A* and *B*). Although contrary, FBF and VC of CL rapidly increased to values similar to baseline (Table 2; Fig. 4, *A* and *B*).

DISCUSSION

Although heart and musculoskeletal hemodynamic response to SS have been recently investigated, the role of sympathetic and parasympathetic activity in relation to heart hemodynamic and musculoskeletal circulation in stretched and contralateral muscles has received so far only little attention. In the present study, we investigated the heart hemodynamics and musculoskeletal blood flow responses to repetitive bouts of unilateral SS of the quadriceps muscle. The contribution of the sympathetic and parasympathetic activity to the heart hemodynamics and local difference in the circulation of stretched and contralateral muscles were also investigated. In accordance with our hypothesis, the main findings of the present study were as follows: *1*) the heart hemodynamic responses to SS seemed to be primarily influenced by the parasympathetic withdrawal rather than the increase in sympathetic activity; *2*) the stretch-induced hyperemia in the passively stretched limb was initially greater in comparison to the contralateral limb; and *3*) this local difference in musculoskeletal hemodynamic response to SS was dependent on the repetitive exposures to SS (number of bouts), indicating that, after a transiently local hyperemia, a peripheral vasoconstriction occurred, presumably triggered by the stretch-induced mechanoreflex.

Interaction between skeletal muscle stretching, autonomic nervous system, and central hemodynamics. The present findings advance the knowledge on the interactions between autonomic nervous system and central hemodynamics in response

Table 2. *Knee joint angle, force output, and peripheral hemodynamics during flexion and extension phases of consecutive bouts of 1-leg SS*

	1st	2nd	3rd	4th	5th
KJA_{SL} ROM, °	112 ± 9	116 ± 11	117 ± 11	118 ± 11	118 ± 1
FO mean (FL), N	52.7 ± 9.7	53.0 ± 9.6	50.7 ± 9.3	51.1 ± 9.7	50.4 ± 10.1
FAD_{SL} mean, mm					
FL	0.85 ± 0.5	0.85 ± 0.4	0.85 ± 0.4	0.85 ± 0.3	0.87 ± 0.3
EX	0.85 ± 0.5	0.85 ± 0.6	0.85 ± 0.3	0.84 ± 0.4	0.84 ± 0.3
FAD_{CL} mean, mm					
FL	0.84 ± 0.4	0.84 ± 0.5	0.84 ± 0.6	0.84 ± 0.4	0.84 ± 0.5
EX	0.84 ± 0.4	0.84 ± 0.5	0.84 ± 0.4	0.85 ± 0.4	0.84 ± 0.5
			FBF_{SL}		
Δpeak, ml/min					
FL	377 ± 95	78 ± 103[c]	−179 ± 99[cd]	−221 ± 89[cde]	−220 ± 91[cde]
EX	464 ± 115	432 ± 123[a]	411 ± 119[a]	525 ± 119[a]	479 ± 111[a]
AUC, ml					
FL	80.6 ± 8.1	−81.5 ± 9.2[c]	−92.3 ± 6.2[cd]	−120.4 ± 9.9[cde]	−121.0 ± 9.8[cde]
EX	73.6 ± 4.1	72.6 ± 5.3[a]	71.6 ± 6.2[a]	84.2 ± 5.9[a]	72.1 ± 5.8[a]
			FBF_{CL}		
Δpeak, ml/min					
FL	−57 ± 48[b]	−132 ± 98[bc]	−160 ± 92[cd]	−153 ± 99[cd]	−190 ± 97[cdef]
EX	114 ± 44[ab]	111 ± 43[ab]	121 ± 42[ab]	133 ± 49[ab]	114 ± 47[ab]
AUC, ml					
FL	−30.2 ± 4.2[b]	−83.9 ± 8.1[c]	−96.7.4 ± 9.2[cd]	−94.3 ± 8.9[cd]	−111.7 ± 8.8[cdef]
EX	23.2 ± 3.3[ab]	23.4 ± 3.1[ab]	25.7 ± 4.1[ab]	27.6 ± 3.9[ab]	22.9 ± 3.8[ab]
			VC_{SL}		
Δpeak, $ml \cdot min^{-1} \cdot mmHg^{-1}$					
FL	3.77 ± 0.05	1.20 ± 0.07[c]	−2.22 ± 0.09[cd]	−2.28 ± 0.08[cd]	−2.67 ± 0.09[cd]
EX	4.82 ± 0.04	4.85 ± 0.05[a]	5.06 ± 0.06[a]	6.16 ± 0.07[a]	5.53 ± 0.08[a]
AUC, $ml \cdot mmHg^{-1}$					
FL	0.84 ± 0.09	−0.54 ± 0.08[c]	−1.20 ± 0.11[cd]	−1.32 ± 0.08[cd]	−1.58 ± 0.08[cd]
EX	0.82 ± 0.08	0.84 ± 0.07[a]	0.85 ± 0.07[a]	0.98 ± 0.09[a]	0.84 ± 0.08[a]
			VC_{CL}		
Δpeak, $ml \cdot min^{-1} \cdot mmHg^{-1}$					
FL	−0.72 ± 0.04[b]	−1.61 ± 0.08[bc]	−1.74 ± 0.09[c]	−1.65 ± 0.08[c]	−2.00 ± 0.08[cdef]
EX	1.59 ± 0.05[ab]	1.52 ± 0.09[ab]	1.70 ± 0.08[ab]	1.89 ± 0.07[ab]	1.63 ± 0.07[ab]
AUC, $ml \cdot mmHg^{-1}$					
FL	−0.30 ± 0.06[b]	−1.02 ± 0.07[c]	−1.07 ± 0.10[c]	−1.04 ± 0.08[c]	−1.21 ± 0.07[cdef]
EX	0.28 ± 0.05[ab]	0.29 ± 0.06[ab]	0.33 ± 0.09[ab]	0.36 ± 0.09[ab]	0.31 ± 0.09[ab]

Data are presented as means ± SE. SS, static stretching; FL, flexion; EX, extension; ROM, range of motion; FO, force output; Δpeak, absolute change; AUC, area under the curve; KJA_{SL}, knee joint angle in stretch leg; FAD_{SL}, femoral artery diameter in stretched leg; FAD_{CL}, femoral artery diameter in control leg; FBF_{SL}, femoral blood flow in stretched leg; FBF_{CL}, femoral blood flow in control leg; VC_{SL}, vascular conductance in stretched leg; VC_{CL}, vascular conductance in control leg. [a]$P < 0.05$ from FL; [b]$P < 0.05$ from stretch leg; [c]$P < 0.05$ from 1st; [d]$P < 0.05$ from 2nd; [e]$P < 0.05$ from 3rd; [f]$P < 0.05$ from 4th.

to SS, during which the sympathetic and parasympathetic activity are partially modulated by the feedbacks that arise from mechanoreceptor activation in the skeletal muscle (1, 10, 42). The data from the present investigation indicate that SS influences the sympathovagal balance. However, the rise of the LF/HF index, describing the sympathovagal balance of the heart, could occur as a result of an increase of the sympathetic activity, a withdrawal of the vagal tone, or a combination of both. Given the decrease of RMSSD, a direct marker of the parasympathetic drive, and the lack of any changes in LF_{SBP}, a marker of the sympathetic activity, it is reasonable that the increase of LF/HF index during SS could be ascribed mainly to the parasympathetic withdrawal in combination with an increase in sympathetic activity (Fig. 2). Similar to the blood pressure effect induced by the exercise pressure reflex, the mean DBP rise found in the present study could be likely due to the increases in intramuscular pressure produced during flexion phases of SS (30). In accordance with a previous study (9), such stretch-induced changes in autonomic nervous system discharge were coupled with significant central hemodynamic responses (Table 1, Fig. 2), supporting the first hypothesis that, not only the cardioacceleration, but also the increases in CO, SV, and MAP are primarily influenced by the parasympathetic withdrawal triggered by the mechanoreflex (9, 30).

Stretch-induced hyper- and hypoemia in the passively stretched and contralateral limb. From the present study, the high-resolution analysis of peripheral circulation of the passively SL revealed a marked hyperemia in response to the first stretching maneuver, which was likely explained by local release of vasoactive substances overcoming sympathetically mediated vasoconstriction that was likely more relevant at the peripheral level compared with the central level (37, 42). Conversely, the concomitant FBF and VC were significantly reduced in the CL. This phenomenon could explain the rise in mean DBP and be likely ascribed to the stretch-induced increases in sympathetic activity, at vascular level, evoked by the mechanoreflex activation triggered by SS in the SL (Table 2 and Fig. 4). Indeed, the present data on the stretch-induced hyperemia are in agreement with previous studies that adopted a similar technical approach (12–14, 26, 38, 42). However, this

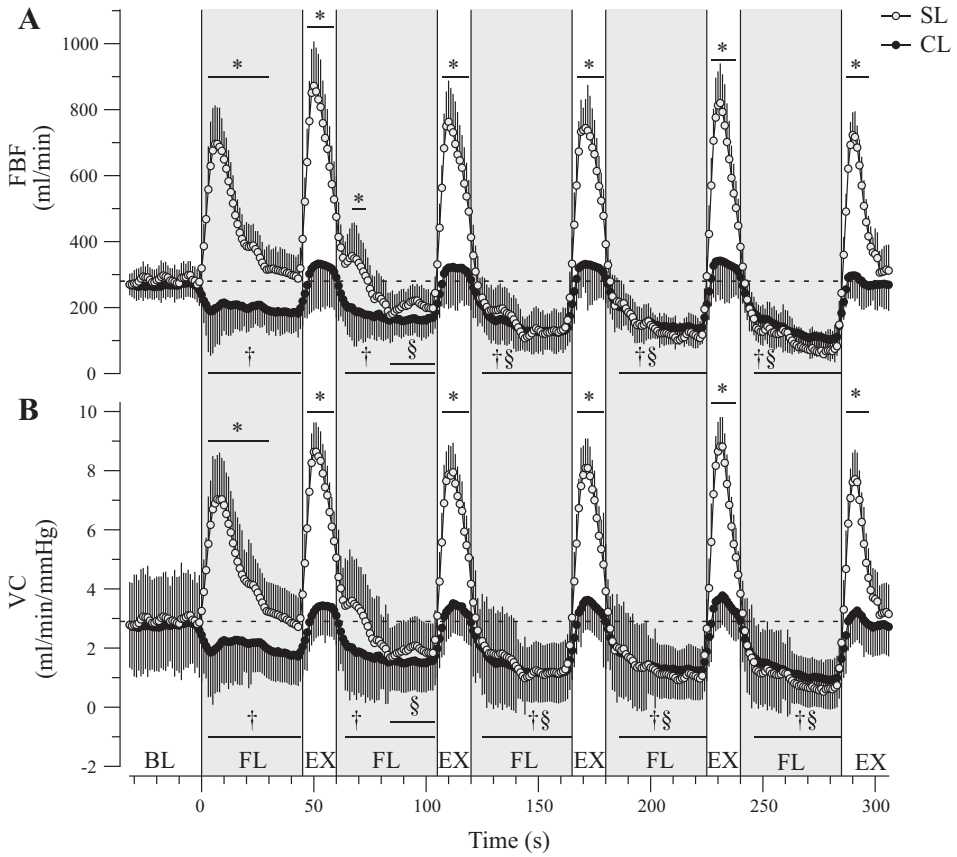

Fig. 4. Changes in femoral blood flow (FBF) and vascular conductance (VC) in the stretched leg (SL) and control resting leg (CL) to 5 sequences of static stretching, at baseline (BL), during knee static passive flexion (FL), and static passive extension (EX), respectively. Data are means ± SE; *significantly increased from BL; †significantly reduced from BL in the CL; §significantly reduced from BL in the SL. Gray areas indicate significantly different values between SL and CL.

hyperemia in the SL is partially in disagreement with the data reported by a recent study (19), in which the investigators observed no detectable change in net blood flow and VC measured at popliteal artery during 5 min of SS on the plantar flexors. This discrepancy could be possibly explained by the volume of the stretched muscle, which, in turn, can generate different NO release and greater hyperemia in a larger muscle (42). However, it could be argued that it would be the same when normalized to muscle mass; thus the concentration of NO in the muscle would be comparable, resulting in similar impact on vasodilation. Alternatively, this discrepancy may be explained by differences in the magnitude of muscle fiber lengthening. Specifically, a change in joint angle of 90° at the knee and ankle may not yield the same change in muscle fiber length of the muscles that span those joints attributable to differences in tendon length and fiber pennation angle. Moreover, in a recent murine study, it has been demonstrated that passive stretching does not increase NO synthase activity in skeletal muscle (17). Therefore, other physiological mechanisms are potentially involved in this phenomenon. To the best of the authors' knowledge, the present investigation is the first study that has documented an SS-induced hypoemia on a remote muscle not involved in the stretching procedure; therefore, a comparison with previous studies is not possible.

Transitory nature of musculoskeletal stretch-induced hyperemia. From the present study, the analysis of peripheral circulation during consecutive bouts of passive quadriceps muscle SS revealed that the hyperemia in response to the first stretching maneuver rapidly disappeared during the second SS procedure. Interestingly, during the third, fourth, and fifth repetitions of SS, the blood flow to the stretched muscle was significantly reduced, overlapping that in the CL. This finding suggests that the hyperemia evidenced in the first SS maneuver was likely mediated by local factors, but, because of the plausible reduced bioavailability of these factors, the systemic sympathetic-mediated vasoconstriction that was activated by the stretch-induced mechanoreflex prevailed thereafter. Because of the transitory and unstable nature of these local vasoactive factors, such as NO, their direct measurements are rather complicated. In the past, NO synthase inhibitor activators were utilized to understand the role of NO during dynamic passive stretching (37). However, to our knowledge, no studies have investigated the role of NO during SS. In a prospective view, future potential studies could explore the individual contributions of peripheral vasodilators, such as NO, and mechanoreceptor activation on this immediate hyperemic response. For instance, the use of NO synthase inhibitor activators and afferent blockade in separate or combined stretching trials would be interesting to compare these influences.

Data from the present study indicate no detectable change in the hyperemia of SL during all five 15-s extensions of the SS protocol. Specifically, this constant FBF response during the extension phases could be likely supported by a mechanical reduction in stretch-induced peripheral resistance and the contribution of some metabolic vasodilatory factors released in response to the reduction of venous return following the muscle stretch of the muscle. Indeed, SS maneuver likely collapsed the venules and veins, thereby reducing venous return. The build of metabolic bioproducts, not attributable to

increased production but rather to decreased clearance, might result in vasodilation and transient hyperemic response observed during the extension phases. These metabolic bioproducts would be quickly washed out, and, therefore, the hyperemic response would be short lived. In a previous investigation (27), it was revealed that FBF was clearly influenced by knee joint angle. In detail, FBF was documented to increase as the knee was extended from the lower (90°) to the middle and upper (0°, full extension) range of knee joint angle. It was concluded that the factors likely involved in this response were muscle length-dependent changes in capillary tortuosity and vessel diameter (24, 33). Overall, our data indicate that, in the absence of the local metabolic perturbation, triggered by voluntary exercise, the balance among neural and local factors contributing to the regulation of skeletal muscle blood flow was likely dependent on the repetitive exposures to SS (number of bouts) and influenced by the reduced bioavailability of local vasoactive factors (e.g., NO) that are released during the initial, passive stretch of the skeletal muscle.

Conclusions. This study documented that heart hemodynamic responses to SS are primarily influenced by the parasympathetic withdrawal rather than the increase in sympathetic activity. The musculoskeletal hemodynamic responses documented in the SL and CL during repetitive exposures to SS suggest an initial limb difference in local circulation response to SS that disappeared during the third repetition of SS. Overall, these results indicate that the balance among neural and local factors contributing to the regulation of musculoskeletal blood flow is dependent on the SS exposure, suggesting that, after a transiently local hyperemia, a systemic sympathetic-mediated vasoconstriction prevailed via the stretch-induced mechanoreflex.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.V., S.R., A.V.B., E.C., and F.E. conceived and designed research; M.V., S.R., E.L., A.V.B., and E.C. performed experiments; M.V., S.R., G.C., E.L., A.V.B., and E.C. analyzed data; M.V., S.R., G.C., E.C., and F.E. interpreted results of experiments; M.V. prepared figures; M.V., G.C., A.V.B., and F.E. drafted manuscript; M.V., G.C., E.C., and F.E. edited and revised manuscript; M.V., S.R., G.C., E.L., A.V.B., E.C., and F.E. approved final version of manuscript.

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