# Static Muscular Stretching Improves Microvascular Function in the Calf Muscles of Patients with Peripheral Artery Disease

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**ABSTRACT**

CHEN L.-S., S. P. LANKA, Y. ALSABBAGH, S. NUSSBAUM, J. SARMIENTO, J. MARAJ, B. SCHEUERMANN, C. ADE, D. N. PROCTOR, H. FARRES, A. HAKAIM, K. HOTTA, and J. MULLER-DELP. Static Muscular Stretching Improves Microvascular Function in the Calf Muscles of Patients with Peripheral Artery Disease. *Med. Sci. Sports Exerc.,* Vol. 58, No. 10, pp. 2167–2175, 2026. **Purpose:** Supervised walking exercise is a critical component of rehabilitation for patients with peripheral artery disease (PAD); however, various barriers could limit participation in such programs, highlighting the need for accessible exercise strategies that may facilitate a gradual transition to more intensive physical activity while improving vascular function and walking performance in this population. We examined the hypothesis that static muscular stretching would improve microvascular function and walking ability in patients with PAD. **Methods:** Thirty-six PAD patients, classified as Fontaine Stage II or III, were randomized into stretching or nonstretching groups. The stretching group performed daily calf stretching using an ankle dorsiflexion splint for 30 min, 5 d a week, for 4 wk. The six-minute walk test (6MWT) and a vascular occlusion test were used to assess changes in walking ability and calf microvascular function before and after the intervention. **Results:** Microvascular hyperemic reperfusion significantly increased in the treatment group (4958 ± 1111–5749 ± 1459%*s, $P$ = 0.034), with no change observed in the control group (5502 ± 1231–5022 ± 1160%*s, $P$ = 0.155). The 6MWT total walking distance increased in all participants (260 ± 88–285 ± 85 m, $P$ = 0.004). A moderate but nonsignificant correlation was observed between the changes in 6MWT and total walking distance and reperfusion index in the treatment group ($r$ = 0.45, $P$ = 0.26). **Conclusions:** Static muscular stretching improves microvascular perfusion in the calf muscles, which may contribute to improvement in walking ability in patients with PAD. This low-cost and time-saving intervention may serve as a gradual transition to traditional supervised walking exercise rehabilitation, especially in patients with severely impaired walking ability.
**Key Words:** MICROCIRCULATION, NEAR-INFRARED SPECTROSCOPY, REHABILITATION, SIX-MINUTE WALK TEST, VASCULAR OCCLUSION TEST

Peripheral artery disease (PAD) affects approximately 6.8–8.5 million individuals in the United States and over 200 million individuals worldwide (1,2). The economic burden of PAD is substantial; total annual costs associated with vascular hospitalizations in PAD patients are estimated at $21 billion in the United States (3). PAD is characterized by the stenosis or occlusion of the arteries in the upper- or lower extremities (4). Because of the stenosed arteries, blood flow and oxygen delivery to the limbs are reduced, leading to intermittent claudication and pain in the lower extremities (5). Consequently, patients with PAD commonly experience impaired walking ability (6).

One physiological factor that has been associated with impaired walking ability among PAD patients is vascular dysfunction, which encompasses impairments in both conduit arteries (macrovascular) and resistance vessels (microvascular). Flow-mediated dilation, a measurement of endothelium-dependent vasodilation of the conduit arteries, reflects the capacity of the endothelial cells to release vasodilators (e.g., nitric oxide)

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in response to shear stress. This endothelial function was significantly lower in patients with PAD and was positively correlated with walking performance (7,8). Similarly, nitroglycerin-induced vasodilation in the conduit artery, an indicator of vascular smooth muscle function (endothelium-independent vasodilation), was also impaired in PAD patients and associated with reduced walking ability (9). While macrovascular function determines the delivery of blood flow, the microcirculation is responsible for regulating tissue perfusion and oxygen exchange. Impaired microvascular function has also been linked to limited walking ability in PAD patients (10). A recent investigation suggested that microvascular reactivity in response to an ischemic stimulus may be a stronger predictor of walking distance compared with macrovascular function (10), highlighting the critical role of microvascular function and tissue perfusion in functional capacity in this population.

Currently, supervised walking exercise remains the gold standard treatment in the exercise prescription for patients with PAD (11,12). A 6-month supervised aerobic rehabilitation program has been shown to significantly improve both ambulatory function and endothelial function in patients with PAD (13). Despite these beneficial effects, several barriers limit patients from participating in the supervised walking exercise programs, including copay costs, transportation or distance challenges, time commitment, and lack of interest in treadmill walking (14,15). Additionally, the presence of pain and the ambiguity regarding the appropriate pain levels during exercise also present limitations to walking among individuals with claudication (16). As a result, the adherence to supervised treadmill exercise remains low among PAD patients (17). These barriers to adherence in supervised walking exercise programs underscore the need for supplemental interventions that may enhance physiological function and better prepare patients with PAD for traditional supervised walking exercise rehabilitation.

Passive muscular stretching may be a promising alternative to improve vascular function and walking ability in patients with PAD. A previous animal study demonstrated that daily passive muscle stretching enhanced blood flow and endothelial function in the skeletal muscle of aged rats (18). Furthermore, our previous study with a randomized crossover design found that daily passive muscular stretching improved macrovascular endothelial function of the popliteal artery and walking ability in patients with PAD (19). Given the emerging evidence linking microvascular function to functional outcomes in patients with PAD (10), and previous works in healthy human subjects demonstrating that an acute bout of passive muscle stretch can alter microvascular oxygenation of muscle both during and after the stretching bout (20,21), further investigation is warranted to determine the effects of static muscular stretching on microvascular function and walking performance in patients with PAD.

The present study aimed to investigate the effects of passive static muscular stretching on microvascular function and walking ability in patients with PAD. We hypothesized that a 4-wk intervention of daily muscular stretching would improve microvascular reactivity in the calf muscles and increase walking distance in PAD patients. We also hypothesized that microvascular function would positively correlate with walking function.

## METHODS

### Participants

Participants diagnosed with PAD were recruited for the present study. Inclusion criteria were: 1) age ≥40 years, 2) resting ankle-brachial index (ABI) ≤0.90 in either leg, and 3) classification as Stage II or Stage III based on the Fontaine classification system for peripheral arterial disease (22). Participant were excluded if they: 1) engaged in habitual exercise, defined as 30 min of continuous activity for three or more days per week, 2) participated in a cardiovascular rehabilitation program in the past 3 month, 3) had below or above-knee amputation or chronic limb threatening ischemia (ulceration or gangrene), 4) had other cardiovascular disease that limited their ability to perform the 6MWT, 5) had insulin-dependent diabetes, 6) had major surgery or lower extremity revascularization during the past 3 months, 7) received treatment for major medical illness within the past 12 months, 8) had central neurological disease, 9) had limited ankle or knee joint range of motion, 10) required supplemental oxygen during activity or exercise, 11) had heart failure more severe than a class II New York Heart Association level of heart failure, 12) were in wheelchair confinement or unable to walk, 13) had cognitive impairment, or 14) had any diagnosed cardiovascular condition including Takayasu arteritis, Buerger disease, collagen disease or Raynaud disease.

Mayo Clinic research staff screened the participants' medical records for eligibility and explained the purpose of the study to eligible patients who expressed interest in participating. All participants provided written informed consent before participating in the study. This study was conducted in accordance with the Declaration of Helsinki. The Institutional Review Board at Mayo Clinic in Jacksonville, Florida, reviewed and approved the study (19-005234 Mayo). This trial was registered at clinicaltrials.gov as NCT04222751.

### Study Design and Experiment Protocol

A single-blinded randomized controlled trial was conducted to examine the effects of the static muscular stretching intervention in patients with PAD. Participants were blinded and randomly assigned by a clinical research coordinator, who was independent of the study, to either the stretching group (treatment

group) or the nonstretching group (sham control group). Microvascular function, measured by vascular occlusion test with near-infrared spectroscopy (NIRS), and walking ability were evaluated before and after 4 wk of intervention. Participants were instructed to maintain their usual daily routines and refrain from engaging in any additional exercise training program during their participation in this study. Participants were asked to refrain from caffeine and food for 4 h and alcohol for 24 h before the laboratory visits. All measurements were performed during the same time of day and in the same testing order.

### Passive Stretching Intervention

A pair of boot-like ankle dorsiflexion splints (Mars Wellness Plantar Fasciitis Posterior Night Splint, Mars Med Supply Lakewood, NJ) was provided to the participants to passively stretch the calf muscles in both legs. This stretching device was equipped with an inflatable insole that connected to a manual pump to adjust the dorsiflexion angle (Fig. 1). Participants were asked to fully extend their knee (0°). In this position, both the gastrocnemius and soleus muscles are stretched, with the tension predominantly placed on the gastrocnemius. Participants were fitted with an appropriately sized splint, and the dorsiflexion angle was adjusted by study staff to achieve a degree of stretching, using side straps and the inflatable insole, that induced a decrease in muscle $O_2$ saturation while also being tolerable to the participant for 15 min. The strap position was fixed, and participants were instructed in proper application of the splints and ergonomic position to assume during stretching and asked to wear them on both legs at a prescribed dorsiflexion angle for 30 min/d, 5 d/wk, for 4 wk. Participants in the nonstretching sham control group were instructed to wear the splints in the same manner as those who experienced stretching, but the side straps were adjusted to a position that did not increase dorsiflexion, and the participants were instructed to use the pump in a manner (only one pump) that also did not produce any further dorsiflexion. As a result, the angle applied to the ankle did not create any muscle stretching in the control group. All participants kept a diary to document their stretching sessions.



**FIGURE 1—Ankle dorsiflexion splints (Mars Wellness Plantar Fasciitis Posterior Night Splint) with side straps and a manual pump to increase the dorsiflexion angle were used to perform static muscular stretching in the calf muscle.**

### Six-minute Walk Test

Walking ability was evaluated by using the 6MWT, following the guidelines of the American Thoracic Society (23). The walk test was administered by trained clinical staff working in the Mayo Clinic cardiac rehabilitation facility. Participants were instructed to walk continuously up and down a 30 m corridor, covering as much ground as possible in 6 min. Participants were allowed to rest if needed and then asked to resume walking as soon as able. Pain-free walking distance (Pain-free WD) was defined as the distance covered before the onset of calf pain. Continuous walking distance (Continuous WD) was measured as the distance covered without stopping or resting. Total walking distance (Total WD) was determined as the total distance covered in 6 min (19). A NIRS device (Moxy Monitor, Fortiori Design LLC, Spicer, MN) was placed on the gastrocnemius muscle during the 6MWT to monitor muscle oxygenation during walking. Muscle oxygen consumption kinetics were modeled by fitting a nonlinear and least-squares mono-exponential model to the time-series data from baseline through 120 s after the onset of the 6MWT (24).

$$Y(t) = Y_{Baseline} + Amplitude\left[1 - e^{-(t-TD)/\tau}\right]$$

Amplitude (AMP) represented the maximum change in skeletal muscle oxygen saturation ($SmO_2$) during the 6MWT. Time delay (TD) was the duration between the start of the 6MWT to the time point when a decrease of $SmO_2$ was observed, and tau ($\tau$) was the time constant. The mean response time (MRT) was calculated as TD + $\tau$ (25).

### Vascular Occlusion Test

Vascular occlusion tests (VOT) were performed on the leg with the lower ABI of each participant to evaluate the changes in microvascular function before and after the stretching intervention. The same NIRS device (Moxy Monitor, Fortiori Design LLC, Spicer, MN) was used to measure skeletal muscle oxygenation during an ischemia–reperfusion protocol. The optodes of the near-infrared photometer were placed over the medial head of the gastrocnemius muscle. A pneumatic thigh blood pressure cuff, connected to a rapid cuff inflation

system (Hokanson, Bellevue, WA), was positioned above the knee to occlude the blood flow to the calf muscles. The cuff pressure was set at 250 mm Hg for VOT. Each VOT consisted of a 5-min baseline measurement, a 5-min occlusion, and a 5-min reperfusion period. NIRS continuously recorded relative changes in $SmO_2$ throughout the test while participants remained supine. Linear regression was used to calculate the desaturation slope (rate of $SmO_2$ decline during occlusion) and reperfusion slope (rate of $SmO_2$ increase during the first 10 s after cuff release). The reperfusion index was defined by the area under the curve of the reperfusion slope within the first 2 min after cuff deflation, as has been used by a previous study (26). The area under the curve value was determined using the trapezoid rule and integrating between the minimum value observed during the occlusion period and each $SmO_2$ value during reperfusion. This particular approach was utilized in the present study to account for the magnitude of the ischemic stimulus, as described previously (26).

Because 70% of the participants were classified as overweight or obese (BMI ≥25), and subcutaneous adipose tissue could affect the NIRS signal, the $SmO_2$ readings that were beyond physiologically normal boundaries were excluded from analysis (27,28). For the vascular occlusion test, data from 14 participants were excluded: five withdrew owing to intolerance to the occlusion procedure, and nine were excluded owing to a poor signal-to-noise ratio that obscured the deoxygenated and hyperemic physiological responses. For NIRS measurements during the 6MWT, data from seven participants were excluded primarily owing to excessive motion artifacts. Limb movement during the walking exercise resulted in nonphysiological signals and a poor data quality that precluded accurate curve fitting for the AMP, TD, and MRT calculations. The final number of participants included in each analysis is indicated in the figure for each measurement.

### Statistical Analyses

Sample size was calculated by using G*Power software. A large effect size of 0.47 on flow-mediated dilation measurement in patients with PAD was observed in our previous crossover trial (19). To account for the variation in study design and a different vascular function measurement, we utilized a conservative effect size with the following inputs: effect size ($f$) = 0.25, ($\alpha$) = 0.05, and power (1–$\beta$) = 0.80. With these parameters, a minimum sample size of 17 participants per group (34 total) was determined to be adequate for the analysis. All statistical analyses were performed using SPSS Version 29.0.1.0 (IBM Corp., Armonk, NY). The Shapiro–Wilk test was used to examine the normality of the data, and Levene test was used to examine the homogeneity of variance. Two-way analysis of variance (ANOVA) with repeated measures was used to evaluate changes in 6MWT distance, NIRS measurements during 6MWT (AMP, TD, and MRT), and VOT parameters (occlusion slope, reperfusion slope, and reperfusion index), before and after the intervention. The Bonferroni post hoc test was used to identify significant differences between the mean values if the $F$ value was significant. Partial eta-squared values ($\eta p^2$) are provided as an indicator of effect size for these multifactorial ANOVA models. Pearson product-moment correlation coefficient analysis (two-tailed) was conducted to examine the relation between changes in reperfusion index and changes in 6MWT parameters in each group. The significance level was set *a priori* at $P < 0.05$. Data are presented as means ± SD unless stated otherwise.

## RESULTS

### Participants

A total of 36 participants provided written informed consent and were enrolled in the present study. Five participants withdrew from the study. Reasons for withdrawal included withdrawal of consent ($n$ = 2), refusal to complete baseline procedures ($n$ = 1), perceived lack of benefit ($n$ = 1), and distance and time constraints regarding follow-up visits ($n$ = 1). Table 1 shows the baseline characteristics of participants who completed the intervention in both the treatment group and control group. There was no difference in age, sex, race, height, weight, BMI, screening ABI in both legs, disease stages, and medication use between groups (all $P > 0.05$). Adherence to stretching, as recorded in the participant diaries, was high, with rates of 99.5% in the treatment group and 96% in the control group. There were no adverse events associated with the stretching protocol.

### Six-minute Walk Test (6MWT)

The results of the 6MWT are summarized in Table 2. When all participants were included in the

TABLE 1. Participants characteristics ($n$ = 31).

| Variables | Treatment ($n$ = 14) | Control ($n$ = 17) | $P$-value |
|---|---|---|---|
| Sex (M/F) | 8/6 | 11/6 | 0.67 |
| Race (White/Asian) | 13/1 | 17/0 | 0.26 |
| Age (yr) | 72 ± 12 | 75 ± 13 | 0.58 |
| Height (cm) | 171 ± 10 | 168 ± 10 | 0.48 |
| Body weight (kg) | 75 ± 18 | 82 ± 17 | 0.31 |
| BMI (kg/m$^2$) | 26 ± 5 | 29 ± 5 | 0.09 |
| ABI (lower of two legs) | 0.55 ± 0.11 | 0.54 ± 0.15 | 0.79 |
| ABI (higher of two legs) | 0.73 ± 0.19 | 0.80 ± 0.21 | 0.33 |
| Bilateral/unilateral PAD ($n$) | 12/2 | 11/6 | 0.24 |
| Fontaine Stage II/Stage III ($n$) | 12/2 | 12/5 | 0.41 |
| Medications | | | |
| Antihypertensive ($n$) | 13 | 16 | 0.89 |
| Antiplatelet ($n$) | 12 | 10 | 0.13 |
| Antidiabetic ($n$) | 2 | 6 | 0.24 |
| Adherence to stretching | | | |
| Adherence rate (%) | 99.5% | 96% | 0.34 |

TABLE 2. Changes in total walking distance, continuous walking distance, and pain-free walking distance before and after the intervention.

| Variables | Treatment | | Control | | ANOVA *P*-value Time | ANOVA *P*-value T*G |
|---|---|---|---|---|---|---|
| | Before | After | Before | After | | |
| Total WD (m) | 295 ± 84 | 314 ± 81 | 234 ± 84 | 262 ± 84 | 0.004 | 0.608 |
| Continuous WD (m) | 273 ± 109 | 293 ± 106 | 217 ± 93 | 251 ± 90 | 0.012 | 0.508 |
| Pain-free WD (m) | 205 ± 77 | 203 ± 87 | 168 ± 72 | 203 ± 87 | 0.318 | 0.232 |
| After excluding participants with baseline walking distance <200 m (1 in treatment group and 7 in control group) | | | | | | |
| Total WD (m) | 295 ± 84 | 315 ± 81 | 292 ± 50 | 304 ± 61 | 0.041 | 0.605 |
| Continuous WD (m) | 273 ± 109 | 293 ± 106 | 269 ± 82 | 290 ± 79 | 0.083 | 0.984 |
| Pain-free WD (m) | 205 ± 77 | 203 ± 87 | 200 ± 76 | 223 ± 67 | 0.339 | 0.260 |

analysis, significant main effects of time were observed in the measurement of Total WD ($F = 9.56$, $P = 0.004$, $\eta p^2 = 0.25$) and Continuous WD ($F = 7.28$, $P = 0.012$, $\eta p^2 = 0.21$), indicating an increase in Total WD and Continuous WD in all participants. No time × group interaction was observed on Total WD ($F = 0.27$, $P = 0.608$, $\eta p^2 = 0.01$), Continuous WD ($F = 0.45$, $P = 0.508$, $\eta p^2 = 0.02$), and Pain-free WD ($F = 1.49$, $P = 0.23$, $\eta p^2 = 0.05$). There were more participants who walked less than 200 m in the control group (seven participants) than in the treatment group (one participant), with a lower baseline walking distance observed in the control group (Table 2). To reduce variability and obtain a more homogeneous sample, participants who walked less than 200 m at baseline were excluded for sensitivity analysis based on the Fontaine classification of PAD (22) (Table 2). In this adjusted model, a significant main effect of time on Total WD remained ($F = 4.75$, $P = 0.041$, $\eta p^2 = 0.18$), suggesting that both groups increased Total WD. No time × group interaction was observed for Total WD ($F = 0.28$, $P = 0.605$, $\eta p^2 = 0.01$), Continuous WD ($F < 0.01$, $P = 0.984$, $\eta p^2 < 0.01$), or Pain-free WD ($F = 1.34$, $P = 0.26$, $\eta p^2 = 0.06$) in this adjusted model.

## NIRS Measurements During 6MWT

The changes in AMP, TD, and MRT before and after the intervention in both groups are displayed in Figure 2. Statistical analysis indicated a time × group interaction for TD (two-way ANOVA interaction: $F = 6.74$, $P = 0.016$, $\eta p^2 = 0.24$; two-way ANOVA main effect of time: $F = 0.019$, $P = 0.89$, $\eta p^2 = 0.001$). Bonferroni post hoc tests indicated that the treatment group had a non-significant increase in TD (23.6 ± 6.4–31.3 ± 11.3 s; $P = 0.066$, 95% CI: [−0.5, 15.9] s) while the control group had a non-significant decrease in TD (29.8 ± 14.6–22.9 ± 9.5 s; $P = 0.096$, 95% CI: [−15.1, 1.3] s). No time × group interaction was observed for AMP (two-way ANOVA interaction: $F = 0.29$, $P = 0.87$, $\eta p^2 = 0.001$; two-way ANOVA main effect of time: $F = 0.97$, $P = 0.335$, $\eta p^2 = 0.\ 0.042$) or MRT (two-way ANOVA interaction: $F = 4.01$, $P = 0.058$, $\eta p^2 = 0.15$; two-way ANOVA main effect of time: $F = 0.432$, $P = 0.518$, $\eta p^2 = 0.019$). Correlation analyses revealed no significant relation between the change in total WD and changes in muscle oxygenation kinetics during walking, including change in AMP ($r = -0.22$, $P = 0.32$), change in TD ($r = -0.14$, $P = 0.51$), and change in MRT ($r = -0.16$, $P = 0.48$). These scatter plots, with distinct labeling for the control and treatment groups, are provided in Supplemental Fig. 1 (Supplemental Digital Content, https://links.lww.com/MSS/D425).

## Vascular Occlusion Test (VOT)

There were no significant differences in baseline $SmO_2$, occlusion slope, and reperfusion slope before and after the intervention in both groups (all $P > 0.05$; Table 3). However, a time × group interaction was observed in the reperfusion index before and after the intervention (two-way ANOVA $F = 7.41$, $P = 0.016$, $\eta p^2 = 0.33$; two-way main effect of time: $F = 0.44$, $P = 0.52$, $\eta p^2 = 0.03$). Bonferroni post hoc tests indicated that reperfusion index increased significantly by 791 %*s in the treatment group (4958 ± 1111–5749 ± 1459%*s; $P = 0.034$, 95% CI: [67, 1516]%*s) while no change was observed in the control group (difference: −480%*s; 5502 ± 1231–5022 ± 1160%*s, $P = 0.155$, 95% CI: [−203, 1163]%*s) (Fig. 3).

## Correlation Between Changes in 6MWT Walking Distance and Reperfusion Index

As shown in Figure 4, when analyzing the pre-to-post intervention changes, a moderate positive but non-significant correlation was observed between the change in total walking distance (6MWT) and the change in calf reperfusion index in the treatment group ($r = 0.452$, $P = 0.261$). Although this relation did not reach statistical significance, potentially owing to the limited sample size, no such trend was observed in the control group ($r = 0.094$, $P = 0.810$).

## DISCUSSION

The present study investigated the effects of passive static muscular stretching on microvascular function and walking ability in patients with PAD. The high adherence rate to the stretching protocol (99.5% in the treatment group) indicates that this intervention is feasible and

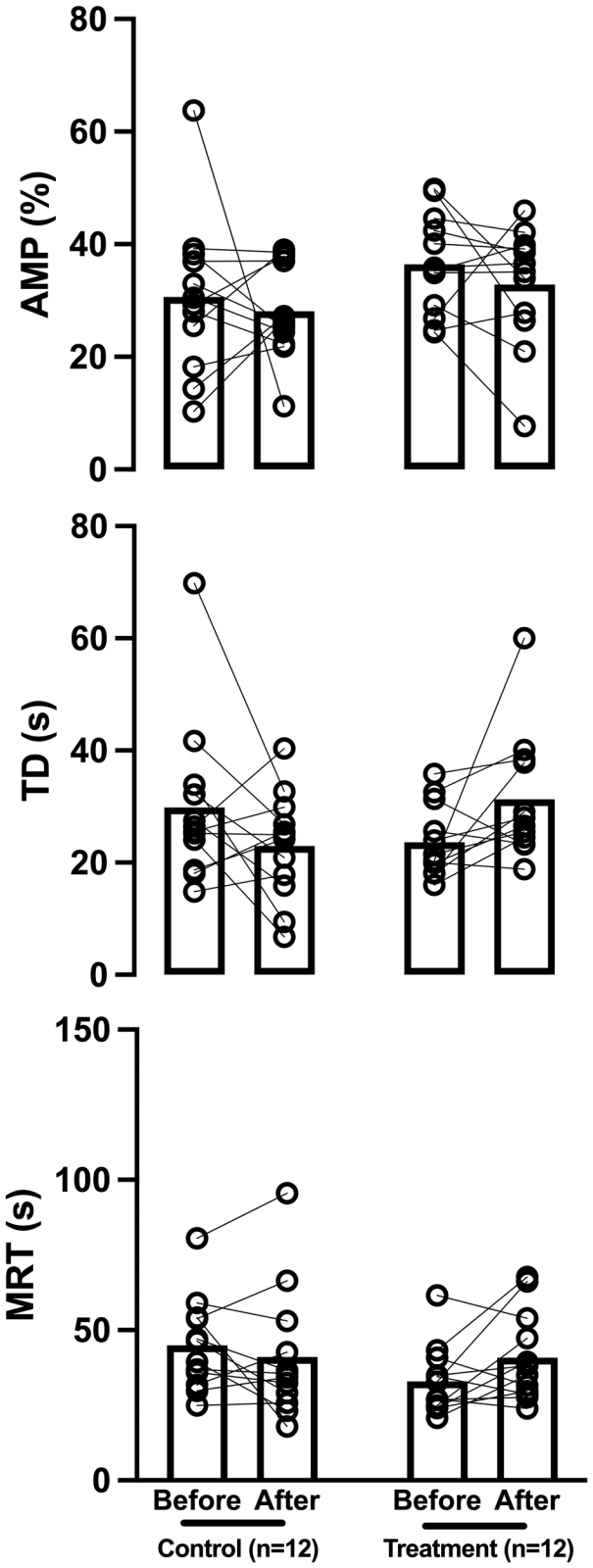



**FIGURE 2—Changes in NIRS measurements during 6MWT.**

well-tolerated in this patient population. The main findings are as follows: 1) the treatment group demonstrated a significant increase in reperfusion index and an trend toward an increase in TD ($P$ = 0.066) after the intervention; 2) Total WD and Continuous WD increased significantly among all participants; 3) a nonsignificant positive trend ($r$ = 0.452) was observed between the change in total walking distance and the change in reperfusion index within the treatment group, while no such trend was observed in the control group ($r$ = 0.094). Together, these results suggest that stretching-induced improvements in microvascular function might contribute to increased walking ability in patients with PAD.

A recent systematic review and meta-analysis reported that stretching exercise significantly improves walking performance in older adults (29). Our previous randomized crossover trial also demonstrated that passive muscular stretching improved total walking distance and continuous walking distance in PAD patients (19). These previous findings suggested the potential for muscular stretching to improve walking ability. In the present randomized controlled trial, however, walking distance improved significantly in all participants while no time by group interaction was observed. This finding should be interpreted with caution because four participants in the treatment group dropped out, which may have reduced the statistical power. Although the change between groups did not reach statistical significance, the direction of improvement supports the potential clinical relevance of stretching as a low-cost and time-saving intervention to enhance walking ability in patients with PAD. Importantly, the observed improvement of 20 meters meets the minimal clinically important difference for 6-min walking distance in this population, which is typically 12–34 m (30).

The present study found a significant time by group interaction for the NIRS measurement of TD during 6MWT. The treatment group demonstrated a trend toward an increase in TD ($P$ = 0.066) while the control group demonstrated a decrease in TD. TD is defined as the time duration from the onset of exercise to the initial observed decrease in $SmO_2$. $SmO_2$ reflects the dynamic balance between oxygen utilization and supply. Previous research showed that PAD patients with greater walking ability exhibited a lower deoxygenation rate and a longer time to reach the lowest $SmO_2$ during exercise (31). Similarly, another study confirmed that PAD patients experience a severe supply-demand mismatch during exercise compared with healthy controls (32). This supply-demand mismatch, owing to reduced oxygen supply, often forces the muscle to rely on rapid oxygen extraction, reflected by fast oxygen consumption kinetics and shorter TD. Taken together, these findings provide insight into the current data, suggesting that the observed increase in TD in the treatment group may be a beneficial physiological adaptation. The increase in TD indicates that daily passive stretching may be effective in delaying the muscle oxygen consumption kinetics during exercise by improving the oxygen delivery to the muscle

TABLE 3. Changes in baseline $SmO_2$, occlusion slope, and reperfusion slope during the vascular occlusion test before and after intervention.

| Variables | Treatment | | Control | | ANOVA *P*-value Time | ANOVA *P*-value T*G |
|---|---|---|---|---|---|---|
| | Before | After | Before | After | | |
| Baseline $SmO_2$ (%) | 47 ± 8 | 51 ± 14 | 47 ± 10 | 46 ± 10 | 0.610 | 0.289 |
| Occlusion slope (%/s) | −0.12 ± 0.04 | −0.12 ± 0.03 | −0.14 ± 0.08 | −0.11 ± 0.04 | 0.373 | 0.493 |
| Reperfusion slope (%/s) | 0.70 ± 0.51 | 0.78 ± 0.48 | 0.88 ± 0.74 | 0.65 ± 0.57 | 0.676 | 0.371 |

tissue, thereby enhancing the balance between oxygen supply and utilization. This concept is supported by our previous data showing that macrovascular function of the popliteal artery, evaluated by flow-mediated dilation, significantly improved after stretching intervention in patients with PAD (19).

The calf reperfusion index increased significantly in the treatment group after 4 wk of stretching. Although not calculated directly in previous studies, data suggested that the reperfusion index was significantly lower in patients with PAD compared with healthy controls during different ischemia–reperfusion protocols (10,33). The reperfusion index reflects how quickly the microcirculation recovers from ischemia induced by the cuff occlusion. The finding of an increased reperfusion index suggests that daily lower-limb muscle stretching improves vasodilatory reserve in skeletal muscle. This improvement likely reflects enhanced endothelial bioavailability of vasodilators (e.g., nitric oxide), improved vascular smooth muscle sensitivity, and/or increased capillarity. Consistent with this mechanism, daily muscle stretching in aged rats has been shown to improve endothelium-dependent vasodilation of intramuscular resistance arterioles and increase capillarity of the soleus muscle (18).

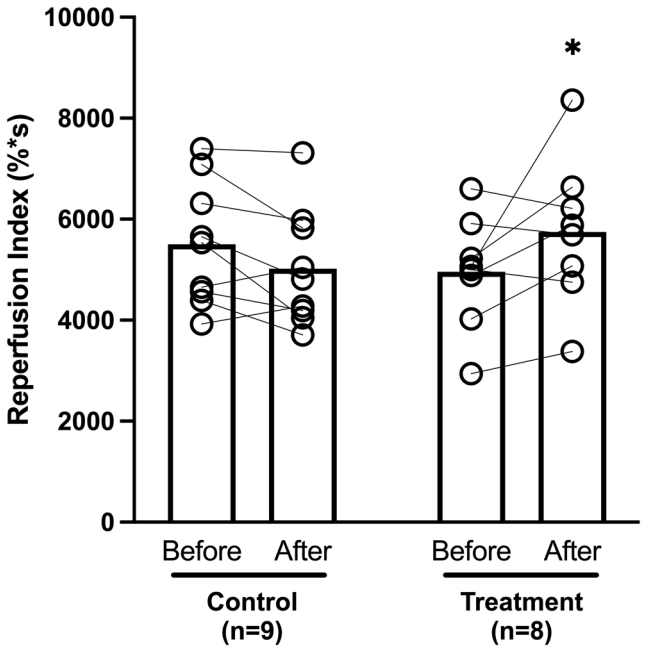



**FIGURE 3—Changes in calf reperfusion index before and after the intervention in both groups.**

Together, data from our previous study and the present study demonstrated that stretching has the potential to improve vasodilatory function and/or reactivity of both conduit and resistance vessels. Future work focused on morphological assessment of stretched muscles, that is, muscle biopsies, could provide insight into these potential microvascular adaptations induced by stretching in patients with PAD.

The present study also found a moderate but nonsignificant correlation ($r$ = 0.45) between the change in reperfusion index and the change in total walking distance, potentially owing to the limited sample size. This result

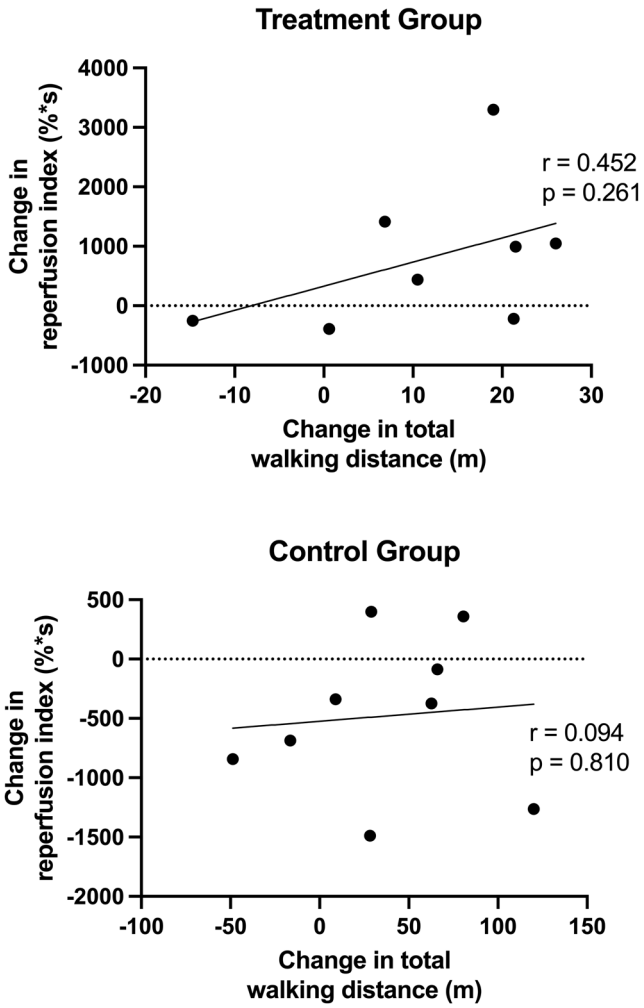



**FIGURE 4—Correlation analysis between the change in calf reperfusion index and change in total walking distance before and after intervention in each group.**

aligns with the findings in the literature that maximal calf conductance, another measurement of microvascular function, and microvascular reactivity were positively associated with 6-min walking distance in patients with PAD (10,34). However, no correlation was observed between TD and total walking distance in 6MWT in the present study (Supplemental Fig. 1B, https://links.lww.com/MSS/D425). From a physiological perspective, while TD reflects the match between oxygen delivery and utilization at the onset of exercise, the reperfusion index serves as a proxy for microvascular vasodilator capacity and perfusion potential. Our results suggest that improvements in walking distance among PAD patients may depend more on enhanced overall microvascular reactivity and the ability to recruit the microvascular bed during sustained exercise, rather than a relatively minor contribution of the initial kinetics of oxygen delivery represented by TD. Future larger-scale randomized controlled trials are needed to elucidate the relation between different microvascular measurements and walking ability.

To our best knowledge, this is the first single-blind randomized controlled trial to evaluate the effectiveness of stretching on microvascular function in patients with PAD. As in any other study, however, the present study is not without limitations. First, participants classified as Fontaine Stage II or Stage III were recruited to increase the generalizability of the findings to a broader range of PAD patients. However, the variability among participants may have complicated data interpretation despite randomization. Future studies should consider focusing on a more homogeneous group, possibly by randomization after stratification of disease severity. Second, adipose tissue thickness might interfere with the NIRS signal and should be taken into account in future experimental design (27,28). Third, the NIRS data during the 6MWT may have been affected by the effort performed by the participants in each trial. Future studies should consider using a treadmill test that could standardize the exercise intensity of the measurement to reduce the potential confounding factors and to better examine the change in microcirculation function and walking performance (35). Fourth, inherent limitations regarding blinding in exercise interventions should be acknowledged. Although we utilized a sham design where the control group wore the same ankle splint with minimal inflation, participants may have been able to distinguish between the intervention and control conditions based on the sensation of muscle tension. Furthermore, the outcome assessors were not blinded to group allocation, which represents a potential source of bias in the interpretation of the results. Fifth, adherence to stretching was determined using self-reported diaries, which may be subject to recall bias or overestimation. Lastly, the sample size of this study was limited because this study was conducted during the COVID-19 pandemic.

## CONCLUSIONS

In summary, the present randomized controlled trial demonstrated that static muscular stretching improves microvascular function, possibly leading to improved walking ability in patients with PAD. Compared with supervised exercise training, using a stretching device at home is a low-cost and convenient option. The findings in the present study suggested that stretching intervention could be a viable or supplementary option for PAD patients to enhance their microvascular function and may provide a gradual transition to more intense exercise training.

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Dr. Judy Muller-Delp holds patents related to the concept of using stretching to improve microcirculation (US Patent Nos. 11,020,054 and 12,115,120). The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

No conflicts of interest were disclosed.

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