Evidence for improved systemic and local vascular function after long-term passive static stretching training of the musculoskeletal system

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Key points

- Vascular function and arterial stiffness are important markers of cardiovascular health and cardiovascular co-morbidity.
- Transitional phases of hypoemia and hyperemia, with consequent fluctuations in shear rate, occurring during repetitive passive stretching administration (passive stretching training) may constitute an effective stimulus to induce an amelioration in vascular function, arterial stiffness and vascular remodelling by improving central and local blood flow control mechanisms.
- Vascular function, arterial stiffness and vascular remodelling were evaluated before and after 12 weeks of passive stretching training and after 6 weeks from training cessation, in the femoral, popliteal (treated with stretching), and brachial arteries (untreated) of both sides.
- After passive stretching training, vascular function and arterial remodelling improved, and arterial stiffness decreased in all the arteries, suggesting modifications of both central and local blood flow control mechanisms. Passive stretching-induced improvements related to central mechanisms seemed to have a short duration, as they returned to pre-training baseline within 6 weeks from training cessation, whereas those more related to a local mechanism persisted in the follow-up.

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A. V. Bisconti and E. Cé contributed equally to the work.
Abstract Acute passive stretching (PS) effects on blood flow (Q), shear rate (Y), and vascular function in the feeding arteries of the stretched muscle have been extensively investigated; however, few data are available on vascular adjustments induced by long-term PS training. We investigated the effects of PS training on vascular function and stiffness of the involved (femoral and popliteal) and uninvolved (brachial) arteries. Our hypothesis was that PS-induced changes in Q and Y would improve central and local mechanisms of Q control. Thirty-nine participants were randomly assigned to bilateral PS (n = 14), monolateral PS (n = 13) or no PS training (n = 12). Vascular function was measured before and after 12 weeks of knee extensor and plantar flexor muscles’ PS training by single passive limb movement and flow-mediated dilatation (FMD). Central (carotid-femoral artery PWV, PWV_{CF}) and peripheral (carotid-radial artery PWV, PWV_{CR}) arterial stiffness was measured by pulse-wave velocity (PWV), together with systolic (SBP) and diastolic (DBP) blood pressure. After PS training, increases of 30%, 25% and 8% (P < 0.05) in femoral ΔQ, popliteal and brachial artery FMD%, respectively, occurred in both PS training groups. A decrease in PWV_{CF}, PWV_{CR}, SBP and DBP (−25%, −17%, −4% and −8%, respectively; P < 0.05) was noted. No changes occurred in controls. Vascular function improved and arterial stiffness reduced in the arteries involved and uninvolved with PS training, suggesting modifications in both central and local Q control mechanisms. PS-induced improvements had a short duration in some of vascular function parameters, as they returned to baseline within 6 weeks of PS training cessation.

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Introduction

Vascular function (Hotta et al. 2013; Nishiwaki et al. 2015), the ability of an artery to dilate and constrict, is an important marker of cardiovascular health and cardiovascular co-morbidity (Qureshi et al. 2007). Alterations in vascular function often precede an increase in arterial stiffness, which is inversely related to cardiovascular health (Kruse & Scheuermann, 2017). Improving and/or maintaining vascular function is crucial for the prevention of cardiovascular disease (Green et al. 2017b).

Blood flow (Q) distribution throughout body vasculature is strongly influenced by the balance of the sympathetic activity (Sandoo et al. 2010; Thijssen et al. 2014; Venturelli et al. 2019) and localized vaso-dilator mechanisms (Widlansky et al. 2003; Wilson et al. 2016). Recent studies have reported that acute passive static stretching (PS), a well-established practice in rehabilitation and sport to increase joint range of motion (ROM) (Esposito et al. 2011; Kay & Blazevich, 2012; Behm et al. 2015), may have a positive effect on vascular function, arterial stiffness and arterial structure (Cortez-Cooper et al. 2008; Kato et al. 2017; Shinno et al. 2017).

Acute PS administration elicits two opposite responses: vasoconstriction with reduced Q in the feeding artery of the stretched muscle (Venturelli et al. 2019), which is triggered by a systemic increase in sympathetic neural tone (Cui et al. 2006) due to PS-induced stress on muscle mechano- and metabo-receptors (Venturelli et al. 2017a); and vasodilatation with subsequent increase in Q in the feeding artery of the stretched muscle potentially due to the release of endogenous vasoactive substances, as a result of the stretch-induced stress on the vessel’s wall that over-whelms systemic sympathetic vasoconstriction (Venturelli et al. 2017a, 2019). During multiple stretch-relaxation cycles, the first acute hyperaemic response to stretching seems to progressively attenuate until it disappears during subsequent stretching bouts, possibly due to the depletion of vasoactive substances (Venturelli et al. 2017a, 2019). Moreover, the relaxation phase in between two subsequent stretches is always characterized by hyperaemia due to a reduction in peripheral vascular resistance after stretch-induced deformation of the vessel (Venturelli et al. 2019). While the effects of acute PS on Q, and vascular function and structure in the feeding arteries of the stretched muscle have been already extensively investigated, some data are also available on vascular function and arterial stiffness ameliorations induced by long-term PS training (Cortez-Cooper et al. 2008; Kato et al. 2017; Shinno et al. 2017). However, the indirect approach used in the previous studies to assess vascular function changes after PS training did not permit evaluation of possible PS training-induced mechanical remodulations of the arterial wall in the vessels directly involved in PS training. A plausible explanation for these phenomena involves the shear rate (Y), which is the frictional or drag force acting on the vessel’s inner lumen that can trigger a chain of reactions, leading to higher endothelial NO synthase activity (Niebauer & Cooke, 2020).
Continuous, repetitive increases in $\dot{Y}$ induced by PS training may indeed act as a sort of ‘vascular training’ leading to endothelial remodelling (i.e. changes in vascular stiffness and structure), thus improving vascular function, in a manner similar to skeletal muscle training (Green et al. 2017a,b; Bisconti et al. 2019). Noticeably, changes in arterial stiffness may be also attributable, at least in part, to changes in the structural properties of the connective tissue. A biomarker to determine changes in human vascular structure in vivo is the maximal arterial dilatation capacity (Naylor et al. 2005), which can be induced by hyperaemia in response to ischaemic exercise (Naylor et al. 2005).

Acute, during a bout of PS, a reduction in $Q$ in the contralateral, unstretched limb was found to promptly recover during the relaxation phase (Venturelli et al. 2019). The authors suggested that recovery was induced by systemic sympathetic-mediated vasoconstriction activated by the stretch-induced mechanoreflex (Venturelli et al. 2019). When PS is applied chronically over a long period, repeated stimulations of the vessel wall (alternating vasoconstriction and vasodilatation) may induce changes in the systemic autonomic control of $Q$ distribution, possibly leading to an increase in vascular function. It is still debated, however, whether PS training may also affect the vascular function in the feeding artery of the contralateral muscle, which is not directly involved in stretching. Together with changes in local control mechanisms, possible PS training-induced changes in the systemic autonomic control of $Q$ distribution have also been reported (i.e. reduced blood pressure and aortic wave reflection magnitude (Wong & Figueroa, 2014)), although its effectiveness remains controversial (Farinatti et al. 2011; Hotta et al. 2013; Williams et al. 2013).

Vascular training, i.e. possible PS training-induced changes in local and systemic mechanisms underlyng vascular function, may have practical implications for maintaining or even improving cardiovascular health in people with limited mobility and/or while bedridden. With this in mind, we investigated the effects of PS training on vascular function and stiffness of the arteries directly involved (i.e. femoral and popliteal arteries) and not directly involved (i.e. contralateral femoral and popliteal arteries and brachial artery) in manoeuvres applied to the plantar flexor, knee extensor and hip flexor muscles. To do this, we measured vascular function and arterial stiffness before and after 12 weeks of PS training. Our hypothesis was that repetitive fluctuations in $Q$ and $\dot{Y}$ during PS bouts would provide effective stimulus to affect the central and local $Q$ control mechanisms, thus improving vascular function and arterial stiffness not only in the feeding arteries of the stretched muscle, but also in those districts not directly involved in PS training.

**Methods**

**Ethical approval**

All participants provided written, informed consent after being informed about the aims of the study and the potential risks derived from tests and methods. The Institutional Review Board of the Università degli Studi di Milano approved the study (CE 27/17). The study was registered at ClinicalTrial.gov (ID: NCT04271241) and performed in accordance with the principles of the Helsinki Declaration. The researchers who analysed the data were blinded to group allocation.

**Participant recruitment**

Figure 1 illustrates the study flow chart. A total of 54 healthy adults volunteered to participate in the study. Exclusion criteria were: neurological, vascular and musculoskeletal disorders of the lower and upper limbs; being on pharmacological therapy related to either neural and/or vascular response, including hormonal contraceptives and oral supplements; being a current or former smoker; having an irregular menstrual cycle (26 to 35 days) up to 3 months before the beginning of the study; contraindications to joint mobilization; regular involvement in a PS training programme. Based on previous research (Hotta et al. 2013; Nishiwaki et al. 2015; Venturelli et al. 2017a), for this study we used changes in blood flow as main outcomes, a two-way analysis of variance (ANOVA; within-group factor: time; between-groups factor: intervention) in the statistical approach, with an $\alpha$ level of 0.05 and a required power ($1-\beta$) of 0.80; the desired sample size, computed using statistical software (G-Power 3.1, Dusseldorf, Germany) resulted in 36 participants. Accordingly, the study sample was 39 participants (19 females and 20 males, age $23 \pm 2$ years, body mass $69 \pm 3$ kg, stature $1.68 \pm 0.11$ m, mean $\pm$ standard deviation (SD)) were enrolled and randomly assigned to one of three groups: bilateral PS ($PS_{bil}$; $n=14$ (7F/7M), age $23 \pm 2$ years, body mass $68 \pm 4$ kg, stature $1.68 \pm 0.09$ m, ankle ROM $25 \pm 3$ deg, knee ROM $145 \pm 9$ deg); monolateral PS ($PS_{mono}$; $n=13$ (6F/7M), age $22 \pm 2$ years, body mass $70 \pm 3$ kg, stature $1.71 \pm 0.11$ m, ankle ROM $24 \pm 4$ deg, knee ROM $145 \pm 12$ deg), and control group (no stretching, Ctr: $n=12$ (6F/6M), age $23 \pm 2$ years, body mass $69 \pm 3$ kg, stature $1.70 \pm 0.08$ m, ankle ROM $26 \pm 6$ deg, knee ROM $146 \pm 12$ deg).

**Study design**

Before testing, a preliminary session was conducted for familiarizing the participants with the procedures to identify maximum isometric voluntary contraction (MVC) of the knee extensor and plantar flexor muscles of both limbs.
Measurements were taken bilaterally: pulse wave analysis (PWA) and pulse wave velocity (PWV) were measured at the femoral and the radial artery by photo-plethysmography as an indirect marker of arterial stiffness (Doupis et al. 2016); single passive limb movement (sPLM), flow-mediated dilatation (FMD), and hypoxic exercise (HEx) tests were performed on the femoral, the popliteal and the brachial arteries, respectively. Knee and ankle flexion ROM were measured.

On completion of measurements, the ultrasound probe position was marked on a transparency sheet, together with skin landmarks (moles, scars, angiomas, etc). Results of the familiarization and the pre-training experimental sessions were used to calculate intersession reliability. All measurements were taken at the beginning (Pre), at 6 weeks (week 6), 12 weeks of PS training (week 12), and then again at 6 weeks post-intervention (follow-up). The participants were tested at the same time of the day in a climate-controlled laboratory (temperature 20 ± 1°C and relative humidity 50 ± 5%) to minimize confounders due to circadian rhythms. For the women, all the tests were conducted on the same day of the menstrual cycle (early follicular phase days 3 ± 3). The female participants recorded their menstrual cycle in a personal...
diary throughout the study, which was used to assess the early follicular phase and allowed the women to be tested on the same menstrual day. On the test days, the participants came to the laboratory after having fasted overnight and refrained from caffeine and other similar substances for at least 12 h and from intensive exercise for at least 48 h prior to the tests. The two stretching groups (PSBil and PSMono) underwent 12 weeks of PS training, 5 sessions per week (60 sessions in total). Each PSBil session lasted 40 min and included two exercises for the knee extensor and the plantar flexor muscles: 45 s elongation and 15 s recovery in the resting position; the set was repeated five times (Venturelli et al. 2019). In the PSMono sessions the exercises were performed using only the right limb and lasted 20 min. The Ctrl group received no PS training (Fig. 2 gives an example of the PS training exercises). To enhance compliance, daily classes were held at different times of the day (morning and afternoon) at the University Sports Centre gym. Each class was led by an expert supervisor who monitored attendance, correct exercise execution and intensity (80% of the point of discomfort) (C`e et al. 2020). Participants failing to attend at least 80% of classes were excluded from the study, and a new participant was recruited to substitute the drop out.

Since the increase in $\dot{V}$ occurring during PS has been advocated as the mechanism possibly triggering the improvements in vascular function, a subsample of 20 participants (10F/10M), age 22 $\pm$ 1 years, body mass 69 $\pm$ 4 kg, stature 1.71 $\pm$ .012 m, ankle ROM 23 $\pm$ 4 deg, knee ROM 143 $\pm$ 13 deg) underwent a third session during which the femoral and popliteal artery $\dot{V}$ were calculated during a stretching bout involving the knee extensor and plantar flexor muscles.

### Measurements and data analysis

Measurements were performed bilaterally in all groups. Data for the PSBil group are presented as the average between the two limbs, while the data for the PSMono group are presented separately for the stretched (PSMonoSL) and the contralateral limb (PSMonoCL); this was done to detect any possible crossover effect. The data for the upper limbs were calculated as the average of the two limbs.

### Range of motion (ROM).
To monitor the changes in knee and ankle joint ROM, a bi-axial electrogoniometer (TSD 130A, Biopac System, Goleta, CA, USA) was utilized. To measure knee ROM, the electrogoniometer was placed with one axis on the external condyles of the knee joint and the other on the external face of the fibula; to measure ankle joint ROM, the instrument was positioned on the external face of the fibula and on the calcaneum.

### Maximum isometric voluntary contraction (MVC).
MVC of the knee extensor muscles was measured with the participant supine on an ergometer and the knee flexed at 90 deg and firmly secured at the ankle with a Velcro strap (Velcro Industries Inc., Manchester, NH, USA) to a load cell (SM-2000N operating linearly between 0 and 2000 N; Interface, Crowthorne, UK) for force signal detection. The MVC of the plantar flexor muscle was measured with the participant prone on the ergometer, with the foot fixed by a Velcro strap to a mobile metal plate instrumented with a load cell (SM-2000 N, Interface). The hips and shoulders were firmly secured to the ergometer. After a standardized warm-up (10 $\times$ 2-s contractions at 50% MVC determined during familiarization), three MVC trials were performed interspersed by at least 3 min of recovery. The participants
were instructed to push as fast and hard as possible for 3 s. The force signal was transmitted to an A/D converter (UM 150, Biopic, Biopac Systems Inc., Goleta, CA, USA), sampled at a fixed sampling rate of 1000 Hz, and stored on a personal computer. The maximum force recorded during the three trials was defined the MVC and entered in the data analysis.

**Pulse wave analysis (PWA) and velocity (PWV).** Blood pressure wave analysis and arterial stiffness were measured by determining the PWA and PWV using an anplanation tonometry technique (Doupis *et al.* 2016). PWA and PWV were measured using a SphygmoCor PX (Atcor Medical Blood Pressure Analysis System, Sydney, Australia) after the participant had rested for at least 20 min in supine position.

Blood pressure wave parameters, i.e. systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), aortic augmentation (AA), augmentation index (Alx), Alx corrected for heart rate of 75 beats·min\(^{-1}\) (Alx75), and tension time index referred to the systolic tension time index (TTI) were measured by means of anplanation tonometry of the radial artery, as described elsewhere (O’Rourke, 1999). Radial artery pressure wave amplitude was recorded non-invasively with a pencil-type probe (tonometer) placed over the right radial artery of the wrist. After a reproducible signal was obtained, 20 sequential waveforms covering a complete respiratory cycle were acquired from the system and used by the software to generate an average peripheral and a line peripheral haemodynamic data collection, followed by a single passive knee flex-extension and not a continuous movement as in the traditional PLM. In sPLM, central haemodynamic responses are minimized, but the manoeuvre still facilitates the assessment of peripheral vascular function. Indeed, sPLM in comparison to PLM, did not evoke a measurable increase in either heart rate or cardiac output, probably as a consequence of the minimal afferent feedback due to the brevity of the sPLM manoeuvre (Venturelli *et al.* 2017b). sPLM was performed in accordance with recommended procedures (Trinity *et al.* 2012; Venturelli *et al.* 2017b; Bisconti *et al.* 2019). Participants rested in an upright-seated position for 10 min before data collection started and remained in this position until the end of the test. The sPLM protocol consisted of 30 s of baseline peripheral haemodynamic data collection, followed by a single passive knee flexion and extension of 1 s, after which the leg was maintained fully extended for the remaining 59 s for post-movement data collection. sPLM was performed by a member of the research team, who moved the participant’s leg through a 90 deg ROM at 1 Hz. Arterial blood velocity (\(V_{\text{mean}}\)) and vessel diameter (\(D\)) were measured at the common femoral artery of the passively moved leg, distal to the inguinal ligament and proximal to the deep and superficial femoral bifurcation by Doppler ultrasound (Logiq-7, General Electric Medical Systems, Milwaukee, WI, USA). Mean blood velocity was measured with a 9 MHz linear array transducer positioned an insonation angle of 60 deg. The sample volume was

To ensure a stable, artefact-free ECG, the skin was properly prepared (hair removed at the electrode site and the skin cleaned with an alcohol wipe). A minimum of 20 s of signal was recorded after a strong, accurate and reproducible pulse wave signal was obtained. The distance from the carotid to the femoral or the radial artery was measured directly between each artery and the suprasternal notch. The measurements were entered into the SphygmoCor software database. PWV was calculated by measuring the time delay between two characteristic time points (i.e. carotid-femoral arteries, PWV\(_{\text{CF}}\), or carotid and radial arteries, PWV\(_{\text{CR}}\)) on two pressure waveforms at a known distance apart. The SphygmoCor method uses the foot of the waveform as an onset point for calculating the time differences between the R wave of the ECG and the pulse waveforms at each site. PWV was automatically calculated by the SphygmoCor software as PWV\(_{\text{CF}}\) or PWV\(_{\text{CR}}\) distance divided by the wave travelling time between the two measurement sites (Pauca *et al.* 2001; Qureshi *et al.* 2007; Doupis *et al.* 2016). PWV\(_{\text{CF}}\) and PWV\(_{\text{CR}}\) were used as central and peripheral arterial stiffness indexes, respectively. PWA and PWV measurements were taken at least 3 times, obtaining an operator index ≥95%, and are expressed as the average.
centred and size-maximized according to vessel diameter (Trinity et al. 2012). Femoral \( Q (Q_{fem}) \) was calculated at baseline and at peak after single passive knee flexion and extension by calculating the \( D \) and mean blood velocity as:
\[
Q_{fem}(\text{ml} \times \text{min}^{-1}) = V_{\text{mean}} \times \pi \times (\text{vessel } D/2)^2 \times 60.
\]

The cumulative \( Q_{fem} \) was integrated (area under the curve, AUC) using the trapezoidal rule and then calculated.

**Flow-mediated dilatation (FMD).** FMD was measured at the popliteal and the brachial artery according to recommended procedures (Harris et al. 2010; Bisconti et al. 2018, 2019). Before FMD, the participants lay supine for approximately 20 min to restore baseline cardio-vascular values. An arterial pressure cuff was placed around the calf muscles (popliteal artery) and on the forearm immediately distal to the olecranon process (brachial artery) to generate an ischaemic stimulus when inflated. Following baseline assessment, the blood pressure cuff was inflated to 280 mm Hg for the popliteal and 250 mm Hg for the brachial artery for 5 min. Arterial \( D \) and \( V_{\text{mean}} \) recordings resumed at baseline, 30 s prior to cuff deflation and continued for 2 min post-deflation, as described elsewhere (Corretti et al. 2002; Harris et al. 2010; Wray et al. 2013). A 9-MHz linear array and a 15-MHz linear array transducer attached to a high-resolution ultrasound machine were used to image the popliteal artery in the distal third of the leg and the brachial artery in the distal third of the upper arm. When an optimal image was obtained, the probe was held stable and longitudinal in B-mode, and images of the lumen-arterial wall interface were acquired. Continuous Doppler velocity was measured, and the data were collected using the lowest possible insonation angle (<60 deg). The FMD data were exported in AVI format and analysed using commercially available software (Brachial Artery Analyzer for Research, Medical Imaging Applications, LLC, Coralville, IA, USA), which is largely independent of investigator bias. FMD was quantified as the maximal change in artery diameter after cuff release, expressed as a percentage increase above baseline:
\[
(D_{\text{peak}} - D_{\text{bas}}) / D_{\text{bas}} \times 100.
\]

Popliteal \( (\hat{Q}_{\text{pop}}) \) and brachial artery blood flow \( (\hat{Q}_{\text{brac}}) \) was calculated as described for sPLM measurement. Popliteal and brachial artery \( \hat{Y} \) was calculated post-cuff release with the equation:
\[
\text{shear rate } (s^{-1}) = 8V_{\text{mean}}/D
\]

The cumulative \( \hat{Y} \), corresponding to the reactive hyperaemia post-cuff release (total \( \hat{Y} \) from cuff release to time-to-peak, \( \hat{Y} \) AUC) was calculated using the trapezoidal rule.

The \( \hat{Y} \) AUC reflects the amount of mechanical stimulus applied to the endothelium during cuff release hyperaemic response until time-to-peak. Given that FMD is primarily dependent on endothelial response to mechanical stimuli, the FMD was divided by the \( \hat{Y} \) AUC (FMD/ \( \hat{Y} \) (Pyke & Tschakovsky, 2005; Padilla et al. 2008).

**Artery dilatation response to ischaemic exercise (IEx).** In order to detected possible arterial structural changes, popliteal and brachial artery dilatation after IEx was tested by applying a protocol described elsewhere (Naylor et al. 2005). IEx was performed after a further 30 min of rest and consisted in 5 min of ischaemia, during which a voluntary isometric contraction of the plantar flexor muscles (popliteal artery) or the handgrip muscles (brachial artery) was over-impressed. The force output was standardized at 3 kg; the contractions lasted 1 s and were performed every 3 s over 3 min (Naylor et al. 2005). As done in the FMD tests, arterial \( D \) and \( V_{\text{mean}} \) were recorded during IEx at baseline, 30 s prior to cuff deflation, and continued for 5 min post-deflation. The same parameters as in the FMD tests were calculated.

**Acute passive stretching.** The participants rested in a supine position during stretching for the knee extensor (Venturelli et al. 2019) and in a prone position for plantar flexor muscles (Kruse et al. 2016) for 20 min before starting the data collection and remained in this position throughout the entire duration of the data collection. The two stretching bouts were separate by 60 min rest, during which the participant remained at rest on a medical bed. PS protocol consisted of 5 min of resting baseline followed by passive static elongations for 45 s and passive relaxations for 15 s, repeated five times. During the entire PS protocol, the muscles were stretched by the same operator up to a point of discomfort similar to those required during the PS training. The knee and ankle joint angles were continuously recorded using a biaxial goniometer (model no. TSD 130A; Biopac Systems). Force output between the passively stretched leg 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 or on the metatarsum of the stretched limb, and a member of the research team pushed perpendicularly the load cell to stretch the leg extensor for 45 s. The passive force output during the 45 s of the consecutive flexion of the PS protocol was displayed on a PC screen so as to maintain constant the passive force throughout the elongations. During the two stretching bouts, the artery’s \( D \) and the antegrade and retrograde \( V_{\text{mean}} \) were measured from the femoral and the popliteal artery. Antegrade, retrograde and mean \( \hat{Y} \) during the 5 elongations and relaxation phases were then calculated as in the FMD tests.
Results

Statistical analysis

Statistical analysis was performed using a statistical software package (IBM-SPSS Statistics v. 26, Armonk, NY, USA). The Shapiro-Wilk test was used to check normal distribution of the data. To determine inter-session reliability in vascular function parameters, intra-class correlation coefficient (ICC) and percentage change of the standard error of the measurement (SEM%) were calculated. The ICC was interpreted as follows: >0.90, very high; 0.89–0.70, high; 0.69–0.50, moderate (Munro, 2004). The minimal detectable change at 95% confidence interval (MDC95%) was used to detect the sensitivity of the effects on vascular function before and after the stretching intervention (Donoghue et al. 2009). To assess significant effects of stretching, two-way ANOVA (within-group factor: time, 4 levels (Pre, week 6, week 12, and follow-up); between-groups factor: intervention, 4 levels (Ctrl, PSBil, PSMonoSL, PSMonoCL)). To calculate the difference in changes between the groups, analysis of covariance (ANCOVA) was performed, entering the baseline (Pre) values as covariate. A one-way ANOVA for repeated measures (within-group factor: time, 5 levels) was applied to check for differences in ROM and MVC during the study in all groups.

Acute passive stretching

Table 2 provides the changes in antegrade, retrograde, and mean $\dot{Y}$ of the femoral and popliteal artery occurring during the 5 elongations and relaxations of the acute passive stretching bout involving the knee extensor and plantar flexor muscles, respectively. Independently from the artery, during the elongation phase the antegrade and mean $\dot{Y}$ increased during the first stretch ($P < 0.001$) and then decrease from baseline ($P < 0.001$), while the retrograde $\dot{Y}$ increased ($P < 0.001$). On the contrary, during the relaxation phase the antegrade and mean $\dot{Y}$ increased ($P < 0.001$) and retrograde $\dot{Y}$ decreased ($P < 0.001$).

PWA and PWV

Figures 3 and 4 present PWA and PWV measurements. ANOVA disclosed a significant time × intervention interaction for SBP ($F = 2.25$, $p = 0.02$), DBP ($F = 8.18$, $P < 0.001$), MAP ($F = 9.12$, $p = 0.002$), HR ($F = 3.15$, $p = 0.002$), PP ($F = 5.67$, $P < 0.001$), AIX75 ($F = 3.04$)

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p = 0.011), TTI (F = 4.48, p = 0.001), PWV_{CF} (F = 4.09, P < 0.001), and PWV_{CR} (F = 4.47, P < 0.001).

A main effect for time was found for SBP (F = 6.81, p = 0.001), DBP (F = 100.15, P < 0.001), MAP (F = 5.01, P < 0.001), HR (F = 5.24, P < 0.001), PP (F = 5.87, P < 0.001), Alx75 (F = 8.21 P < 0.001), AA (F = 7.19, P < 0.001), and PWV_{CR} (F = 6.94, P < 0.001).

SBP, DBP and MAP were decreased significantly in the PS_{bil} group at week 6 (MAP: −4.6 ± 0.9 mmHg, d = −1.06 (−1.95/−0.17), p = 0.004) and in the PS_{mono} group at week 12 (MAP: −4.8 ± 1.0 mmHg, d = −1.20 (−2.15/−0.25), P < 0.001) and returned to baseline at follow-up assessment. No changes in HR were observed in any group. PP, Alx75 and TTI were decreased in the PS_{bil} and the PS_{mono} group at week 12 (TTI: PS_{bil}: −23 ± 5.6 ms, d = −1.70 (−2.64/−0.77), P < 0.001; PS_{mono}: −12 ± 6.6 ms, d = −0.74 (−1.57/−0.08), p = 0.004). PP remained reduced at follow-up, whereas Alx75 and TTI returned to baseline values. AA was decreased in the PS_{bil} and the PS_{mono} group at week 6 (PS_{bil}: −2.4 ± 0.6 mmHg, d = −1.59 (−2.51/−0.67), p = 0.003; PS_{mono}: −2.5 ± 0.9 mmHg, d = −1.14 (−2.00/−0.28), p = 0.002) and at week 12 (PS_{bil}: −2.9 ± 0.7 mmHg, d = −1.88 (−2.84/−0.92), P < 0.001; PS_{mono}: −3.7 ± 0.9 mmHg, d = −1.72 (−2.65/−0.78), P < 0.001) and returned to baseline at follow-up. PWV_{CF} and PWV_{CR} were significantly decreased in all PS training groups at week 6 and week 12, respectively (P < 0.001). They remained reduced in the PS_{bil} and PS_{mono} group at follow-up (p = 0.002 and 0.005). Changes in SBP, DBP and MAP at any time were greater in the PS_{bil} and the PS_{mono} group than in the Ctrl group (P < 0.001). The changes in PWA and in PWV_{CR} at week 12 were greater in the PS_{bil} and the PS_{mono} group than in the Ctrl group (P from 0.005 to <0.001).

### sPLM

Figure 5 presents the results of femoral artery vascular function testing. ANOVA disclosed significant time × intervention interactions for \( Q_{fem,bas} \) (F = 6.11, P < 0.001), \( Q_{fem,peak} \) (F = 5.37, P < 0.001), \( \Delta Q_{fem} \) (F = 5.54, P < 0.001), and AUC (F = 6.66, P < 0.001). Main effects for time were found for \( Q_{fem,bas} \) (F = 5.34, P < 0.001), \( Q_{fem,peak} \) (F = 7.82, P < 0.001), \( \Delta Q_{fem} \) (F = 7.56, P < 0.001), and AUC (F = 8.15, P < 0.001). In all PS training groups, the other parameters except for
Table 2. Changes in the antegrade, retrograde and mean shear rate values in the femoral and popliteal artery during the elongation and relaxation phases of the acute stretching bout administered to the knee extensor and plantar flexor muscle

<table>
<thead>
<tr>
<th>Shear rate (s⁻¹)</th>
<th>Elongation phase</th>
<th>Relaxation phase</th>
<th>One-way ANOVA RM</th>
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<tr>
<td></td>
<td>Baseline</td>
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<tr>
<td>Femoral artery</td>
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<tr>
<td>Antegrade</td>
<td>79 ± 17</td>
<td>117 ± 14</td>
<td>70 ± 17</td>
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<tr>
<td>Retrograde</td>
<td>−3 ± 1</td>
<td>−6 ± 3</td>
<td>−5 ± 2</td>
</tr>
<tr>
<td>Mean</td>
<td>76 ± 15</td>
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<td>65 ± 15</td>
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<td>Popliteal artery</td>
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<tr>
<td>Antegrade</td>
<td>47 ± 5</td>
<td>70 ± 14</td>
<td>42 ± 9</td>
</tr>
<tr>
<td>Retrograde</td>
<td>−5 ± 1</td>
<td>−6 ± 2</td>
<td>−7 ± 3</td>
</tr>
<tr>
<td>Mean</td>
<td>42 ± 4</td>
<td>64 ± 13</td>
<td>35 ± 8</td>
</tr>
</tbody>
</table>

*P < 0.05 higher than baseline; †P < 0.05 lower than baseline (n = 20). Data are presented as mean ± standard deviation.

D_fem,bas were significantly increased at week 6 (ΔQ_fem PSBil: +52 ± 11.2 ml·min⁻¹, d = 1.34 (0.67/1.77), P < 0.001; ΔQ_fem PSMono: +31 ± 5.6 ml·min⁻¹, d = 1.14 (0.28/2.00), P < 0.001) and remained elevated up to follow-up (P < 0.001 for all parameters). Changes in Q_fem,bas at week 6 and week 12 for the PSBil and the PSMono SL group were greater than for the Ctrl group (P from 0.003 to < 0.001). Changes in Q_fem,peak were greater for the PSBil and the PSMono SL group than for the Ctrl and the PSMono CL group (P from 0.004 to < 0.001). Changes in ΔQ_fem were greater for the PSBil and the PSMono SL group than for the Ctrl group at any time and at week 12 and follow-up than for the PSMono CL group (P from 0.01 to < 0.001). Changes in AUC_fem were greater at week 6 for the PSBil and the PSMono SL group than for the Ctrl group, and at week 12 for the PSBil, the PSMono SL, and the PSMono CL than for the Ctrl group (P from 0.009 to < 0.001).

FMD

Figures 6 and 7 present FMD test results for the popliteal and the brachial artery, respectively. For the popliteal and the brachial artery, significant time × interventions interaction were observed in Dbas (F = 7.03 and 6.57, P < 0.001), Dpeak (F = 4.58 and 4.46, P < 0.001), FMD% (F = 2.34 and 2.80, p = 0.017 and 0.005), AUC (F = 7.40 and 7.47, P < 0.001), Y_AUC (F = 3.59 and 3.91, P < 0.001), FMD/Y (F = 2.56 and 2.02, p = 0.010 and 0.042), and Q_peak (F = 7.11 and 7.35, P < 0.001). Main effects for time were found in Dbas (F = 6.27 and 6.62, P < 0.001), Dpeak (F = 7.02 and 7.44, P < 0.001), FMD% (F = 3.56 and 8.99, p = 0.022 and < 0.001), AUC (F = 7.37 and 7.41, P < 0.001), Y_AUC (F = 7.07 and 7.35, P < 0.001), and Q_peak (F = 7.34 and 6.73, P < 0.001).

D_pop,bas and Q_pop,bas were significantly increased in the PSBil and the PSMono SL group at week 12 (P from 0.01 to 0.04). D_pop,peak and FMD_pop% (p = 0.002) were significantly increased in the PSBil and the PSMono SL group at week 6 (D_pop,peak PSBil: +0.16 ± 0.024 mm, d = 0.89 (0.22/1.52), P < 0.001; D_pop,peak PSMono SL: +0.17 ± 0.015 mm, d = 0.99 (0.31/1.71), P < 0.001) in the PSMono CL group at week 12 (D_pop,peak PSMono CL: +0.18 ± 0.06 mm, d = 0.71 (−0.11/1.54, p = 0.02), before returning to baseline at follow-up. Q_pop,peak and AUC_pop were increased in the PSBil and the PSMono SL group at week 6 (Q_pop,peak PSBil: +33 ± 1.5 ml·min⁻¹, d = 1.21 (0.57/1.72), P < 0.001; Q_pop,peak PSMono SL: +34 ± 2.0 ml·min⁻¹, d = 1.24 (0.62/1.81), P < 0.001) and week 12 in the PSMono CL group up to follow-up (Q_pop,peak PSMono CL: +29 ± 2.6 ml·min⁻¹, d = 1.01 (0.45/1.44, p = 0.003), Y_AUC was decreased in the PSBil and the PSMono SL group at week 6 until follow-up (Y_pop AUC PSBil: −2.91 ± 0.61 s⁻¹, d = −0.74 (−1.56/−0.09), p = 0.03; Y_pop AUC PSMono SL: −2.57 ± 0.78 s⁻¹, d = −0.76 (−1.59/−0.60), p = 0.005). Except for D_pop,bas changes in all the other parameters were greater at week 6 and week 12 in the PSBil and the PSMono SL group than in the Ctrl group (P from 0.004 to < 0.001).

D_brach,bas and Q_brach,bas remained unchanged in all groups, whereas D_brach,peak was significantly increased in the PSBil and the PSMono SL at week 12 until follow-up.
(\(D_{\text{brach, peak PSBil}}: +0.22 \pm 0.041 \text{ mm, } d = 0.88 (0.04/1.71), p = 0.004; D_{\text{pop, peak PSMono SL}}: +0.18 \pm 0.056 \text{ mm, } d = 0.82 (0.01/1.65), p = 0.007\)). FMD_{\text{brach, %}} was increased at week 12 in the PSBil and the PSMono \((p = 0.006\) and 0.01), before returning to baseline at follow-up. \(Q_{\text{brach, peak}}\) and AUC_{\text{brach}} were increased in the PSBil and the PSMono group at week 12 up to follow-up \((p \text{ from 0.02 to 0.002}). \ Y_{\text{brach}}\) AUC was decreased in the PSBil and the PSMono group at week 12, returning to baseline at follow-up \((Y_{\text{pop}, AUC PSBil: } -30 \pm 5.1 \text{ s}^{-1}, d = -1.21 (-2.08/-0.34), p = 0.003; Y_{\text{pop}, AUC PSMono SL: } -23 \pm 5.1 \text{ s}^{-1}, d = -1.30 (-2.18/-0.34), p = 0.001))\). FMD/\(Y_{\text{brach}}\) was increased in the PSBil and the PSMono group in week 12 returning to baseline at follow-up \((p = 0.002\) to 0.004). Changes in \(Y_{\text{brach}}\) AUC were greater for the PSBil and the PSMono than for the Ctrl. group at week 12 and follow-up \((P < 0.001 \text{ for both comparisons}).\)

### Pulse wave analysis

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<th>PSMono</th>
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<tr>
<td>Pulse pressure (mmHg)</td>
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<td><img src="image8" alt="Graph" /></td>
<td><img src="image9" alt="Graph" /></td>
</tr>
<tr>
<td>Augmentation index (75 \text{ bpm}) (%)</td>
<td><img src="image10" alt="Graph" /></td>
<td><img src="image11" alt="Graph" /></td>
<td><img src="image12" alt="Graph" /></td>
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</tbody>
</table>

**Figure 3. Pulse wave analysis**

Individual data for systolic blood pressure, diastolic blood pressure, pulse pressure, and augmentation index normalised at 75 beats min\(^{-1}\) in the control (Ctrl), the bilateral passive stretching (PSBil), and the unilateral passive stretching group (PSMono) at baseline (Pre), at weeks 6 and 12, and at week 6 of follow-up. Histograms report the percentage changes in respect to Pre for the systolic pressure, systolic blood pressure, diastolic blood pressure, pulse pressure and augmentation index normalised at 75 beats min\(^{-1}\) in the control (Ctrl), the bilateral passive stretching (PSBil), and the unilateral passive stretching group (PSMono) at weeks 6 and 12, and at week 6 of follow-up (*\(P < 0.05\) vs Pre, #\(P < 0.05\) vs Ctrl).
IEx

Figure 8 reports the IEx test results for the popliteal and the brachial artery, respectively. Time × interventions interaction was observed in the brachial artery the IEx% (F = 2.89, p = 0.004). In the popliteal and the brachial artery, main effects for time were found in IEx % (F = 3.47 and 8.90, p = 0.024 and < 0.001). IEx%pop, was significantly increased at week 12 in the PSbil, the PSMono-SL, and the PSMonoCL group (P from 0.005 to < 0.001), before returning to baseline at follow-up. IEx%pop was increased in all groups at week 12 (P from 0.004 to 0.003), before returning to baseline at follow-up. Changes in IEx%pop and IEx/Ypop were greater for the PSbil and the PSMonoSL than for the Ctrl group at week 12 (P from 0.003 to < 0.001).

IEx%brach was increased in the PSbil and the PSMono group (P < 0.001 for both groups) at week 12 before returning to baseline at follow-up. IEx/Ybrach was increased in all groups at week 12 (P from 0.005 to 0.003), before returning to baseline at follow-up. Changes in IEx%brach and IEx/Ybrach were greater for the PSbil and the PSMono than for the Ctrl group at week 12 (p = 0.003 and to 0.004, respectively).

Correlations

Table 3 provides the correlations between the changes in ankle and knee joint ROM and in PWA, PWA, and in the variables calculated in sPLM, FMD and IEx tests. Inverse correlations ranging from moderate to high were found between ankle and knee joint ROM and the DBP, PP, AIX75, PWVCF and PWVCR. Direct correlations spanning from moderate to high were retrieved between ankle and knee joint ROM and several variables in sPLM, FMD and IEx variables.

Discussion

Improvement in blood pressure, arterial stiffness and vascular function was noted in the arteries of the body parts directly and not directly involved in PS training of the lower limbs. Blood pressure was decreased, central and peripheral arterial stiffness was reduced, and vascular function was increased after 12 weeks of PS training. Such changes suggest PS training-induced local and systemic cardiovascular adjustments. Interestingly, systemic changes, in particular in the vessels not directly
involved in PS training, seemed to have a shorter duration in comparison to local adaptations, which are maintained in the arteries directly involved in PS training even after 6 weeks from its cessation.

**Preliminary considerations**

After PS training, ankle and knee joint ROM was increased in the stretched but not in the contralateral, unstretched limb. Increased ROM with stretching is a well-described phenomenon that is more likely the result of a rise in stretch tolerance (reduced mechanoreceptor and nociceptor activity during stretching) of the muscles than a decrease in muscle/tendon stiffness or a change in muscle architecture (Freitas et al. 2018). At follow-up assessment 6 weeks after stretching cessation, ROM was noted to have returned to pre-training levels. As this is the first study to investigate the effects of chronic PS cessation on joint ROM, no comparison with previous studies can be made. Furthermore, our observation of no effect of PS training on maximum isometric force of plantar flexor and knee extensor muscles is shared by previous reports (Medeiros & Lima, 2017).

**PS training effects on blood pressure and central and peripheral arterial stiffness**

We observed positive readjustment in blood pressure and central and peripheral arterial stiffness after PS training. Arterial stiffness was decreased in both limbs of the PS training groups, albeit with some time differences between central and peripheral readjustment: PWV\text{CE} was decreased starting at week 6 of PS training, while PWV\text{CR} and other blood pressure parameters were reduced starting at week 12. Several mechanical and neural adaptations may elucidate these changes. The reduction in central artery stiffness may be explained by the reduction in central pressure in view of the strong two-way street relation between the two variables. Indeed, PS training, by inducing repetitive stimulation of transient sympathetic

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**Figure 5. Single passive limb movement test**

Individual data for baseline blood flow, peak blood flow, and area under the curve in femoral artery in the control (Ctrl), the bilateral passive stretching (PS\text{BL}), and the unilateral stretching group (stretched limb, PS\text{MSL}), and contralateral limb (PS\text{MCCL}) at baseline (Pre), at weeks 6 and 12, and at week 6 of follow-up. Histograms report the percentage changes in respect to Pre for the baseline blood flow, peak blood flow, and area under the curve in femoral artery in the control (Ctrl), the bilateral passive stretching (PS\text{BL}), and the unilateral stretching group (stretched limb, PS\text{MSL}), and contralateral limb (PS\text{MCCL}) at weeks 6 and 12, and at week 6 of follow-up (*$P < 0.05$ vs. Pre, # $P < 0.05$ vs. Ctrl, § $P < 0.05$ vs. PS\text{MCCL}).
excitation (Cui et al. 2006), may have chronically reduced the central autonomic mechanism of $\dot{Q}$ control, thus also decreasing arterial stiffness in the vessels not directly involved in PS training and aortic blood pressure with related parameters (PP, MAP and TTI). Moreover, the decrease in TTI, an indirect index of myocardial oxygen consumption (Chemla et al. 2008) may be indicative of a reduction in afterload and, probably, in overall cardiac work. Although the present study does not provide direct measurement of a PS-induced reduction in the

![Flow mediated dilatation in the popliteal artery](image)

**Figure 6. Flow mediated dilatation in the popliteal artery**

Individual data for baseline blood flow, baseline diameter, flow-mediated dilatation, area under the curve, and flow-mediated dilatation/shear rate in the popliteal artery in the control (Ctrl), the bilateral passive stretching (PSBl), the unilateral stretching group (stretched limb, PSMonoSL), and contralateral limb (PSMonoCL) at baseline (Pre), at weeks 6 and 12, and at week 6 of follow-up. Histograms report the percentage changes in respect to Pre for the baseline blood flow, baseline diameter, flow-mediated dilatation, area under the curve, and flow-mediated dilatation/shear rate in the popliteal artery in the control (Ctrl), the bilateral passive stretching (PSBl), the unilateral stretching group (stretched limb, PSMonoCL), and contralateral limb (PSMonoCL) at weeks 6 and 12, and at week 6 of follow-up ($^*P < 0.05$ vs. Pre, $^#P < 0.05$ vs. Ctrl).
Figure 7. Flow mediated dilatation in the brachial artery

Individual data for baseline blood flow, baseline diameter, flow-mediated dilatation, area under the curve, and flow-mediated dilatation/shear rate in the brachial artery in the control (Ctrl), the bilateral passive stretching (PSBl), the unilateral stretching group (stretched limb, PSMonoSL), and contralateral limb (PSMonoCL) at baseline (Pre), at...
central autonomic mechanism of $Q$ control, its possible contribution to reducing arterial stiffness and blood pressure cannot be ruled out. The changes in arterial stiffness and blood pressure are partially in agreement with previous work (Williams et al. 2013; Wong & Figueroa, 2014). Similar changes were noted in an elderly but not in a young population (Yamamoto et al. 2009; Williams et al. 2013; Wong & Figueroa, 2014; Shinno et al. 2017). Such a discrepancy may be attributed to PS training duration and sample size. Previous studies had shorter training periods and/or training sessions than our study, with a lower total amount of stretching (Wong & Figueroa, 2014; Shinno et al. 2017). Additionally, previous studies did not mention participant adherence to the protocol (Williams et al. 2013; Wong & Figueroa, 2014; Shinno et al. 2017), which in our case was set at 80% to ensure a minimum of involvement by all participants. PS training may have reduced arterial stiffness by inducing an increase in elastin and collagen content in the arterial wall as a result of sustained axial elongation of the arteries involved in chronic stretching (Jackson et al. 2002; Nichols et al. 2011; Marti et al. 2012). This explanation could be supported by evidence for a further increase in vasodilatation capacity after IEx and by the correlations between the changes in ankle and knee joint ROM, and the changes in central and peripheral arterial stiffness, suggesting mechanical remodulation of the arterial wall in the vessels directly involved in PS training (Naylor et al. 2005). Independently from the mechanisms underlying the PS training-induced changes in arterial stiffness and blood pressure, blood pressure returned to baseline within 6 weeks after training cessation, while the central and peripheral arterial stiffness were still reduced at the end of the follow-up. The different time course between blood pressure and arterial stiffness after PS training cessation may suggest a different relative weight played by the central and local (mechanical) mechanisms underlying their decrement.

**PS training-induced changes in vascular function**

The present findings provide novel evidence for the impact of PS training on vascular function, as assessed by FMD (Harris et al. 2010) and by the more recent sPLM methodology (Venturelli et al. 2017b). FMD is a well-recognized means to assess vascular function and estimate cardiovascular risk (Harris et al. 2010; Broxterman et al. 2019). FMD response is dependent on both reduction in sympathetic outflow towards the vessel (Hijmering et al. 2002) and on bioavailability of local vasodilatory molecules such as NO (Green, 2005; Wray et al. 2013). In contrast, sPLM response is more representative of a microvascular assessment and closely related to local factors (see Methods). Thus, its response seems to be only marginally influenced by the sympathetic activity (Venturelli et al. 2017a,b). Previous studies reported enhanced vascular function of the stretched limb after PS training only indirectly as measured with the reactive hyperaemia peripheral tonometry index (Hotta et al. 2013; Kato et al. 2017; Shinno et al. 2017), a technique mainly related to better NO bioavailability due to shear stress-induced vasodilatation (Matsuzawa et al. 2015). FMD results in the present study are in line with the previously published data: indeed, an improvement in vascular function in the arteries directly involved with PS training was noted for both training groups under investigation. The improvement may stem from repeated changes in $\dot{Y}$ during PS and from its effect on NO bioavailability. PS training repeatedly exposed the vessels to increased levels of $\dot{Y}$ (Kruse et al. 2016; Venturelli et al. 2017a, 2019), thus stimulating endothelial mechanotransduction signalling in smooth muscle cells that results in vasodilatation (2017, Green et al. 2017a,b). Such repeated alterations in $\dot{Y}$ over time can be considered as a kind vascular preconditioning that may lead to increase NO bioavailability (Green et al. 2017a; Bisconti et al. 2019). It was demonstrated that vascular function increases during skeletal muscle contraction because of an increase in mean $\dot{Y}$ due to elevated antegrade $\dot{Y}$ (Tinken et al. 2009). A previous study characterized the oscillatory nature of $\dot{Y}$ during elongation and relaxation cycles intrinsic in stretching manoeuvres, and reported that a marked hyperaemic response occurred immediately after a bout of stretching, which coincided with elevated antegrade and mean $\dot{Y}$ and attenuated retrograde $\dot{Y}$ (Kruse et al. 2016). The increment of antegrade and mean $\dot{Y}$ and the attenuation of retrograde $\dot{Y}$ shown in our study during a single PS administration further support this mechanism, suggesting a pivotal role for $\dot{Y}$ in triggering an adjustment in vascular function after PS training. Additionally, some studies reported that these positive effects depend on $\dot{Y}$ magnitude (Green et al. 2017a). In our study, the FMD (%) and its relative AUC were significantly increased in the popliteal artery of the involved limb, as well as in the popliteal and the brachial artery of the limbs not involved in PS training, indicating a widespread systemic effect. Notably, the changes in vascular function in the arteries directly involved in PS training occurred earlier than in those not involved. Nevertheless, after 12 weeks...
of PS training, no significant differences in FMD response were observed between the arteries directly involved in PS training and the contralateral arteries (non-involved), suggesting a major contribution of the systemic/central mechanisms in explaining the increase in FMD response. This increase returned to baseline within 6 weeks from PS training cessation, indicating a limited duration of the aforementioned mechanisms.

Our study demonstrated a clear improvement in all sPLM parameters at week 12 of PS training of the involved and uninvolved limbs, thus reinforcing the hypothesis for a systemic effect. As stated above, the sPLM response relates to microvasculature reactivity induced by increased perfusion pressure and peripheral vasodilatation (Mortensen et al. 2012; Trinity et al. 2012). Enhancement of the microvasculature, together with lower
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<td>&lt;0.001</td>
<td>0.017</td>
<td>0.001</td>
<td>&lt;0.001</td>
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</tr>
<tr>
<td></td>
<td>(0.291/0.718)</td>
<td>(0.501/0.803)</td>
<td>(0.135/0.574)</td>
<td>(0.561/0.845)</td>
<td>(0.347/0.755)</td>
<td></td>
</tr>
<tr>
<td><strong>Knee ROM</strong></td>
<td>R (CI95%)</td>
<td>0.389</td>
<td>0.541</td>
<td>0.482</td>
<td>0.658</td>
<td>0.453</td>
</tr>
<tr>
<td>p</td>
<td>0.014</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.131/0.608)</td>
<td>(0.340/0.717)</td>
<td>(0.232/0.687)</td>
<td>(0.450/0.797)</td>
<td>(0.187/0.668)</td>
<td></td>
</tr>
<tr>
<td><strong>FMD brachial artery</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Ankle ROM</strong></td>
<td>R (CI95%)</td>
<td>0.275</td>
<td>0.652</td>
<td>0.194</td>
<td>0.611</td>
<td>0.117</td>
</tr>
<tr>
<td>p</td>
<td>0.090</td>
<td>&lt;0.001</td>
<td>0.236</td>
<td>&lt;0.001</td>
<td>0.477</td>
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<tr>
<td></td>
<td>(0.011/0.497)</td>
<td>(0.462/0.791)</td>
<td>(−0.033/0.384)</td>
<td>(0.428/0.773)</td>
<td>(−0.380/0.533)</td>
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</tr>
<tr>
<td><strong>Knee ROM</strong></td>
<td>R (CI95%)</td>
<td>0.407</td>
<td>0.541</td>
<td>0.084</td>
<td>0.650</td>
<td>0.322</td>
</tr>
<tr>
<td>p</td>
<td>0.010</td>
<td>&lt;0.001</td>
<td>0.612</td>
<td>&lt;0.001</td>
<td>0.045</td>
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</tr>
<tr>
<td></td>
<td>(0.186/0.614)</td>
<td>(0.302/0.731)</td>
<td>(−0.239/376)</td>
<td>(0.419/0.809)</td>
<td>(0.037/0.556)</td>
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<tr>
<td><strong>IEx popliteal/brachial artery</strong></td>
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<td></td>
</tr>
<tr>
<td><strong>Ankle ROM</strong></td>
<td>R (CI95%)</td>
<td>0.369</td>
<td>0.600</td>
<td></td>
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<tr>
<td>p</td>
<td>0.021</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>(0.067/0.582)</td>
<td>(0.424/0.757)</td>
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<td></td>
</tr>
<tr>
<td><strong>Knee ROM</strong></td>
<td>R (CI95%)</td>
<td>0.274</td>
<td>0.373</td>
<td></td>
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</tr>
<tr>
<td>p</td>
<td>0.100</td>
<td>&lt;0.001</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>(−0.060/0.547)</td>
<td>(0.058/0.653)</td>
<td></td>
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</table>

*R* coefficient with 95% confidence interval and *P* values are provided for each correlation (*n* = 39). PWA, pulse wave analysis; PWV, pulse wave velocity; CF, carotid-femoral; CR, carotid-radial; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; AIx, augmentation index normalised to 75 bpm; Q_bas, baseline blood flow; Q_peak, peak blood flow; AUC, area under the curve; D_bas, baseline diameter; Y shear rate; IEx%, maximum dilatation capacity.
A significant increase in angiogenesis was observed after cannot be ruled out. In addition to these mechanisms, in vessel tortuosity, a reduction following PS training. While our study did not supply direct evidence for changes for long-term vascular adaptation, induced by PS training. Acute vessel distortion constitutes an important stimulus as well as of $\dot{Q}$ and $\dot{O}_2$ supply (Poole et al. 1997). Such acute vessel distortion constitutes an important stimulus for long-term vascular adaptation, induced by PS training. While our study did not supply direct evidence for changes in vessel tortuosity, a reduction following PS training cannot be ruled out. In addition to these mechanisms, a significant increase in angiogenesis was observed after PS training (30 min day$^{-1}$, 5 days week$^{-1}$, for 4 weeks) in a murine model (Hotta et al. 2018), a period far shorter than our study protocol. Further studies are needed to clarify the occurrence of this mechanism also in humans. Interestingly, the change in sPLM response was higher in the artery of the limb directly involved in PS training than in the contralateral artery (non-involved). This difference may underline a larger increase in vascular function in the area where a greater shear stress stimulus occurred. Moreover, an increased sPLM response was also observed at follow-up, with the maintenance of greater response in the artery of the limb directly involved compared to the contralateral (non-involved) artery, probably suggesting more persisting effects of the local mechanisms.

Lastly, femoral artery resting $\dot{Q}$ was increased in all training groups in the limbs directly and not directly involved in PS training. Such a finding suggests that not only vascular function but also muscle perfusion improved after PS training. The correlations found in the present study between the changes in ankle and knee ROM and the resting $\dot{Q}$ in all the three investigated arteries may further support this possible occurrence.

**Study limitations**

The present study has several limitations. First, direct assessment of muscle sympathetic nerve activity and of NO bioavailability would have evidenced possible stretch-induced remodulation of sympathetic vessel tone and endothelial function, respectively. The lack of a direct assessment of these measurements did not allow us to establish a clear balance between central and local mechanisms underlying the positive changes in vascular function. Second, measurements on female participants were always made during the early follicular phase of the menstrual cycle, implying that tests may have occurred with a $\pm 5$ day-dispersion from the exact testing week. However, in view of the strong vascular responsiveness fluctuation induced by the menstrual cycle, we preferred to test female participants during the same period of the menstrual cycle. Lastly, a larger sample size may have helped to detect possible differences in PS training response between female and male participants.

**Conclusions**

The present study clearly demonstrates that 12-week PS training is effective in improving vascular function and decreasing stiffness of the directly involved arteries (i.e. femoral and popliteal arteries of the stretched limbs) and the arteries not directly involved (i.e. contralateral femoral and popliteal arteries and brachial artery) in PS training. These improvements encompassed both the modification of central and local $\dot{Q}$ control mechanisms. However, the central mechanisms influencing blood pressure and vascular function (i.e. PWA and FMD) had a shorter duration compared to those mainly driven by local control (i.e. sPLM), as the former returned to baseline within 6 weeks from PS training cessation, while the latter were still present at the end of follow-up. PS has been shown to be an effective means to improve vascular function, with practical implications for its use as a novel non-pharmacological treatment for improving vascular health, reducing the overall cardiovascular risk, especially in individuals with limited mobility.

**References**


Vascular function improvement after passive stretching training


Additional information

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare no professional relationship with companies or manufacturers that might receive help from the results of the
present study. The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

**Authors contributions**

All experiments were conducted at the Physiology Labs of the School of Sport Science, Università degli Studi di Milano. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed. Conception or design of the study: A.V.B., E.C., S.L., F.E. Acquisition, analysis or interpretation of data for the study: A.V.B., E.C., S.L., M.V., G.C., E.L., C.D., S.R., F.E. Drafting the manuscript or revising it critically for important intellectual content: A.V.B., E.C., S.L., M.V., G.C., E.L., C.D., S.R., F.E. All the authors have approved the final version of the manuscript and agree to be accountable for all aspects of the study in ensuring that questions related to the accuracy or integrity of any part of the study are appropriately investigated and resolved.

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**Keywords**

arterial stiffness, endothelial function, flow-mediated dilatation, muscle stretching, single passive limb movement

**Supporting information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Statistical Summary Statement**

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