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Reduced post-exercise muscle microvascular perfusion with compression is offset by increased muscle oxygen extraction: Assessment by contrast-enhanced ultrasound

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Abstract

The microvasculature is important for both health and exercise tolerance in a range of populations. However, methodological limitations have meant changes in microvascular blood flow are rarely assessed in humans during interventions designed to affect skeletal muscle blood flow such as the wearing of compression garments. The aim of this study is, for the first time, to use contrast-enhanced ultrasound to directly measure the effects of compression on muscle microvascular blood flow alongside measures of femoral artery blood flow and muscle oxygenation following intense exercise in healthy adults. It was hypothesized that both muscle microvascular and femoral artery blood flows would be augmented with compression garments as compared with a control condition. Ten recreationally active participants completed two repeated-sprint exercise sessions, with and without lower-limb compression tights. Muscle microvascular blood flow, femoral arterial blood flow (2D and Doppler ultrasound), muscle oxygenation (near-infrared spectroscopy), cycling performance, and venous blood samples were measured/taken throughout exercise and the 1-hour post-exercise recovery period. Compared with control, compression reduced muscle microvascular blood volume and attenuated the exercise-induced increase in microvascular velocity and flow immediately after exercise and 1 hour post-exercise. Compression increased femoral artery diameter and augmented the exercise-induced increase in femoral arterial blood flow during exercise. Markers of blood oxygen extraction in muscle were increased with compression during and after exercise. Compression had no effect on blood lactate, glucose, or exercise performance. We provide new evidence that lower-limb compression attenuates the exercise-induced increase in skeletal muscle microvascular blood flow following exercise, despite a divergent increase in femoral artery blood flow. Decreased muscle microvascular

Abbreviations: CEU, contrast-enhanced ultrasound; NIRS, near-infrared spectroscopy; NMRF, nuclear magnetic resonance flowmetry; PET, positron emission tomography; RSE, repeated-sprint exercise.

James R. Broatch and Shane F. O'Riordan are equal contribution and shared first authorship.

perfusion is offset by increased muscle oxygen extraction, a potential mechanism allowing for the maintenance of exercise performance.

KEYWORDS

capillary–myocyte interface, ergogenic, microvasculature, NIRS, sprint-interval exercise

1 | INTRODUCTION

Blood flow plays a vital role in the delivery of nutrients and hormones to many tissues in the body, including skeletal muscle.[1-3] During exercise, total limb and microvascular (capillary) blood flow increases to augment oxygen delivery and uptake to the contracting muscle—a phenomenon that has been known for over 100 years.[3,4] Oxygen uptake increases in skeletal muscle in an exercise intensity-dependent fashion to meet the increased metabolic demand of the contracting muscle.[5-7] Thus, it is expected that exercise with a higher metabolic demand (eg, repeated-sprint exercise) will concurrently demand larger volumes of blood flow distribution to the working muscles to maintain exercise performance.[3,8] However, it is the microvasculature that lies downstream of the cardiovascular and arterial vascular networks that is in direct contact with the myocyte and is therefore ultimately responsible for nutrient and hormone exchange including the delivery and uptake of oxygen and substrates. In support, microvascular dysfunction is a hallmark feature of many clinical populations who suffer from exercise intolerance including type 2 diabetes,[9,10] heart failure,[11,12] and peripheral arterial disease.[13] Thus, it is not surprising that the extent of microvascular blood flow in response to exercise is considered a rate limiting step for exercise capacity, at least in clinical populations characterized by microvascular dysfunction.[9-13] However, the link between microvascular blood flow and exercise performance in recreationally active and healthy people is less clear, a likely consequence of the indirect methods used to estimate or measure muscle blood flow.[14-20]

Compression garments have previously been assessed as a potential method to augment exercise-induced increases in limb blood flow and/or skeletal muscle perfusion, with mixed results.[15-22] This may be important considering the proposed role of limb blood flow in moderating muscle regeneration,[23] and exercise capacity in both healthy[24-26] and clinical populations that exhibit vascular dysfunction.[9-13] As such, compression-induced alterations in blood flow may be a potential strategy for improving exercise capacity and post-exercise recovery. It is thought that the external compression applied by the garment reduces arteriolar transmural pressure by an amount approximately equivalent to the level of pressure applied,[19] subsequently causing a reflex vasodilation of the arterioles and increased blood supply to the capillary network.[18] Additionally, compression may improve blood supply to the microvasculature via increased arterial blood flow,[27] greater venous return,[28] and a subsequent increase in stroke volume.[29,30] In support of these mechanisms we have reported that lower-limb compression tights increase muscle blood flow by ~18% during exercise in healthy individuals, as measured by near-infrared spectroscopy (NIRS) during brief venous occlusion.[14] Similarly, compression garments have been reported to increase limb perfusion both at rest and during exercise as measured by strain-gauge plethysmography,[18,21] nuclear magnetic resonance flowmetry (NMRF),[18-20] and radioactive ^{133}Xe isotope clearance.[22] Conversely, compression garments have also been reported to have either no effect on limb perfusion as measured by NIRS,[15,16] or to even reduce limb perfusion as measured by positron emission tomography (PET).[17] These contradictory reports likely stem not only from variations in exercise protocol, the application of compression, and population demographics, but also variations in techniques used to measure limb perfusion.

The vast majority of techniques used to measure compression-induced changes in blood flow are unable to distinguish between macrovasculature flow responsible for feeding the capillary network (ie, large arteries, feed arteries, and arterioles), and the microvasculature flow feeding the muscle bed (ie, capillaries) that is responsible for nutrient and hormone exchange. This is important, as it has been shown that macrovascular and microvascular blood flow responses to muscle contraction,[9,31] insulin infusion,[32,33] and meal ingestion,[34,35] can be altered independent of each other. One of the most common methods for estimating muscle microvascular perfusion is NIRS. However, NIRS measurements represent a weighted average of arterial, capillary, and venous heme O_2 saturations,[36] and the distribution of heme units among these vascular beds is largely unknown.[37] In addition, the NIRS signal is influenced by factors like adipose tissue thickness,[38] skin melanin content,[39] and cutaneous blood flow/volume[40]; all of these will affect NIRS-derived measures of blood flow. More sophisticated blood flow measurement techniques like NMRF and PET provide high-resolution measurements of regional blood flow, but are unable to spatially distinguish the microvasculature from larger conduit and feed arteries within the muscle.[41] With these limitations in mind, it is unknown if previously reported compression-induced changes in blood flow are representative of muscle microvascular blood flow occurring at the capillary-tissue interface, or whether they are due to changes in macrovascular blood flow supplying the microvascular network.

Our team, along with our collaborators, have optimized a technique known as real-time contrast-enhanced ultrasound

(CEU) to directly measure microvascular blood volume, velocity, and flow in human skeletal muscle.[10,33-35,42,43] This technique measures microvascular hemodynamics via the intravenous infusion of a contrast agent composed of hemodynamically inert lipid microspheres, which are echogenic and sufficiently small in size to perfuse capillaries. This is a widely used method for the evaluation of microvascular blood flow (perfusion) in various human tissues,[44] including skeletal muscle in vivo.[1,2,42] The main feature of CEU that makes it unique from other techniques is that it can quantify changes in: (i) the number of capillaries that are active/open (ie, microvascular blood volume), (ii) the filling rate of blood through the capillary bed (ie, capillary flow velocity), and (iii) the overall extent of blood flow through the capillary bed (ie, microvascular blood flow) which is the product of volume and velocity. While this technique has been used in the context of muscle contraction and exercise,[9,10,42] no research to date has utilized it to assess the effects of compression on microvascular blood flow.

The aim of this study was to use modern ultrasound techniques to comprehensively assess, for the first time, the effects of compression garments on both macro (femoral artery) and microvascular (capillary) blood flow in skeletal muscle following intense exercise and throughout the post-exercise recovery period. Considering the divergent roles of the macro- (ie, supplying the microvasculature) and microvasculature (ie, capillary-muscle nutrient and hormone exchange), these assessments will provide novel information to better characterize the effects of compression on the different vascular networks. It was hypothesized that both femoral artery and muscle microvascular blood flow would be augmented with compression garments compared with a control immediately after and 1-hour post-exercise.

2 | MATERIALS AND METHODS

2.1 | Participants and experimental design

The study employed a within-subject crossover design, in which participants completed two exercise sessions under two separate conditions: (1) wearing full-length, lower-limb compression tights (2XU Elite MCS Tights, Melbourne, Australia; COMP), and (2) wearing normal loose-fitting exercise shorts (control; CON). Ten (8 male and 2 female) recreationally active participants, performing at least 90 minutes of moderate- to high-intensity aerobic exercise per week, completed the study (mean ± SD: age, 27.4 ± 6.3 y; height, 1.81 ± 0.06 m; body mass, 74.5 ± 14.0 kg; body-mass index, 22.6 ± 3.5 kg/m^2). Written informed consent was obtained prior to participation. Participants were screened for contraindications for study participation including smoking, personal or family history of type 2 diabetes and cardiovascular disease, critical limb ischemia (including peripheral artery disease), microvascular disease, and other factors limiting exercise capacity (eg, arthritis or other musculoskeletal complications). All procedures were approved by the Deakin University Human Research Ethics Committee (2018-177) and were conducted in accordance with the Declaration of Helsinki.

2.2 | Familiarization and experimental sessions

Participants reported to the laboratory on three separate occasions. The first session involved a familiarization session where participants underwent two full sets of the exercise protocol and were familiarized with the ultrasound equipment and blood flow measurement methods. The study utilized a repeated-sprint exercise (RSE) protocol in an attempt to maximize the effects of compression on blood flow. For example, supra-maximal intensity exercise with short (<60 s) recovery periods places considerable metabolic stress on components of aerobic metabolism, including blood flow and oxygen uptake and delivery.[3,8,14,45]

A minimum of 48 hours after the familiarization session, participants reported to the laboratory for their first randomized experimental session (CON or COMP). Participant preparation included mark-up of ultrasound probe location and NIRS placement on the thigh, insertion of a catheter into a forearm antecubital fossa vein, and fitting/pressure assessment of the compression tights (COMP condition only) as previously described.[14] Garment pressure was assessed (Kikuhime Pressure Monitor; mediGROUP, Melbourne, Australia) at the medial aspect of the maximal calf girth, and on the anterior aspect of the thigh at the mid-point between the inguinal crease and superior-border of the patella.[14,46] Specifically, the garments in the COMP condition elicited 20.6 ± 3.7 mm Hg of pressure at the maximal calf, and 11.1 ± 3.1 mm Hg of pressure at the mid-thigh. After 30 minutes of rest on a bed, baseline blood samples were collected and baseline vascular measures were taken. Participants then completed the RSE protocol, which was followed by 60 minutes of rested recovery on a bed. Muscle microvascular blood flow was measured at baseline, immediately after completion of the RSE protocol (immediate transfer to bed), and after 60 minutes of post-exercise recovery in the Semi-Fowler's position (supine with the head and trunk raised to 30˚). Femoral arterial blood flow was measured at baseline, after RSE sets 1, 2 and 3, and at 60 minutes post-exercise. Due to potential Doppler interference associated with intravenous microsphere infusion, femoral arterial blood flow could not be measured after RSE set 4. Venous blood samples were acquired at baseline, after RSE sets 1, 2 and 3, and at 15, 30, 45, and 60 minutes post-exercise (Figure 1). Due to intravenous infusion of the ultrasound contrast agent, a venous blood sample could not be taken after RSE set 4.

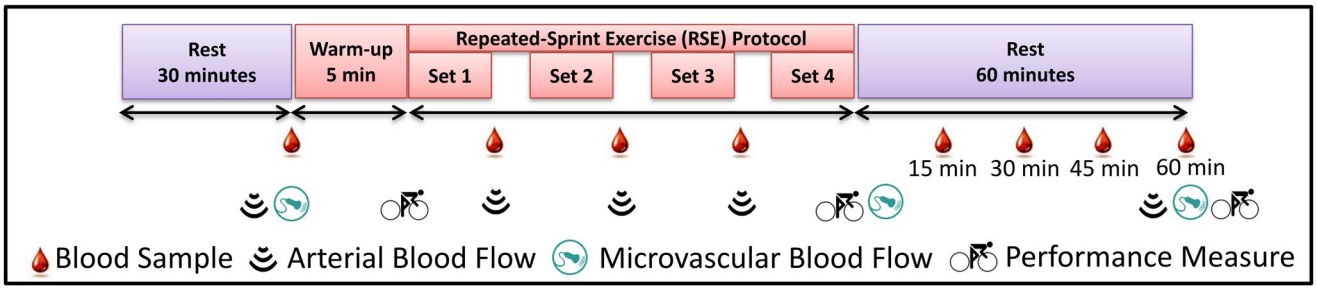

FIGURE 1 Experimental Design. In a randomized, cross-over, repeated-measures design, participants completed the experimental session twice; once wearing loose clothes, and once wearing lower-limb compression garments. Venous blood and vascular measures were taken before, during, and after the repeated-sprint exercise protocol, and throughout the 60-minute post-exercise recovery period

Within 2 to 7 d after completing the first experimental session, participants attended the research laboratory to undergo their second alternative experimental session. The order in which participants performed the CON and COMP sessions was randomized, counter-balanced, and performed at the same time of day to minimize diurnal variation. All participants were asked to be well fed (last meal 2-3 hours prior to testing), hydrated, and refrain from strenuous exercise (24 hours) and caffeine (12 hours) prior to each testing session. Participants completed a 24-hour diet diary in the lead up to their first experimental session which they were then asked to replicate prior to their second session.

2.3 | Repeated-sprint exercise (RSE) protocol

Prior to the RSE protocol, participants performed a warm-up procedure consisting of a 5-min self-selected warm-up (134 ± 41 W), and three practice sprints at 75%, 90%, and 100% of perceived maximal effort. Participants then performed 4 sets of 10 × 6-s maximal sprints on a wind-braked cycle ergometer (Wattbike Pro/Trainer, Wattbike Ltd, UK). Each 6-s sprint was interspersed by 24 s of passive recovery and each set interspersed by 2 minutes of seated recovery. Approximately 5 s before starting each sprint, participants were asked to assume the ready position (dominant foot slightly above parallel to the ground) and to wait for the start signal to be announced by the researcher. The flywheel was required to be motionless before each sprint, such that each effort commenced from a stationary start. Verbal encouragement was given consistently during the protocol by the same tester, and participants were reminded of the number of intervals remaining in each RSE set at regular intervals. Subjective ratings of perceived exertion (RPE) were assessed at the end of each RSE set using Borg's 6-20 scale.[47] The peak power and average power achieved during each sprint was used as a measure of exercise performance. In addition, participants performed a single 6-s maximal cycling sprint immediately after the 60-min post-exercise microvascular measurement (and a repeat of the warm-up procedure), which was used as a measure of post-exercise recovery performance. The heart rate was measured throughout the RSE protocol and recovery period using a heart rate strap (Polar H10, Polar, USA).

2.4 | Vastus lateralis muscle microvascular blood volume, velocity, and flow

Microvascular blood flow in the *Vastus Lateralis* was measured using real-time CEU during intravenous infusion of a commercially available ultrasound contrast agent (Definity, Lantheus Medical Imaging, USA), as previously performed.[34] The *Vastus Lateralis* muscle was chosen as the predominant and preferred site for measuring skeletal muscle microvascular blood flow due to its accessibility (ie, it is superficial and provides a large region of interest for analysis), which affords a clear uninterrupted ultrasound image, and importantly its correlative role in force production during repeated-sprint cycling exercise.[48] A steel measuring tape was used to create a line between the anterior superior iliac spine (ASIS) and the superior border of the patella. A linear array ultrasound probe (L9-3) interfaced to an ultrasound machine (iU22 Philips, Bothell, WA, USA) was then initially placed in cross-section over the *Vastus Lateralis* muscle approximately two thirds distal along the ASIS-patella line. The contrast enhanced ultrasound image of the *Vastus Lateralis* muscle was checked during the first two minutes of contrast agent infusion, and if necessary minor adjustments were made to the probe location to avoid interference from substantial arterioles and fascia artefacts. The precise probe location was marked on the skin with a permanent marker and measured with a steel tape measure (relative to the ASIS-patella) to ensure accurate replication within and between testing sessions. Depth and focus were adjusted for each participant in their first testing session to maximize the *Vastus Lateralis* region of interest and kept consistent for their subsequent testing session. For the compression session, the probe location and mark-up were completed prior to participants adorning the compression garments. A small incision (~6 cm) was then made in the garment

over the marked probe position to ensure the probe was in direct contact with the participant's skin. Pilot data confirmed that this incision had no measurable impact on the level of compression applied to the participant's skin by the garment.

The contrast agent solution (1 mL of Definity suspended in 29 mL of saline solution [0.9% NaCl]) was intravenously infused at a rate of 192 mL/min using a standard syringe pump (TE-311, Terumo, Japan). Infusion of the contrast agent was commenced 4 minutes before each time point to allow the blood pool microsphere concentration to reach steady state. To ensure steady-state whole-body equilibrium of the contrast agent for the post-exercise measure, infusion commenced between sprints 4 and 5 of the last sprint set for this time point. After 4 minutes of infusion, six 45-s digital captures were acquired. All digital recordings were preceded by a high mechanical index flash to disrupt all microspheres within the probe line of sight to measure muscle microsphere re-appearance kinetics. Settings for gain (75%-76%) and mechanical index (0.11 for continuous and 1.30 for flash) were kept identical for all participants and testing sessions.

To calculate muscle microvascular blood volume, velocity and flow, all digital images were analyzed offline using the Qlab software (QLAB, Philips Healthcare, Andover, MA, USA). The background acoustic intensity (0.5-s frame for the baseline and 60-min post-exercise time points, and 0.25-s frame for the post-exercise time point) was subtracted from the raw data to eliminate signal from larger vessels and tissue artefacts.[34] The acoustic intensity measured from a region of interest was exported for each individual 45-s video capture. The average of the six 45-s captures for each time point was then plotted over time and curve fitted using the equation $y = A(1 - e^{-\beta(t-tb)})$, where "$y$" is the acoustic intensity, "tb" is the background time, "t" is time, "β" is the rate constant (a measure of microvascular capillary refilling rate), and "A" is the plateau of acoustic intensity (a measure of microvascular blood volume). The muscle microvascular blood flow was calculated by $A \times \beta$. Due to the specialized nature and training required to accurately, reliably, and consistently analyze and process contrast-enhanced ultrasound images, the same individual analyzed all ultrasound files.

2.5 | Superficial femoral artery diameter, velocity and flow

A high frequency L12-5 linear array transducer was used to measure the diameter and blood velocity of the superficial femoral artery, as previously described.[34] The diameter was assessed using 2D ultrasound and measured at the peak of the QRS complex using a three-lead electrocardiograph system interfaced to the ultrasound machine. This was completed to ensure that all femoral artery diameter measurements were taken at the same phase within the cardiac cycle. Blood velocity (time-averaged mean velocity) was assessed by Doppler ultrasound. The femoral artery blood flow (mL/min) was calculated as $\Pi r^2 \times$ mean velocity $\times$ 60 minutes, where the radius (r) is in cm and the mean velocity is in cm/s. Femoral artery ultrasound measurements were taken in triplicate. All settings were recorded and kept consistent within and between sessions.

2.6 | NIRS-derived muscle oxygen extraction

Markers of muscle oxygen extraction were assessed using the NIRS technique[49] continually during the entire experimental session (ie, baseline, warm-up, RSE protocol, and 60-min recovery period). This technique provides continuous, non-invasive monitoring of the relative changes in oxyhemoglobin (O_2Hb) and de-oxyhemoglobin concentration (HHb). Changes in O_2Hb and HHb of the right *Vastus Lateralis* were monitored using NIRS oximetry (Oxymon MKIII Near Infrared Spectrophotometer, Artinis Medical Systems, The Netherlands) with data transmitted simultaneously to a personal computer and acquired using the Oxysoft software (V3.0.53, Artinis Medical Systems, The Netherlands). The oximeter's optodes were housed in a plastic holder to ensure their position was fixed, and then secured on the cleaned (hair shaved off and skin swabbed with an alcohol swab) skin surface with tape. The oximeter was positioned at the same location on the contralateral leg to the ultrasound muscle microvascular blood flow measurements. Muscle oxygen extraction was calculated as changes in O_2Hb and HHb, relative to a 60-s average taken during passive bed rest prior to exercise. To account for muscle blood volume changes following exercise and recovery which may influence NIRS-derived measures of muscle oxygen extraction, changes in muscle O_2Hb and HHb were normalized to changes in total hemoglobin (tHb), ie, O_2Hb–tHb and HHb–tHb, respectively. This was performed as NIRS-derived measures of tHb are indicative of changes in regional blood volume.[50] All NIRS measures were calculated at baseline (average of last 60 s of bed rest prior to exercise), during RSE (average of each RSE set including all exercise and rest periods), and at 15, 30, 45 and 60-min post-exercise (average of the 60 s preceding each time point). A 3-s moving average was applied to smooth all NIRS signals before analyses.[51]

2.7 | Blood collection and analysis

Venous blood samples were collected at baseline, immediately after RSE sets 1, 2 and 3, and at 15, 30, 45, and 60 minutes post-exercise. Venous blood was collected using safePICO Blood Gas syringes containing EDTA (Radiometer Medical, Denmark) and analyzed immediately for blood lactate and glucose (ABL800 FLEX Blood Gas Analyzer, Radiometer, Denmark).

2.8 | Statistical analyses

Data were checked for normality and analyzed using Prism statistical analysis software (Graphpad Prism 8.4.3). Non-normally distributed data was first log-transformed to approximate normal distribution prior to statistical analysis. Comparisons of multiple means were analyzed using a two-factor repeated measures mixed model analysis of variance (ANOVA) with Time (before, during and after exercise) and Condition (CON and COMP) as the within-subjects factors. Significant interaction and main effects were explored post-hoc with Fisher's Least Significant Difference test. Average sprint data was analyzed using a two-tailed paired *t*-test. Statistical analysis was conducted at the 95% level of significance ($P \leq .05$).

3 | RESULTS

3.1 | Muscle microvascular blood volume, velocity, and flow

Main effects of time ($P < .001$) and condition ($P = .023$) were detected for muscle microvascular blood volume (Figure 2A). Compared with baseline, microvascular blood volume increased in both conditions immediately post-exercise ($P < .001$) and remained elevated above baseline at 60 minutes post-exercise ($P = .002$). When data are averaged over the time points measured, microvascular blood volume was lower in the COMP condition as compared with the CON condition (main condition effect of ~14%). There was no interaction effect for microvascular blood volume ($P = .215$).

Interaction effects were detected for muscle microvascular blood velocity ($P = .025$; Figure 2B) and flow ($P = .026$; Figure 2C). Compared with baseline, muscle microvascular blood velocity and flow increased immediately post-exercise and remained elevated at 60 minutes post-exercise in both conditions (all $P < .01$). However, the increase in microvascular blood velocity occurred to a lesser extent in the COMP condition as compared with the CON condition immediately after exercise (−14% lower peak blood velocity, $P = .004$) and 60 minutes post-exercise (−27% lower peak blood velocity, $P < .001$). Likewise, the increase in muscle microvascular blood flow was lower in the COMP condition as compared with the CON condition immediately after exercise (−29% lower peak blood flow, $P = .009$) and 60 minutes post-exercise (−40% lower peak blood flow, $P < .001$).

Representative CEU images of muscle microvascular perfusion and microsphere flow dynamics are presented in Figures 3 and 4, respectively. The average background acoustic intensity of all ultrasound images in contrast enhanced mode was similar between the CON and COMP conditions

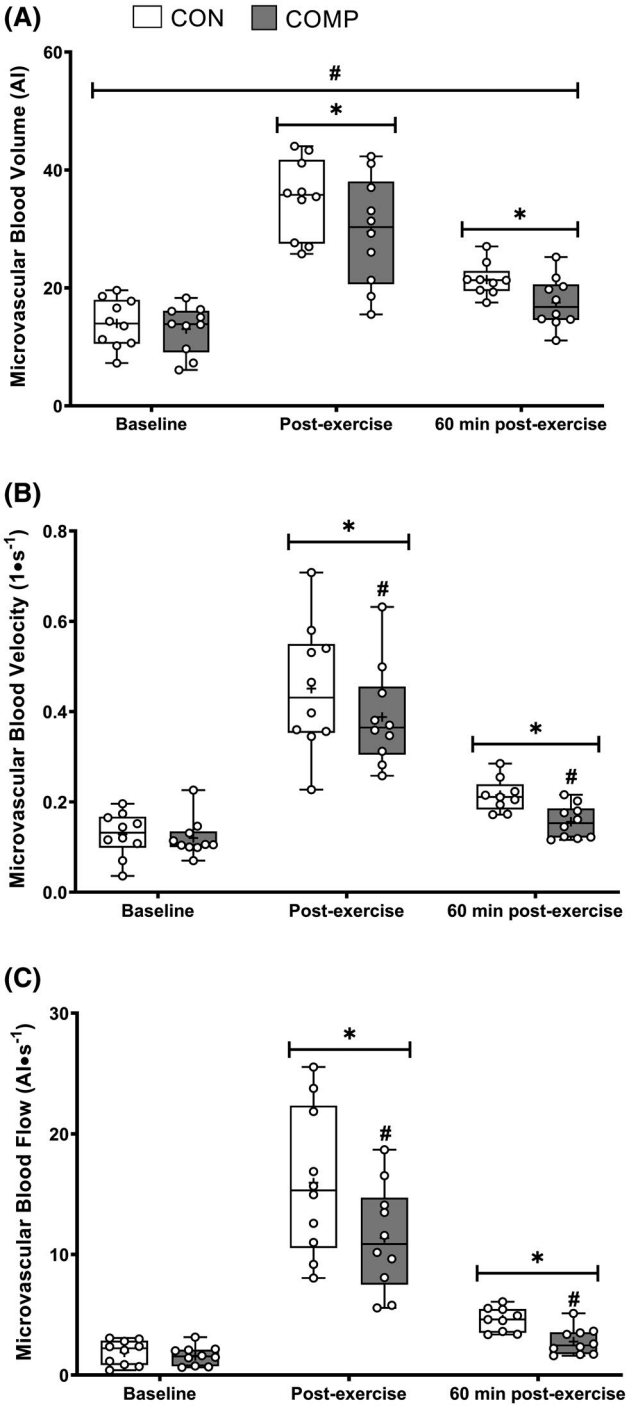

FIGURE 2 The effects of compression garments on repeated-sprint, exercise-induced muscle microvascular blood volume (A), velocity (B) and flow (C). Data are presented as box and whisker plots. The box represents the interquartile range alongside the median (line) and mean (plus symbol). The whiskers represent the minimum and maximum range of the data. n = 10 participants. $^{*}P < .05$ compared to baseline. $^{\#}P < .05$ compared to the same time-point in the CON session or indicates a main effect of condition. MBV is expressed as acoustic intensity (AI); microvascular filling rate, or microvascular blood velocity, is expressed as $1{\cdot}s^{-1}$; Microvascular blood flow is expressed as acoustic intensity/s ($AI{\cdot}s^{-1}$)

(25.4 ± 6.7 versus 24.3 ± 6.4 AI units; $P = .307$), indicating that the background tissue and artefact signal for the analyzed regions of interests were similar between conditions.

3.2 | Femoral artery diameter, blood velocity, and flow

Main effects of condition ($P = .014$) and time ($P < .001$) were detected for femoral artery diameter (Figure 5A). Compared with baseline, femoral artery diameter increased in both conditions after the RSE sets and remained dilated at 60-min post-exercise ($P < .001$). Femoral artery diameter was greater in the COMP condition as compared with the CON condition when data were averaged over the entire session (main condition effect of ~2%). There was no interaction effect for artery diameter ($P = .109$).

A main effect of time ($P < .001$) was detected for femoral artery blood velocity (Figure 5B). Femoral artery blood velocity increased above the baseline in both conditions after the RSE sets and remained elevated above baseline, although to a lesser extent, at 60 minutes post-exercise (all $P < .001$). There was a non-significant increase for velocity in the COMP condition as compared with the CON condition over the entire session (main condition effect of ~11%, $P = .063$). There was no interaction effect ($P = .335$) for femoral artery blood velocity.

An interaction effect ($P = .031$) was detected for femoral artery blood flow (Figure 5C). Compared with baseline, femoral artery blood flow was elevated in both conditions after the RSE sets and remained elevated to a lesser extent at 60 minutes post-exercise (all $P < .001$). The increase was greater in the COMP condition as compared with the CON condition after Set 1 ($P = .009$) and Set 2 ($P = .001$), but not Set 3 ($P = .101$) or 60 minutes post-exercise ($P = .930$).

Femoral artery diameter, blood velocity and flow data when averaged over the three RSE sets were elevated in the COMP condition as compared with the CON condition ($P = .027$, $P = .080$, and $P = .025$; Figure 5A-C, respectively).

3.3 | NIRS-derived tHb, O_2Hb, and HHb

An interaction effect ($P = .026$) was detected for muscle tHb (Figure 6A). Compared with baseline, tHb was elevated after the RSE sets and post-exercise time-points in both conditions (all $P < .01$). Muscle tHb was lower in the COMP condition as compared with the CON condition after set 1 ($P = .053$) and sets 2–4 (all $P < .05$).

An interaction effect ($P = .025$) was detected for normalized muscle O_2Hb (Figure 6B). Compared with baseline, normalized O_2Hb was lower after the RSE sets in both conditions (all $P < .001$). Compared with the CON group, normalized O_2Hb was lower with the COMP condition throughout the post-exercise recovery period (all $P < .05$).

An interaction effect ($P = .041$) was detected for normalized muscle HHb (Figure 6C). Compared with baseline, normalized muscle HHb was higher after the RSE sets with COMP only (all $P < .01$) and lower throughout the recovery period in both conditions (all $P < .001$). Compared with the CON group, normalized muscle HHb was higher in the COMP condition after the RSE sets (all $P < .05$) and at 60 minutes post-exercise ($P < .05$).

3.4 | Forearm venous blood lactate and glucose

An interaction effect ($P = .012$) was detected for blood lactate (Figure 7A). Compared with baseline, blood lactate was elevated after each RSE set and remained elevated above baseline throughout the 60-min post-exercise recovery period in both conditions (all $P < .01$). Blood lactate was lower after the first set in COMP as compared with CON ($P = .002$), and similar between the two conditions at all other time points (all $P > .171$).

A main effect of time ($P < .001$) was detected for blood glucose (Figure 7B). Compared with baseline, blood glucose levels were elevated after RSE sets 2 and 3 ($P < .001$) in both conditions, which remained elevated 15 and 30 minutes post-exercise ($P < .05$). No condition ($P = .696$) or interaction effects ($P = .477$) were detected for blood glucose.

3.5 | RSE performance, RPE, and heart rate

A main effect of time was detected for peak power, mean power, ratings of perceived exertion (RPE), and heart rate ($P < .001$). Compared with RSE set 1, peak and mean power were lower, and RPE was higher, after RSE sets 2–4 in both conditions (Figure 8A,B, all $P < .001$; Figure 8C, all $P < .01$). Compared with baseline, heart rate was elevated after each RSE set and remained elevated above baseline throughout the 60-min post-exercise recovery period (Figure 8D, $P < .001$). There were no condition (all $P > .257$) or interaction (all $P > .310$) effects for peak power, mean power, RPE, or heart rate.

3.6 | Sprint performance (single 6-s sprint) before, immediately after, and 60 minutes after the RSE protocol

Main effects of time ($P < .001$) were detected for single-sprint performance peak power, mean power, and peak heart

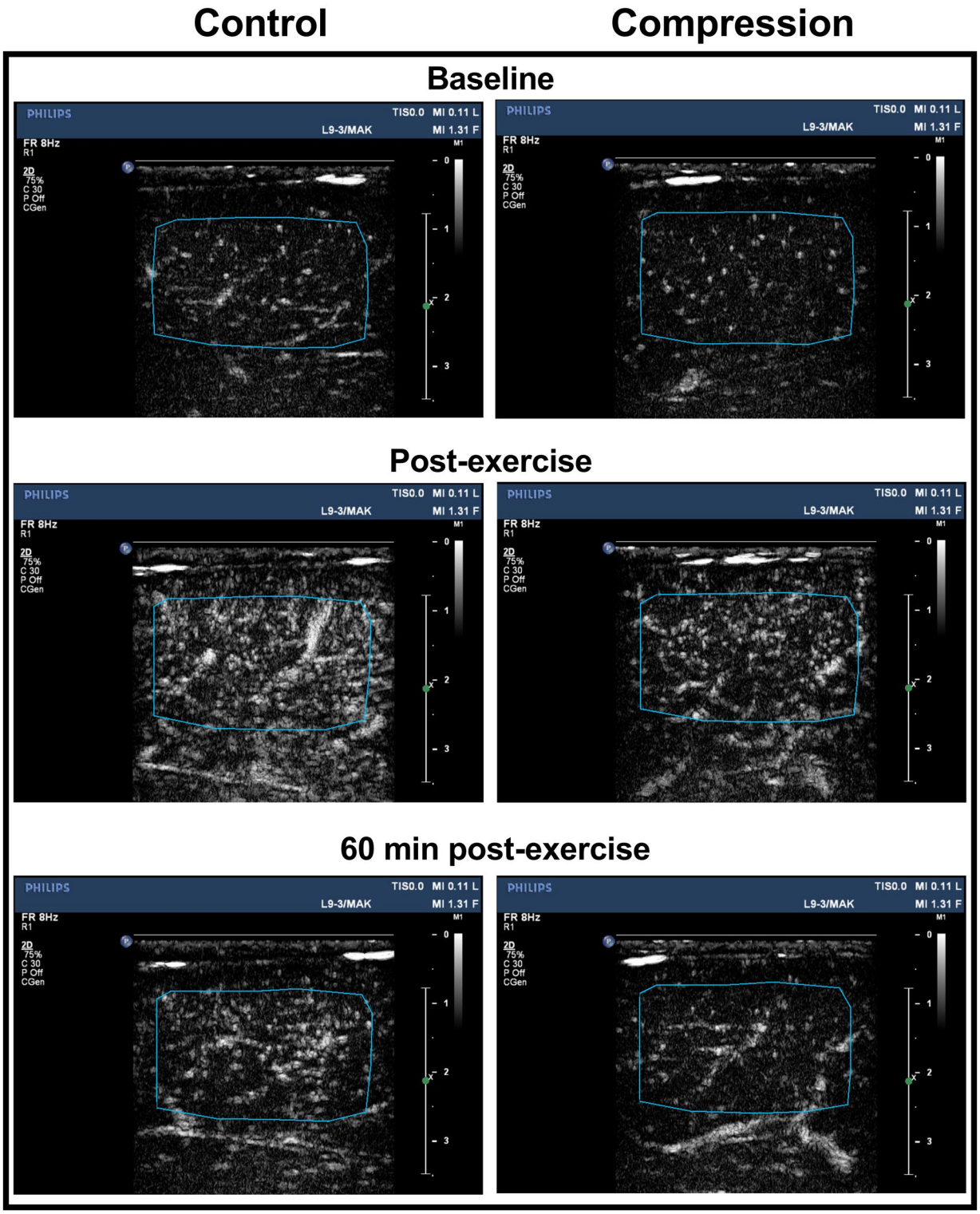

FIGURE 3 Representative contrast enhanced ultrasound images of skeletal muscle microvascular perfusion in cross-section of the Vastus Lateralis from a single participant at baseline, immediately after repeated-sprint exercise, and 60 minutes post-exercise with and without compression garments. The infused contrast agent contains echogenic microspheres which circulate within the muscle microvasculature and can be measured by contrast enhanced ultrasound. The blue box indicates the selected region of interest used to measure microspheres within the muscle microvasculature. The number of microspheres can be seen to increase after RSE with both CON and COMP and remains elevated above baseline at 60 minutes post-RSE. However, the exercise-induced increase in the number of microspheres immediately after RSE and 60 minutes post-RSE is attenuated with COMP

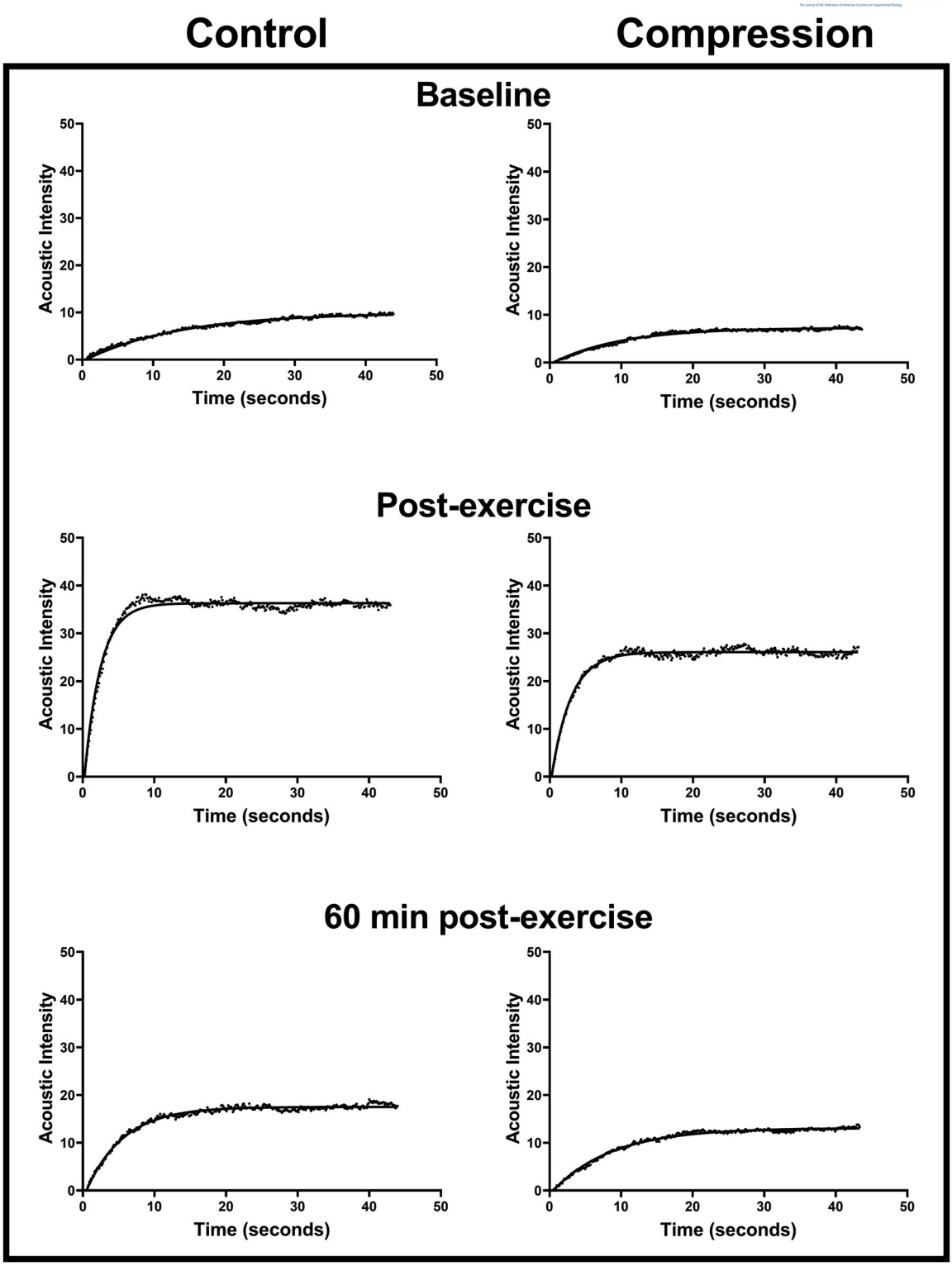

FIGURE 4 Representative curve fits for skeletal muscle microvascular perfusion from a single participant at baseline, immediately after RSE, and 60 minutes post-exercise with and without compression garments. A high-mechanical index flash from the ultrasound probe is used to destroy all current microspheres within the ultrasound probe line of sight. The reappearance kinetics of the microspheres, and their magnitude, are used to calculate muscle microvascular blood volume, velocity and flow

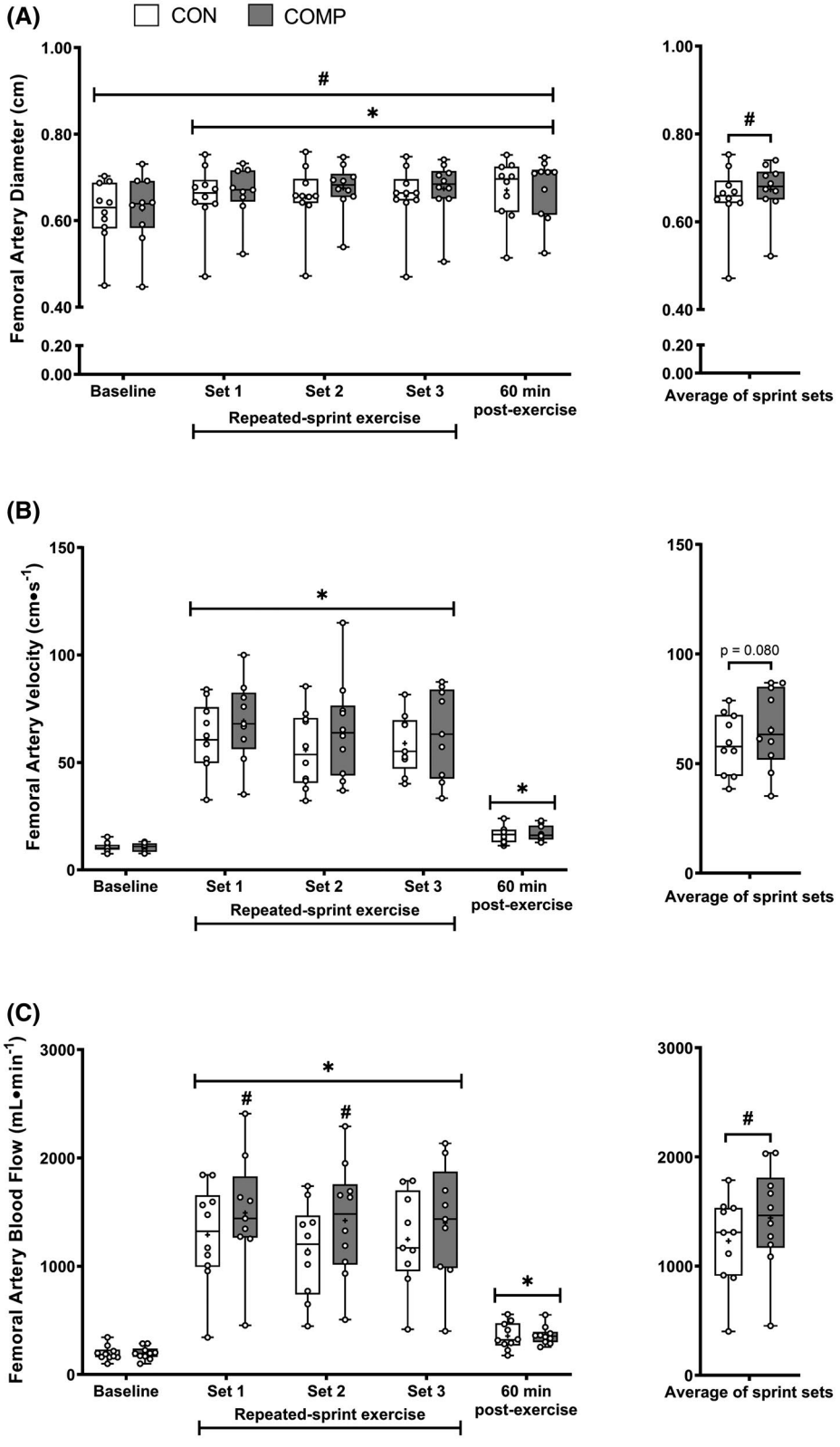

FIGURE 5 The effects of compression garments and repeated-sprint exercise on femoral artery diameter (A), blood velocity (B), and blood flow (C). Data are presented as box and whisker plots. The box represents the interquartile range alongside the median (line) and mean (plus symbol). The whiskers represent the minimum and maximum range of the data. n = 10 participants. *$P < .05$ compared to baseline. $^{\#}P < .05$ compared to the same time-point in the CON session or indicates a main effect of condition

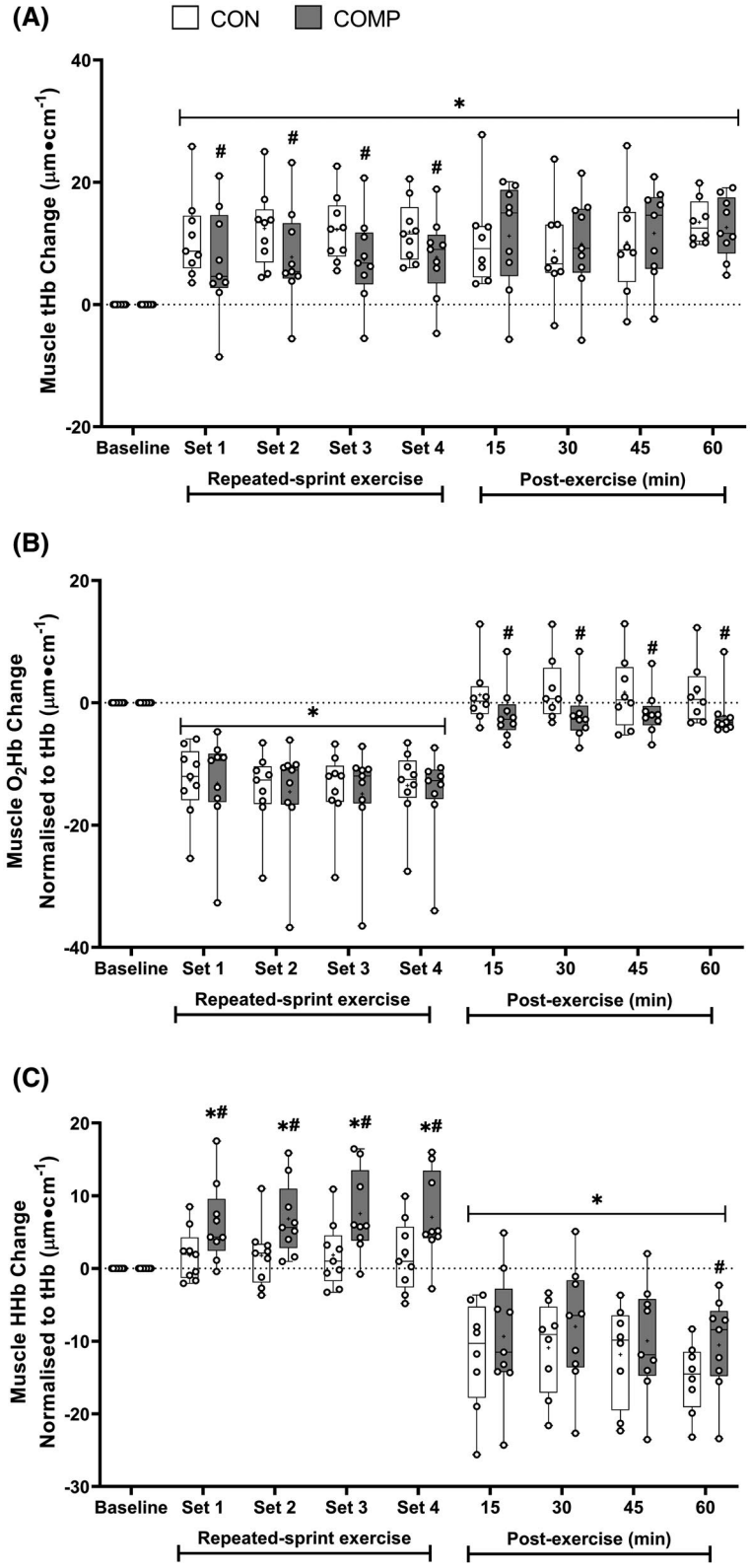

FIGURE 6 The effects of compression garments on repeated-sprint exercise-induced muscle total hemoglobin (tHb; A), oxyhemoglobin (O_2Hb) normalized to tHb (B), and deoxyhemoglobin (HHb) normalized to tHb (C). Data are presented as box and whisker plots. The box represents the interquartile range alongside the median (line) and mean (plus symbol). The whiskers represent the minimum and maximum range of the data. n = 9 participants. $^{*}P < .05$ compared to baseline. $^{\#}P < .05$ compared to the same time-point in the CON session

rate (Table 1). Peak and mean power were lower, and peak heart rate higher, for the last RSE sprint as compared with both the first RSE sprint and recovery sprint (60 minutes post-RSE) in both conditions. There were no condition (all $P > .888$) or interaction effects (all $P > .486$).

4 | DISCUSSION

We provide novel evidence that lower-limb compression tights attenuate the exercise-induced increase in skeletal muscle microvascular blood flow following RSE, and that

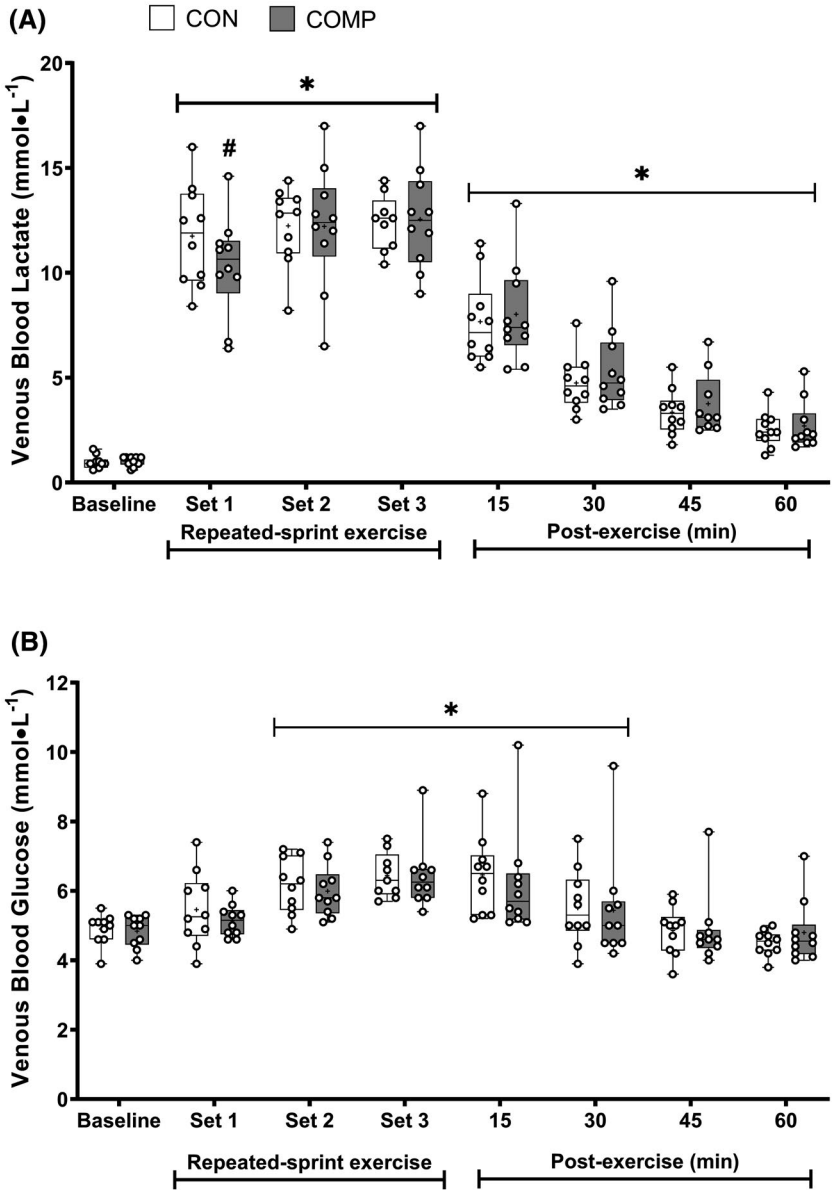

FIGURE 7 The effects of compression garments on repeated-sprint exercise-induced venous blood lactate (A) and glucose (B) concentrations. Data are presented as box and whisker plots. The box represents the interquartile range alongside the median (line) and mean (plus symbol). The whiskers represent the minimum and maximum range of the data. n = 10 participants. $^{*}P < .05$ compared to baseline. $^{\#}P < .05$ compared to the same time-point in the CON session

these reductions persist for up to 1 hour post-exercise. This attenuation of microvascular blood flow occurred despite an increase in femoral artery blood flow, indicating for the first time that there are divergent effects of compression on leg macro- and microvascular blood flow during and following exercise. The compression-induced attenuation in muscle microvascular perfusion did not influence exercise performance. However, compression led to an increase in measures of blood oxygen extraction, which may indicate a compensatory mechanism to conserve exercise capacity in healthy individuals during conditions of reduced muscle microvascular blood flow. We conclude that the microvasculature in skeletal muscle has considerable built-in redundancy (ie, a large capacity to adapt to an attenuation in blood flow to maintain adequate gas and nutrient exchange) in healthy, recreationally active people such that RSE performance is not impaired when microvascular blood flow is reduced with lower-limb compression garments.

This study demonstrates, for the first time, that compression exerts divergent effects on macrovascular and microvascular blood flow following exercise. Consistent with previous reports of an increase in femoral arterial blood velocity and flow,[27] as well as total-limb blood flow,[14,18-22] compression tights augmented the exercise-induced increase in femoral artery diameter, velocity, and blood flow in the current study. This has been hypothesized to occur due to a compression-induced reduction in transmural pressure and a subsequent arterial/arteriolar vasodilatory response.[19] While confirming an increase in upstream large artery blood flow, we add the novel observation that compression attenuated the exercise-induced increase in muscle microvascular blood velocity, volume, and flow. These findings were supported by a decrease

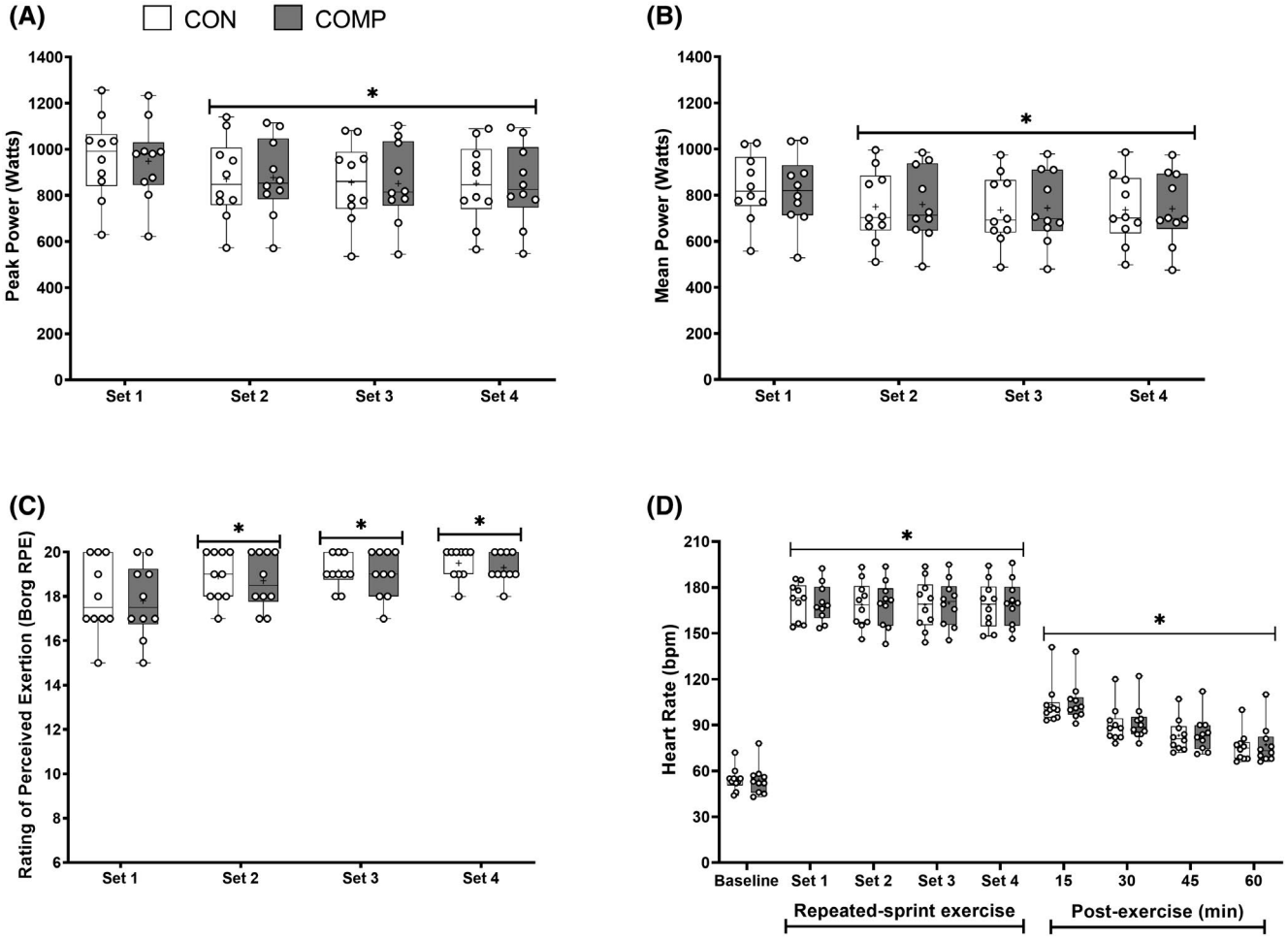

FIGURE 8 The effects of compression garments on repeated-sprint exercise peak power (A), mean power (B), RPE (C) and heart rate (D). Displayed power values represent average peak power and average mean power from the 10 sprints in the corresponding set. Data are presented as box and whisker plots. The box represents the interquartile range alongside the median (line) and mean (plus symbol). The whiskers represent the minimum and maximum range of the data. n = 10 participants. *$P < .05$ compared to set 1 (panels A, B and C) or baseline (panel D)

TABLE 1 The effects of compression garments on single sprint performance before and after repeated-sprint exercise

	First RSE sprint		Final RSE Sprint		Recovery Sprint	
	CON	COMP	CON	COMP	CON	COMP
Mean power (Watts)	994 ± 204	994 ± 204	739 ± 142*	738 ± 153*	983 ± 204	997 ± 202
Peak power (Watts)	1116 ± 233	1125 ± 230	862 ± 154*	839 ± 183*	1100 ± 242	1095 ± 216
Peak heart rate (bpm)	158 ± 18	156 ± 16	174 ± 15*	173 ± 15*	160 ± 16	163 ± 16

Note: Multiple means were analyzed using a two-way linear mixed model (ANOVA) with Time and Condition (CON vs COMP) as the within-subjects' factors. Data are presented as Mean ± SD.

*$P < .001$ compared to both the first repeated-sprint exercise (RSE) sprint and recovery sprint (60 minutes post-RSE).

in NIRS-derived muscle tHb, suggesting a decrease in exercise-induced muscle (regional) blood volume with compression. Decreased muscle microvascular perfusion may be the result of a mechanical hindrance in muscle blood flow, as reported by others using PET imaging.[17] Capillaries are more susceptible to compression than larger blood vessels, due to their lack of vascular smooth muscle and connective tissue. As such, compression may reduce microvascular blood flow through an elevation in interstitial fluid pressure and subsequent microvascular compression. Collectively, we propose that compression causes a vasodilation of the major 'feed' arteries/arterioles and subsequently increases limb arterial blood flow, but concurrently compresses the microvasculature. Others have also reported divergent responses of arterial and microvascular blood flow to muscle contraction and other pharmacological, hormonal, and metabolic stimuli.[31-35]

These observations may reflect redistribution of blood within the limb to other non-myocyte tissues, such as adipose tissue, skin, bone, fascia, and connective tissue.

The regulation and co-ordination of vascular tone and blood flow within and between the cardiac, macrovascular, and microvascular systems is critical for the moderation of exercise capacity, performance, and recovery.[23-26] In support, we have reported that impaired muscle microvascular perfusion in type 2 diabetes patients is associated with reduced exercise capacity, independent of cardiac functional reserve.[10] Others have similarly linked muscle microvascular dysfunction to reduced exercise capacity in heart failure patients[11,12] and peripheral arterial disease.[13] In sedentary rodents, decreasing skeletal muscle nutritive microvascular blood flow with serotonin in the constant-flow perfused rat hind limb markedly impaired skeletal muscle oxygen consumption and force production during muscle contraction elicited by sciatic nerve stimulation despite total leg blood flow remaining constant.[52] In healthy adults, the influence of muscle microvascular blood flow on exercise capacity and performance is less clear. However, the observation of decreased muscle microvascular blood flow alongside the maintenance of exercise capacity suggests there is considerable redundancy built into the muscle capillary–myocyte interface system. This muscle microvascular blood flow reserve likely allows for the maintenance of exercise performance during conditions of reduced muscle microvascular blood flow, at least in healthy individuals. Indeed, total-limb perfusion does not seem to be the rate-limiting factor for oxygen transport, even during intense exercise.[53] Furthermore, basic measures of exercise metabolism (glucose and lactate), heart rate, and RPE, were all similar between compression and control. This suggests alternative hemodynamic or metabolic compensatory mechanisms may be involved in maintaining exercise performance during conditions of decreased muscle perfusion.

To elucidate potential compensatory mechanisms, we measured the effects of lower-limb compression tights on NIRS-derived indicators of muscle oxygen extraction (ie, O_2Hb and HHb) relative to changes in regional flow (ie, tHb). Muscle HHb normalized to changes in regional blood volume was higher with compression, as compared with control during the RSE sets and at 60 minutes post-exercise. This suggests that, in the presence of decreased muscle microvascular blood flow and blood volume during exercise and recovery, blood oxygen extraction is increased with compression to maintain oxygen delivery and availability to the myocyte. The mechanism of a compression-induced increase in oxygen extraction is unclear; however, it may relate to the observed reduction in microvascular blood flow and volume that results in the muscle extracting more oxygen (per unit of blood). In support of this, oxygen[54,55] and glucose[56] extraction have been reported to increase when limb blood flow is decreased via nitric oxide and/or cyclooxygenase inhibition. We propose that compression garments compress low-resistance capillaries allowing flow to be carried by high-resistance capillaries, which improves flow homogeneity and oxygen extraction. Isolated constant-flow muscle systems show that increasing perfusion pressure with certain vasoconstrictors improves flow homogeneity and enhances oxygen delivery and uptake by skeletal muscle during rest or contraction.[57-59] However, considering the paucity of research that has directly measured microvascular blood flow with compression garments, the precise mechanism behind the observed increase in measures of muscle oxygen extraction during and after exercise with compression garments, and the potential link to microvascular blood flow, warrant further investigation.

Muscle blood flow is also critical for aspects of muscle recovery and exercise training adaptations, including muscle protein synthesis (MPS) and angiogenesis. Muscle blood flow is positively associated with rates of MPS,[60,61] and, as such, repeated transient reductions in skeletal muscle microvascular blood flow may impair MPS. This may have implications for resistance training adaptations, including strength and skeletal muscle mass.[62,63] Blood flow to the microvasculature has also been implicated in angiogenesis, which may contribute to increases in skeletal muscle microvasculature capillarization and endothelial enzyme content, and improved insulin sensitivity and glycemic control.[43,64-66] The signals prompting such vascular remodeling responses are not entirely clear but are linked to hemodynamic stimuli, including vascular shear stress and transmural pressure.[64,66,67] As such, a potential implication of compression attenuating the exercise-induced increase in microvascular flow could be an impairment in muscle vascular remodeling, which may ultimately compromise long-term training adaptations that otherwise lead to improved muscle oxygen delivery and aerobic exercise capacity. Considering the relationship between muscle microvascular dysfunction and exercise intolerance in diseased populations,[10-13] this may have important implications for longer-term, vascular-related exercise training adaptations in healthy individuals. Research investigating the long-term effects of compression garments on muscle microvascular remodeling and function are warranted.

The post-exercise reduction in microvascular blood flow with compression reported in the current study is comparable to those reported in clinical populations. For example, clinical populations such as those with type 2 diabetes and peripheral arterial disease display a ~25% to 60% lower microvascular blood flow response to exercise as compared with controls when assessed with contrast-enhanced ultrasound. Specifically, we have reported that exercise-intolerant type 2 diabetes participants have ~25% lower exercise-stimulated microvascular blood flow (stress testing on treadmill) compared with exercise tolerant type 2 diabetes participants.[10] Peripheral arterial disease patients with type 2 diabetes display ~60% reduction in exercise-stimulated microvascular

blood flow (plantar-flexion exercise) as compared with healthy controls.[68] Exercise-mediated increases in microvascular blood flow (forearm contraction) is ~60% lower in people with type 2 diabetes and microvascular disease (neuropathy or retinopathy) when compared with healthy controls, despite a similar stimulation in brachial artery blood flow.[9] In the current study, we reported a ~29% reduction in exercise-stimulated microvascular blood flow when recreationally active healthy participants wore compression. However, this reduction in microvascular blood flow is the result of mechanical compression, whereas reductions in microvascular blood flow in clinical populations during exercise are mostly driven by a lower capillary density in skeletal muscle.[69]

Both muscle contraction and exercise have been reported to increase microvascular blood flow in skeletal muscle.[42,56,70] Forearm skeletal muscle contraction (hand grip exercise at 80% maximal handgrip strength) in healthy adults humans increases forearm microvascular blood volume by ~46% when measured after a short, 12-min, intermittent contraction protocol.[42] Peak microvascular perfusion measured by CEU in the *gastrocnemius* muscle also increases in healthy adults by ~57% after walking treadmill exercise (60% heart rate reserve for 10 minutes).[13] We have also reported that light-intensity knee extensor exercise (3-min intermittent contraction protocol at 25% 1RM) increases microvascular blood flow in vastus lateralis muscle of healthy adults,[71] which readily returns to basal levels within minutes post-contraction. We contribute to the literature by providing evidence that an intense RSE protocol leads to a substantial increase in muscle microvascular blood flow in the vastus lateralis muscle of healthy adults immediately after exercise (~889%), which remains elevated for up to at least 1 hour post-exercise (~146%). These observations reflect similar findings of elevated muscle microvascular blood flow for up to 3 hours after moderate-intensity cycling exercise in healthy adults (1 hour at ~75% VO_{2peak}),[72] for up to 1 hour after moderate-intensity treadmill exercise in healthy young and older adults (45 minutes at ~40% VO_{2peak}),[70] and for up to 4 hours after intense single-legged knee extensor exercise in healthy adults (1 hour of contractions at 80% W_{peak}, with three 5-min intervals at 100% W_{peak}).[56] As such, RSE is a potent stimulus for increasing muscle microvascular blood flow in healthy young adults.

This study provides an indirect comparison between CEU and other techniques aimed at measuring skeletal muscle microvascular blood flow. For example, using an identical RSE exercise protocol and similar cohort (ie, young and recreationally active adults), we previously reported that lower-limb compression tights increased muscle blood flow by ~11% immediately post-exercise,[14] as measured by NIRS during venous occlusion. In contrast, we report a decrease of ~29% in microvascular blood flow as measured directly by CEU. Although NIRS is thought to only measure [Hb] in blood vessels <1 mm,[73] our current findings support the notion that NIRS-derived measures of microvascular blood flow are likely influenced by factors like heme distribution and/or skin and adipose tissue blood flow.[37] This is further supported by the lack of effect of lower-limb compression garments on NIRS-derived measures of blood flow following long-distance trail running,[15,16] whereby any potential effect may have been masked by the inability of NIRS to measure muscle microvascular perfusion. In the only study to use PET to investigate the effect of compression garments on muscle blood flow, compression shorts (high-level of compression of ~37 mm Hg) reduced *quadriceps femoris* blood flow by ~50% when measured 10 minutes after a high-intensity cycling session.[17] This is consistent with the reduction in microvascular perfusion reported in the current study, and the difference in magnitude may be explained by the level of compression pressure applied (ie, ~37 mm Hg vs ~11 mm Hg) and/or the timing of the post-exercise measurement of microvascular blood flow (ie, 10 minutes post-exercise vs immediately post-exercise). Furthermore, although PET provides a direct measurement of muscle perfusion, it is unable to differentiate between capillaries and arterioles/venules that reside within the muscle.[41] We contribute new knowledge by demonstrating that muscle femoral artery blood flow is increased, whereas microvascular perfusion in muscle is reduced from external compression as low as ~11 mm Hg at the mid-thigh. We propose that future research should employ more sensitive techniques to measure muscle microvascular blood flow, such as CEU, that can better compartmentalize macro and microvascular blood flow in skeletal muscle.

The finding that performance was unchanged with compression garments contradicts the ~5% increase in repeated-sprint cycling power reported in our previous study using the same RSE protocol.[14] This is not unexpected given the equivocal findings of previous research investigating the effects of compression garments on exercise performance.[74,75] Performance has numerous psychological determinants, in that any potential effect of compression may have been masked by factors like external motivation, priming, mental fatigue, and the placebo effect.[76,77] This may be further exacerbated by the nature of the exercise protocol chosen, as performance during supra-maximal exercise relies heavily on participant motivation.[78] Another potential explanation for these discordant findings is the different methods used to calculate power. The Wattbike ergometer used in the current study calculates power using a load cell located next to the chain, whereas the SRM power meter used in Broatch et al[14] calculates power using strain gauges located between the crank axle and chain rings.[79] Although #Wattbike provides close agreement in power as compared with the 'gold-standard' SRM power meter, it has been reported to be less accurate at high power outputs (>700 W),[79] which may have contributed to the differences observed between studies.

Regardless, performance was not reduced when wearing compression tights in the current study, despite the lower microvascular blood flow. Mean power was ultimately maintained between conditions, suggesting that the compression-induced attenuation in microvascular blood flow has minimal effect on exercise performance, at least at supra-maximal intensities in the conditions tested. It is also important to note that due to logistical limitations with the equipment used, microvascular blood flow and arterial blood flow measures were collected immediately after exercise, as opposed to dynamic measures taken during exercise, which may not directly reflect what is occurring during muscle contraction.[3]

This study aimed to assess and differentiate the effects of compression garments on macro and microvascular blood flow in skeletal muscle following RSE. Contrary to our hypothesis, we report that lower-limb compression tights impair the exercise-induced increase in muscle microvascular blood flow immediately following RSE, which was also evident 1 hour into the post-exercise recovery period. Conversely, compression tights increased macrovascular blood flow as assessed at the femoral artery, which is consistent with the majority of research reporting compression-induced increases in total-limb blood flow.[14,18-22] As such, our findings highlight a novel divergence between post-exercise macro and microvascular blood in muscle with compression garments (Figure 9), further highlighting the necessity to distinguish between macrovascular and microvascular blood flow in future exercise physiology and sports science research. Despite the lack of effect on high-intensity exercise performance and the recovery, the observed compression-induced reduction in microvascular blood flow may have implications for glucose disposal and exercise capacity in pathological populations (eg, type 2 diabetes and cardiovascular disease), as well as long-term skeletal muscle adaptations reliant on adequate muscle perfusion (eg, MPS and angiogenesis). The authors also acknowledge the specificity of the cohort and exercise protocol used in the current study, which may limit the translation of findings to certain cohorts (eg, elderly, sedentary, and clinical populations) and more conventional exercise interventions (eg, moderate-intensity continuous exercise and resistance exercise). Future research investigating the effects of compression garments on these factors is warranted.

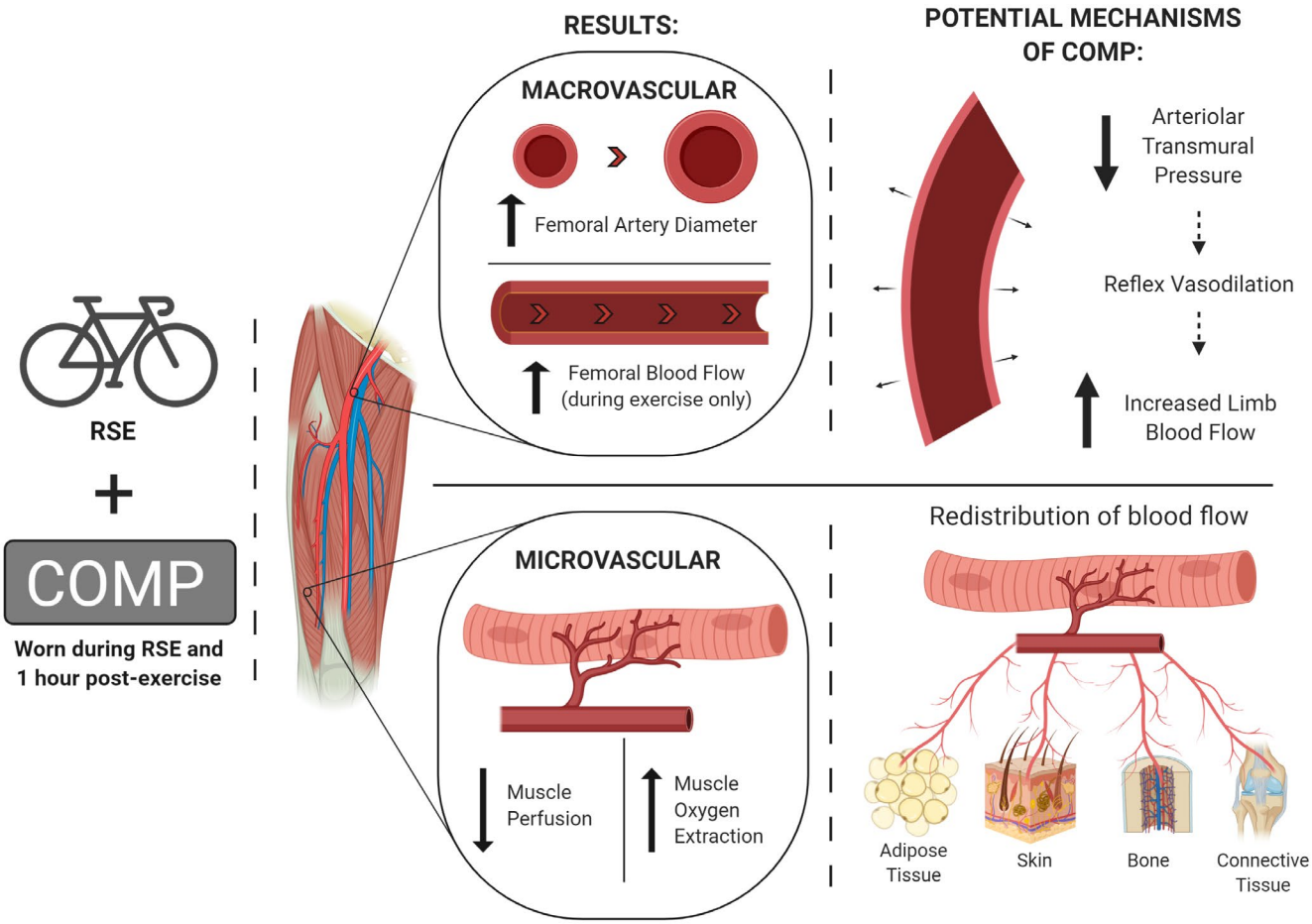

FIGURE 9 Potential mechanisms by which lower-limb compression garments (COMP) may alter femoral artery macrovascular blood flow, and skeletal muscle microvascular blood flow, during repeated-sprint exercise (RSE) and the 1-hour post-exercise recovery period. Created with BioRender.com

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CONFLICT OF INTEREST

All authors have no financial or other interest in the production and/or distribution of 2XU products. For the remaining authors, no conflicts of interest were declared. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

AUTHOR CONTRIBUTIONS

J.R. Broatch, M.A. Keske, A.C. Betik, D.J. Bishop, S.L. Halson, and L. Parker designed the research. J.R. Broatch, S.F. O'Riordan, and L. Parker performed the research. M.A. Keske, A.C. Betik, and L. Parker contributed ultrasound analytic tools; J.R. Broatch, S.F. O'Riordan, and L. Parker analyzed the data. J.R. Broatch, S.F. O'Riordan, and L. Parker wrote the paper. J.R. Broatch, S.F. O'Riordan, M.A. Keske, A.C. Betik, D.J. Bishop, S.L. Halson, and L. Parker provided critical review of the paper and approved the final version.

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