A Single Bout of Continuous Blood Flow Restriction Walking Does Not Negatively Affect Lower Limb Microvascular Function

Original Research

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Abstract

Introduction: Diminished macrovascular vasodilatory function has been reported following one blood flow restriction (BFR) walking bout. The primary aim was to investigate the potential effect that walking exercise (control, BFR) and cool-down (supine, rolling massage [RM], walk) had on microvascular function.

Methods: Fourteen participants walked for 15 minutes at a 3/10 rating of perceived exertion without (Session 1) and with (Sessions 2-4) BFR (60% limb occlusion pressure, LOP). Participants either rested supine, had RM performed on the thigh, or actively walked for the first two minutes of the cool-down. Microvascular functions of the quadriceps and calf muscles of a randomized leg were measured before and 20 minutes after each walking bout using the vascular occlusion test (VOT) via near-infrared spectroscopy. Between session intraclass correlations (ICC) and minimal detectable change (MDC) were calculated for microvascular function of each muscle. Two-way repeated measures analysis of variance was performed to assess if condition (CON+Supine, BFR+Supine, BFR+RM, BFR+Walk) and/or time (pre-exercise, post-exercise) affected microvascular function. Statistical significance was set at $p<0.05$.

Results: Quadriceps (ICC=0.891, MDC=0.60 %/s) and calf (ICC=0.839, MDC=0.63 %/s) microvascular function demonstrated good between session reliability. Microvascular function statistically increased in the quadriceps (0.88±0.74 %/s vs 1.07±0.70 %/s) and calf (0.56±0.57 %/s vs 0.75±0.70 %/s) muscles after exercise; however, the changes (quadriceps=0.13 %/s, calf=0.19 %/s) did not exceed the MDC. No significant differences in microvascular function were observed between conditions.

Conclusions: Microvascular function was maintained following fifteen minutes of continuous BFR walking with 60% LOP, regardless of the cool-down procedure.

Key Words: Aerobic Exercise, Near-Infrared Spectroscopy, Vascular Function

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Introduction

The use of blood flow restriction (BFR) techniques along with various training modalities have demonstrated positive performance outcomes when using low-intensity loads. More specifically, BFR walking interventions have been shown to improve maximal aerobic capacity (VO_{2MAX}) [1], functional ability [2], maximal minute ventilation [1], muscular strength [2–4], and muscular hypertrophy [2–5] following training. Thus, BFR walking interventions might be an alternative training method that practitioners consider when trying to improve various aspects of physical fitness with low-intensity loads. However, the safety of the participants is of utmost importance, especially when considering the potential impacts BFR exercise and training interventions might have on vascular function [6].

When investigating vascular function, both the macrovascular function (endothelial-mediated dilation capacity within an artery) and microvascular function (tissue oxygenation saturation [StO_2] reperfusion within capillaries) can be non-invasively assessed. The flow mediated dilation (FMD), using Doppler ultrasound, and vascular occlusion test (VOT), using near-infrared spectroscopy (NIRS), are two non-invasive assessments that can measure macrovascular and microvascular function, respectively [7]. There is conflicting evidence regarding the potential effects that BFR walking has on macrovascular function and no evidence to our knowledge for how BFR walking might impact microvascular function. One bout of BFR intermittent walking exercise has been shown to diminish macrovascular function (endothelial-mediated dilation within the popliteal artery) twenty minutes after the walking bout was completed in healthy, sedentary young adults [8]. Whereas following a 10-week continuous walking intervention, arterial compliance in the carotid arteries were improved from baseline values in elderly adults who completed either the BFR walking (50% improvement) or non-BFR walking (59% improvement) interventions [4]. Both of these previous investigations [4,8] have studied the effects that BFR walking has on macrovascular (artery) function and thus further investigation into the potential impact that BFR walking has on microvascular (capillary) function is warranted.

Despite the advantageous physical fitness adaptations reported subsequent to a BFR walking intervention [1–5], it would be beneficial to understand the potential impacts that BFR walking might have on the microvascular function so that practitioners can better determine if BFR walking is warranted for their clients or patients. Therefore, the first aim of the investigation was to examine the between session test-retest reliability of the 100% limb occlusion pressure (LOP) measurements and the pre-exercise microvascular function measurements obtained from the VOT. From this between session reliability analysis, the minimal detectable change (MDC) value for each variable (LOP and microvascular function) could be calculated thus helping practitioners in determining if a real change occurred in these measurements after an intervention [9]. It was hypothesized that both the 100% LOP measurements and the pre-exercise microvascular function measurements would provide good between session test-retest reliability. In an effort to address BFR safety considerations, the second aim of the investigation was to determine if an acute bout of BFR continuous walking and a variety of cool-down methods (passive supine, rolling massage [RM], and active walking) might impact the microvascular function within the exercising lower extremity muscles (vastus lateralis [VL] and medial gastrocnemius [MG]). It was hypothesized that the microvascular function would decrease following BFR walking and passive supine recovery but would increase following BFR walking and all other cool-down methods (RM, active walking).

Methods

Participants

Based upon the microvascular function results of a previous investigation [10], an a priori power analysis indicated that twelve participants were needed for the present investigation (power = 0.80, alpha = 0.05). Therefore, fourteen healthy recreationally active adults (Table 1) participated in the present investigation. Participants were informed of the investigation's purpose, procedure, and possible risks. All participants completed an informed consent that was approved by the university's Institutional Review Board for Human Subject Research and was in accordance with the Declaration of Helsinki. Any individual who self-reported a history of metabolic, neurological, cardiopulmonary disease (including blood clotting), or an orthopedic musculoskeletal related injury in the past 12 months was excluded from participation within the investigation. Recreationally active was defined as participating in at least 150 minutes of physical activity per week for the last three months [11]. Two participants reported only performing resistance training while twelve participants reported performing a mix of aerobic and resistance training. Additionally, participants were asked to abstain from consuming caffeine and using tobacco products for at least eight hours prior to all data collection sessions. Lastly, participants were asked to abstain from performing lower body exercise for 24 hours prior to all data collection sessions.

Protocol

Participants reported to the laboratory for four data collection sessions with each session lasting approximately ninety minutes. Height and weight were collected using a stadiometer and calibrated scale, respectively. Participants were then asked to lie in a supine position on a padded table for five minutes, after which their resting heart rate and resting blood pressure were recorded [11]. The resting measurements were measured in a supine position as participants would be in a supine position during the performance of all VOT. Resting heart rate was measured using a pulse oximeter (500 DL Pro, Zacurate, Stafford, Texas, USA) placed on the index finger, while resting blood pressure was measured manually from the brachial artery using a sphygmomanometer and stethoscope by the same experienced investigator. Next, skinfold measurements from the VL and MG were measured using a skinfold caliper (Lange Skinfold Calipers, Beta Technology, Cambridge, Maryland, USA). Two skinfold measurements were taken from each muscle. If the two

measurements from a single muscle differed by more than two millimeters then additional skinfold measurements were taken until two measurements were obtained that differed by no more than two millimeters from one another. The two skinfold measurements from a single muscle were then averaged together and recorded. The VL skinfold was measured midway between the anterior superior iliac spine and the proximal patella over the VL muscle belly. The MG skinfold was measured midway between the proximal and distal ends of the MG over the muscle belly.

Table 1. Participant demographics.

Variable	Data	Variable	Data
Sex	8 Female, 6 Male	Resting Heart Rate	66 ± 11 bpm
Age	22 ± 1 years	Resting Blood Pressure	121/74 ± 10/9 mmHg
Height	1.70 ± 0.07 m	Mean Arterial Pressure	90 ± 8 mmHg
Weight	66.0 ± 9.6 kg	VL Skinfold	19 ± 7 mm
Body Mass Index	22.8 ± 2.0 kg/m^2	MG Skinfold	14 ± 7 mm

Data is reported as average ± standard deviation. VL = Vastus Lateralis. MG = Medial Gastrocnemius.

Continuous wave wireless NIRS sensors (PortaMon MKII, Artinis Medical System, The Netherlands) were placed over the muscle bellies of the VL and MG of a randomized leg and used to measure the StO_2 response at wavelengths of 760 nm and 850 nm. All StO_2 data were sampled at 10 Hz. The skin was shaved (if needed) and cleansed with an alcohol preparation pad prior to placing the NIRS sensors on the participants. The NIRS sensors were placed midway between the anterior superior iliac spine and the proximal patella over the VL muscle belly and midway between the proximal and distal ends of the MG muscle belly. Both NIRS sensors were secured with tape and wrapped with an elastic strap to help limit movement artifact and prevent stray visible light from impacting the NIRS signal. A 5-cm thick foam pad with a cutout for the MG NIRS sensor was placed under the participant's lower legs so that the MG NIRS sensor could be slightly elevated above the padded table while the participant was lying in a supine position during the VOT. Lastly, an inflatable elastic cuff (Rapid Cuff Inflation System, Hokanson, Bellevue, Washington, USA) was applied to the proximal thigh of the randomized test leg.

Once the NIRS sensors and elastic cuff were applied to the test leg, the pre-exercise microvascular function of the VL and MG muscles were assessed using a previously established VOT protocol [7]. Specifically, the StO_2 responses from both the VL and MG muscles were measured simultaneously during a two-minute resting baseline, five-minute arterial occlusion, and eight-minute reperfusion recovery. During the VOT the participant remained at rest in a supine position on a padded table. During the five-minute arterial occlusion portion of the VOT, 300 mmHg of pressure was applied to the proximal thigh using the inflatable elastic cuff. Figure 1 displays the timeline of events throughout the investigation.

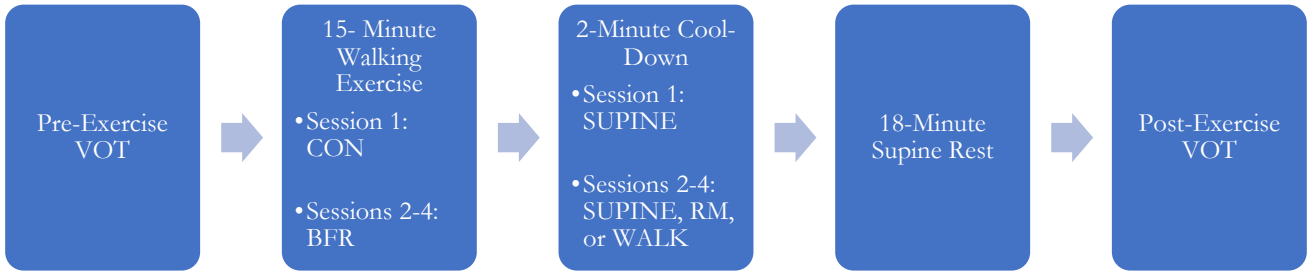

Figure 1. Investigation timeline.
VOT = Vascular Occlusion Test. CON = Control. BFR = Blood Flow Restriction. SUPINE = Supine Recovery Condition. RM = Rolling Massage Recovery Condition. WALK = Active Walk Recovery Condition.

Following the completion of the pre-exercise VOT during session 1, participants were then asked to walk on a treadmill and the speed was gradually increased until the participant stated that they were exercising at a rating of perceived exertion (RPE) of 3/10 (0 = "resting", 10 = "maximal exertion") [11]. The speed from session 1 was recorded in km/h and used as the walking speed for all other data collection sessions. During session 1, participants walked at their pre-

determined speed for 15 minutes without any BFR applied to their legs. Following the completion of the pre-exercise VOT during sessions 2-4, limb occlusion pressures (LOP) were assessed on each leg while the participant was standing upright by placing a handheld Doppler ultrasound over the posterior tibial artery and gradually increasing the cuff pressure until no auditory pulse was detected [12–14]. The Doppler ultrasound remained positioned over the posterior tibial artery after the occlusion cuff was deflated to confirm that blood flow returned to the limb after cuff deflation and that the head of the Doppler ultrasound was not moved during the LOP assessment [12]. The lowest occlusion pressure that resulted in no auditory pulse detected by the Doppler ultrasound was considered 100% LOP. The LOP was assessed while the participant was in a standing position since body position can influence the LOP measurement [14] and the participant would be in a standing position during the walking exercise. Following the LOP assessment during sessions 2-4, participants then walked at the same speed from session 1 for 15 minutes while BFR was applied bilaterally to their proximal thighs at 60% LOP of the randomized test leg. 60% LOP was chosen as the exercising occlusion pressure for the present investigation based upon previous BFR recommendations [15].

Immediately following the completion of the 15-minute walking bouts, participants completed one of the following cool-down methods: passive supine rest (SUPINE), rolling massage (RM), or active walking with no BFR (WALK) for the first two minutes of the twenty-minute recovery. During the SUPINE cool-down, participants would lay supine on a padded table immediately following the completion of the walking bout. During the RM cool-down, the hamstring muscle group and quadriceps muscle group were massaged using a wooden massage stick for one minute per muscle group. Similar to a previous investigation [16], the investigators applied pressure using the massage stick to elicit a 7/10 perceived pain score and used a consistent massaging pace (two seconds up, two seconds down) during the RM cool-down. Throughout the RM cool-down, the participant rated their perceived pain every five seconds using a visual analog scale (0 = no pain, 10 = intolerable pain) and the investigator would adjust the massage pressure as needed to maintain a 7/10 perceived pain score. Previous findings [16] report that microvascular function significantly improves after performing this RM protocol for 30 seconds. During the WALK cool-down, participants continued to walk for two minutes at the same speed that elicited a 3/10 RPE during session 1 without any BFR applied to their proximal thighs.

During session 1, the control (CON) walking bout, participants always performed the SUPINE recovery. During sessions 2-4, the BFR walking bouts, participants would be randomly assigned one of the cool-down methods (SUPINE, RM, or WALK) to complete during the recovery for each session. Regardless of session, each cool-down method was performed for the first two minutes of the twenty-minute recovery and then participants passively rested in a supine position on a padded table for an additional eighteen minutes. Twenty minutes after completion of the walking bout (two-minute cool-down and eighteen-minute passive supine rest), the post-exercise microvascular function of the VL and MG muscles were measured simultaneously using the previously established VOT protocol [7] to allow for easy comparison to a previous BFR walking investigation [8]. Microvascular function for the VL and MG muscles were assessed as the linear, upward slope from the first ten seconds of the StO_2 reperfusion response during the VOT [7]. The StO_2 reperfusion slope was therefore measured in units of percentage per second (%/s).

Statistical Analysis

Relative and absolute between session test-retest reliability of the StO_2 reperfusion slope for the VL and MG muscles as well as the 100% LOP for the left leg and right leg were assessed by mixed absolute agreement intraclass correlation coefficients (ICC) and standard error of the measurement (SEM), respectively. Additionally, 95% confidence intervals (95% CI), coefficients of variation (%CV), and minimal detectable change (MDC) values were assessed for the StO_2 reperfusion slope and 100% LOP. MDC was calculated at a 95% level of confidence [9]. ICC were interpreted as 0.90-1.00 = "excellent", 0.75-0.89 = "good", 0.50-0.74 = "moderate", and < 0.50 = "poor".

Two-way repeated measures analysis of variance (ANOVA) was used to examine if condition (CON+SUPINE, BFR+SUPINE, BFR+RM, BFR+WALK) and/or time (pre-exercise, post-exercise) affected the microvascular function (StO_2 reperfusion slope) of the VL or MG muscles. When appropriate, significant main effects and/or interactions from the ANOVA were further analyzed using Tukey's post-hoc test to identify significant pairwise differences. Effect sizes (ES) were calculated as the difference between the means divided by the pooled standard deviation and were interpreted as 0.00-0.19 = "trivial", 0.20-0.59 = "small", 0.60-1.19 = "moderate", 1.20-2.00 = "large", and > 2.00 = "very large" [17]. The *a priori* power analysis was performed using G*Power 3.1.9.7 [18]. All other statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 23 (IBM Corporation, Armonk, New York, USA). Statistical significance was set at $p < 0.05$. All data are reported as average ± standard deviation.

Results

Between Session Reliability

The pre-exercise microvascular function, as measured via the StO_2 reperfusion slope, for the VL and MG muscles demonstrated good relative (ICC_{VL} = 0.891, ICC_{MG} = 0.839) and absolute (SEM_{VL} = 0.22 %/s, SEM_{MG} = 0.23 %/s) between session reliability (Table 2). Additionally, the 100% LOP for the left leg and right leg reported excellent relative (ICC_{LEFT} = 0.902, ICC_{RIGHT} = 0.923) and absolute (SEM_{LEFT} = 12 mmHg, SEM_{RIGHT} = 11 mmHg) between session reliability (Table 2).

Table 2. Between session reliability results.

Statistic	VL StO_2 Reperfusion Slope	MG StO_2 Reperfusion Slope	Left Leg 100% LOP	Right Leg 100% LOP
Session 1	1.10 ± 0.80 %/s	0.65 ± 0.70 %/s	N/A	N/A
Session 2	0.86 ± 0.47 %/s	0.61 ± 0.49 %/s	244 ± 43 mmHg	215 ± 45 mmHg
Session 3	0.79 ± 0.63 %/s	0.44 ± 0.59 %/s	231 ± 39 mmHg	209 ± 40 mmHg
Session 4	1.00 ± 0.72 %/s	0.55 ± 0.51 %/s	226 ± 31 mmHg	204 ± 30 mmHg
%CV	14.8%	16.3%	4.0%	2.6%
ICC	0.891*	0.839*	0.902*	0.923*
95% CI	0.758-0.961	0.639-0.942	0.751-0.966	0.814-0.973
SEM	0.22 %/s	0.23 %/s	12 mmHg	11 mmHg
MDC	0.60 %/s	0.63 %/s	33 mmHg	29 mmHg

Data from sessions 1-4 are reported as average ± standard deviation. *Denotes significant intraclass correlation coefficients ($p < 0.001$). VL = Vastus Lateralis. MG = Medial Gastrocnemius. LOP = Limb Occlusion Pressure. %CV = Percentage Coefficient of Variation. ICC = Intraclass Correlation Coefficient. 95% CI = 95% Confidence Interval. SEM = Standard Error of the Measurement. MDC = Minimal Detectable Change.

Walking Exercise

Participants completed all four data collection sessions reporting no adverse events. The walking speed (4.0 ± 0.6 km/h) that elicited a 3/10 RPE without the use of BFR was used during all four exercise sessions. The 100% LOP of the randomized test leg in a standing position was 222 ± 41 mmHg. During the BFR walking sessions, participants walked (4.0 ± 0.6 km/h) with 60% LOP (133 ± 24 mmHg) applied bilaterally to the proximal thighs.

Exercise And Cool-Down Effects On Microvascular Function

A significant main effect of time was found for the StO_2 reperfusion slope of the VL ($p = 0.045$). Further examination indicated that there was a trivial (ES = 0.19) increase in the StO_2 reperfusion slope within the VL subsequent to the walking and cool-down bouts (pre-exercise = 0.94 ± 0.66 %/s, post-exercise = 1.07 ± 0.70 %/s, $p = 0.045$). However, the difference in the StO_2 reperfusion slope within the VL from pre-exercise to post-exercise (0.13 %/s) did not exceed the MDC value calculated for the StO_2 reperfusion slope within the VL (0.60 %/s, Table 2). No significant condition main effect ($p = 0.385$) or condition x time interaction ($p = 0.719$) were observed.

A significant main effect of time was found for the StO_2 reperfusion slope of the MG ($p < 0.001$). Further examination showed that there was a small (ES = 0.30) increase in the StO_2 reperfusion slope within the MG subsequent to the walking and cool-down bouts (pre-exercise = 0.56 ± 0.57 %/s, post-exercise = 0.75 ± 0.70 %/s, $p < 0.001$). However, the difference in the StO_2 reperfusion slope within the MG from pre-exercise to post-exercise (0.19 %/s) did not exceed the MDC value calculated for the StO_2 reperfusion slope within the MG (0.63 %/s, Table 2). No significant condition main effect ($p = 0.884$) or condition x time interaction ($p = 0.329$) were observed.

Discussion

In accordance with the hypothesis of the study's first aim, the 100% LOP measurements were found to have excellent between session test-retest reliability. The present between session test-retest reliability results (Table 2) are similar to previously published 100% LOP reliability results (ICC = 0.953) when using an automated tourniquet occlusion cuff

in a standing position [14]. According to the present MDC results, practitioners can be confident that a change occurred between sessions when the 100% LOP in the lower extremities changes by ~30 mmHg (29-33 mmHg) while in a standing position. Additionally, in agreement with our hypothesis, the pre-exercise microvascular function measurements were found to have good between session test-retest reliability (Table 2). The between session %CV of the present investigation (14.8%-16.3%) was slightly higher than the within session %CV (11.0%) of a previous microvascular function investigation [7]. The slightly higher between session %CV observed could be explained by the methods of the present study in which the NIRS sensors were removed from the subjects following the completion of each data collection session. However, as stated earlier, practitioners can be confident that a change in microvascular function occurred between sessions within the VL and MG muscles when the StO_2 reperfusion slope changes by 0.60 %/s or 0.63 %/s, respectively (Table 2).

Contrary to the hypothesis of the study's second aim, the novel findings demonstrate that microvascular function was maintained subsequent to 15 minutes of continuous BFR walking. Although, a trivial to small (ES = 0.19-0.30) statistically significant difference in the microvascular function was observed subsequent to all walking exercises and recoveries (CON+SUPINE, BFR+SUPINE, BFR+RM, BFR+WALK) in both exercising muscle groups (VL, MG), it is important to mention that the change in microvascular function observed within the VL (Δ = 0.13 %/s) and MG (Δ = 0.19 %/s) did not exceed the microvascular function MDC values calculated for each muscle group (MDC_{VL} = 0.60 %/s; MDC_{MG} = 0.63%/s; Table 2). Therefore, the researchers cannot be confident that a real improvement in microvascular function occurred subsequent to the walking exercises and recoveries. However, the present findings do suggest that the exercise types (CON, BFR) and recovery methods (SUPINE, RM, WALK) did not negatively affect microvascular function following 15 minutes of continuous walking in healthy young recreationally active adults. Evidence for this conclusion includes a) microvascular function was maintained and did not decrease after any of the walking and recovery conditions (Table 2) and b) there were no significant differences in microvascular function observed between the CON+SUPINE and BFR+SUPINE walking conditions. The present findings conflict with a previous report of diminished macrovascular function, specifically endothelial-mediated dilation of the popliteal artery, subsequent to a single bout of BFR walking [8].

The different exercising occlusion pressure methodologies used in the present investigation and previous investigation [8], might help explain some of the variance in the conflicted vascular function findings. More specifically, the exercise occlusion pressures (relative 60% LOP versus absolute 160 mmHg) and the timing of when the occlusion cuff was inflated prior to the walking exercise (inflated immediately prior to exercise versus inflated three minutes prior to exercise) might help explain the different vascular function responses subsequent to a BFR walking bout. A recent BFR systematic review suggests that BFR research has undergone a transition from prescribing absolute occlusion pressures during exercise to now using relative occlusion pressures during exercise, with many authors citing the change to relative occlusion pressures, such as a percentage of LOP, due to safety considerations [19]. A previous investigation [20] found that 79%-87% of the variance in 100% LOP measurements within the lower limbs can be explained by sex, systolic blood pressure, limb size, cuff size, and body composition. Therefore, the use of relative occlusion pressure methods could better account for potential anthropometric and sex differences within a group of heterogeneous participants, leading to a more relatively consistent BFR stimulus during exercise. This is in contrast to the prescription of absolute occlusion pressures, which might cause a higher variance in the BFR stimulus during exercise among a group of heterogeneous participants potentially leading to diminished vascular function subsequent to exercise. Additionally, inflating the occlusion cuff minutes prior to the initiation of exercise has been shown to elicit a greater local ischemia (as measured by an increased microvascular deoxygenated heme response via NIRS) within the exercising muscle during low-intensity BFR resistance exercise compared to when the occlusion cuff is inflated immediately prior to the start of exercise [21]. Three minutes of pre-exercise occlusion might have led to a higher level of local ischemia during the present BFR walking bout which could have contributed to the declined vascular function previously observed subsequent to a BFR walking bout [8].

The present research investigation is not without limitations. First, the present investigation examined the potential impact of BFR walking on microvascular function using the VOT rather than macrovascular function using the FMD test. Microvascular function was chosen as the primary dependent variable of the present investigation because it is correlated with macrovascular function [7] and the VOT provides an opportunity to assess multiple sites (VL, MG) simultaneously. Additionally, previous investigations already examined the impact that BFR walking exercise [8] and training [4] had on macrovascular function, while microvascular function had yet to be investigated. Second, heterogeneous StO_2 responses have been recorded within the same exercising muscle when measured via NIRS [22]. In an attempt to mitigate this limitation, the current investigation measured microvascular function in two separate muscle

groups (VL, MG) within the same test leg. Next, the amount of oxidative stress was not measured subsequent to each walking and cool-down session. It could be possible that microvascular function was maintained in the present investigation because the walking exercises did not elicit a high amount of oxidative stress as exercise-induced reductions of macrovascular function have been associated with oxidative stress [23]. Lastly, the present investigation used continuous walking for 15 minutes at a constant speed rather than interval walking (five intervals of two-minute walking and one-minute passive resting) for 15 minutes [3,8]. Fifteen minutes of continuous walking was chosen so that the exercise time can be consistent with previous BFR interval walking investigations [3,8] while replicating the continuous walking that has demonstrated improved carotid arterial compliance following a BFR walking intervention [4].

Conclusions

In conclusion, the present investigation demonstrated that microvascular function was maintained following 15 minutes of continuous walking, with or without BFR. Additionally, it is important to note that the use of BFR walking did not affect microvascular function any differently than the CON walking. Therefore, the present results do suggest that a 15-minute bout of continuous BFR walking using 60% LOP does not negatively affect microvascular function in healthy young recreationally active adults.

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Conflict of Interest

The authors declare no conflicts of interest.

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