J Appl Physiol 126: 51–59, 2019.
First published October 18, 2018; doi:10.1152/japplphysiol.01082.2017.

RESEARCH ARTICLE

The combined effect of sprint interval training and postexercise blood flow restriction on critical power, capillary growth, and mitochondrial proteins in trained cyclists

Emma A. Mitchell, Neil R. W. Martin, Mark C. Turner, Conor W. Taylor, and Richard A. Ferguson
School of Sport, Exercise, and Health Sciences, Loughborough University, Loughborough, United Kingdom

Submitted 11 December 2017; accepted in final form 14 October 2018

Mitchell EA, Martin NRW, Turner MC, Taylor CW, Ferguson RA. The combined effect of sprint interval training and postexercise blood flow restriction on critical power, capillary growth, and mitochondrial proteins in trained cyclists. *J Appl Physiol* 126: 51–59, 2019. First published October 18, 2018; doi:10.1152/japplphysiol.01082.2017.—Sprint interval training (SIT) combined with postexercise blood flow restriction (BFR) is a novel method to increase maximal oxygen uptake ($\dot{V}O_{2max}$) in trained individuals and also provides a potent acute stimulus for angiogenesis and mitochondrial biogenesis. The efficacy to enhance endurance performance, however, has yet to be demonstrated. Trained male cyclists ($n = 21$) ($\dot{V}O_{2max}$: 62.8 ± 3.7 ml·min^{-1}·kg^{-1}) undertook 4 wk of SIT (repeated 30-s maximal sprints) either alone (CON; $n = 10$) or with postexercise BFR ($n = 11$). Before and after training $\dot{V}O_{2max}$, critical power (CP) and curvature constant (W') were determined and muscle biopsies obtained for determination of skeletal muscle capillarity and mitochondrial protein content. CP increased ($P = 0.001$) by a similar extent following CON (287 ± 39 W to 297 ± 43 W) and BFR (296 ± 40 W to 306 ± 36 W). $\dot{V}O_{2max}$ increased following BFR by 5.9% ($P = 0.02$) but was unchanged after CON ($P = 0.56$). All markers of skeletal muscle capillarity and mitochondrial protein content were unchanged following either training intervention. In conclusion, 4 wk of SIT increased CP; however, this was not enhanced further with BFR. SIT was not sufficient to elicit changes in skeletal muscle capillarity and mitochondrial protein content with or without BFR. However, we further demonstrate the potency of combining BFR with SIT to enhance $\dot{V}O_{2max}$ in trained individuals.

NEW & NOTEWORTHY This investigation has demonstrated that 4 wk of sprint interval training (SIT) increased critical power in trained individuals; however, postexercise blood flow restriction (BFR) did not enhance this further. SIT, with or without BFR, did not induce any changes in skeletal muscle capillarity or mitochondrial protein content in our trained population. We do, however, confirm previous findings that SIT combined with BFR is a potent stimulus to enhance maximal oxygen uptake.

angiogenesis; BFR; mitochondrial biogenesis; power-duration relationship

INTRODUCTION

Well-trained individuals are typically accustomed to high training volumes across a broad spectrum of intensities. However, it is generally accepted that eliciting further adaptations within this population becomes challenging (30, 31). Early research has highlighted the reduced plasticity of skeletal muscle in the trained state (22, 44), and a body of literature exists demonstrating that an increase in traditional endurance training volume alone is insufficient to improve aerobic performance or associated physiological determinants in well-trained and "physically active" individuals (12, 13). This blunting of the adaptive scope in trained individuals is also reflected at a molecular level (14, 37) as demonstrated by an attenuated acute transcriptional response as individuals become accustomed to a specific exercise bout (37). Therefore, the development of effective novel training methods is of particular relevance to this population.

We have recently demonstrated the potency of combining sprint interval training (SIT) with blood flow restriction (BFR) in enhancing the adaptive responses in well-trained individuals (48). The addition of BFR elicited an increase in maximal oxygen uptake ($\dot{V}O_{2max}$) of ~4.5% compared with no change with SIT alone. Alongside this, we presented preliminary mechanistic evidence that SIT combined with BFR led to enhanced angiogenic signaling, suggesting the potential for a greater capillary growth with this novel intervention. Increased muscle capillarity is a critical adaptation to enhance oxygen and substrate delivery (28). Classic work in skeletal muscle has shown that the number of capillaries per fiber is proportional to the oxidative activity of that fiber (43) and subsequently demonstrates a strong correlation with $\dot{V}O_{2max}$ (44). An enhanced capillary network also facilitates the greater removal of metabolic end products, which would improve submaximal exercise tolerance (28). Both SIT combined with BFR and SIT alone were also potent in upregulating PGC-1α expression, suggesting the potential for mitochondrial biogenesis (48). Therefore, it was surprising that despite the gains in $\dot{V}O_{2max}$, Taylor et al. (48) did not observe any improvements in exercise performance, measured through a 15-km time trial. This might be because the relative contribution of central and peripheral factors to performance of such a self-paced exercise is task-dependent with increased contribution of central fatigue within longer low intensity time trials (>30 min) and a greater degree of peripheral fatigue after shorter high-intensity efforts (~6 min) (49). Therefore, the peripheral adaptations that we have hypothesized may not be reflected in the performance measure selected but would be more relevant to shorter high-intensity efforts.

Since its introduction by Monod and Scherrer (33), the critical power (CP) concept has been used to describe the relationship

Address for reprint requests and other correspondence: R. A. Ferguson, School of Sport, Exercise, and Health Sciences, Loughborough Univ. LE11 3TU UK (e-mail: R.Ferguson@lboro.ac.uk).

between the tolerable duration that high-intensity exercise can be maintained at a given power output for whole body exercise, (i.e., the power-duration relationship) (39). This hyperbolic relationship can be described by two constants: the asymptote CP, which is considered to represent the greatest rate of oxidative metabolism that can be maintained in the absence of a progressive loss of muscle metabolic homeostasis, and the curvature constant (W') representing the capacity for work performed above CP (27, 34, 50). Accordingly, performance within the severe intensity domain is a function of CP and W', which makes them important determinants of performance (27, 52). CP is enhanced under conditions of increased oxygen delivery and is linked to the ability to maintain a metabolic steady-state (38, 50); therefore, CP could be hypothesized to be sensitive to changes in skeletal muscle capillarity. Indeed, we recently demonstrated a strong positive association between CP and skeletal muscle capillarity in well-trained individuals (32). Furthermore, since CP represents the highest sustainable rate of oxidative metabolism, it is likely to be closely related to mitochondrial content and could therefore be sensitive to an increase in the content of mitochondrial enzymes.

Therefore, the present investigation assessed the potency of SIT combined with BFR in enhancing CP. Furthermore, the angiogenic and mitochondrial biogenesis potential of SIT combined with BFR was also assessed. It was hypothesized that SIT combined with BFR would result in a greater increase in CP, which would be associated with a greater increase in skeletal muscle capillarity and mitochondrial protein content compared with SIT alone.

METHODS

Participants

Healthy men (n = 21; age: 23 ± 5 yr, height: 179.5 ± 6.4 cm, body mass: 75.5 ± 7.9 kg) volunteered to take part in the study. Participants were competitive cyclists or triathletes and had to achieve the inclusion criteria of $\dot{V}O_{2max}$ ≥60 ml·min^{-1}·kg^{-1}. All participants completed a health and biopsy screening questionnaire before participation to mitigate for contraindications to maximal exercise, muscle biopsy procedures, and BFR. Participants did not have a history of neuromuscular, hematological, or musculoskeletal abnormalities and were not using pharmacological treatments during the study period. All experimental procedures were approved by the Loughborough University Ethics Approval (Human Participants) Sub-Committee and conformed in all respects with the Declaration of Helsinki. Participants were fully informed of the risks and discomforts associated with all experimental trials before providing written, informed consent.

Experimental Protocol

The study used an independent-groups design in which participants were assigned to 1 of 2 groups to perform 4 weeks of SIT, either on its own (CON, n = 10) or combined with postexercise BFR (n = 11). Participants were pair-matched between groups based upon initial $\dot{V}O_{2max}$, maximal aerobic power (MAP), and CP. Participants were initially familiarized to the testing and training procedures during preliminary visits. Pretraining outcome measures were assessed over a period of 10 days. $\dot{V}O_{2max}$ was tested initially to ensure participants attained the appropriate inclusion criteria. After approximately 2 days, the pretraining muscle biopsy was obtained. After a further 2 days, the power-duration relationship for determination of CP and W' was assessed, with a minimum of 24 h separating each test. Participants then embarked on the 4-wk supervised training program. After a maximum of 4 days following the final training session, the posttraining outcome measures were assessed in the same order and over a similar time period.

All performance tests were conducted on an electronically braked cycle ergometer (Lode Excalibur Sport, Lode B.V. Gronigen, the Netherlands). Ergometer saddle and handle bar dimensions were recorded for each participant during preliminary testing and remained standardized for the rest of the study. Participants were instructed to maintain a normal diet during the pretraining testing and replicate that diet during the posttraining measures. Participants were instructed to refrain from ingesting alcohol and caffeine during the 48 h preceding testing. Exercise trials were undertaken at approximately the same time each day (±2 h). Laboratory conditions during pre- and posttraining exercise measurements remained constant (19°C–21°C, 40%–50% humidity).

Pre- and Posttraining Outcome Measures

$\dot{V}O_{2max}$ and MAP. Participants performed an incremental test to exhaustion to establish $\dot{V}O_{2max}$ and MAP. Participants began cycling, at a freely chosen, constant pedal cadence for 1 min at 50 W, after which power increased 25 W every 60 s until volitional exhaustion or when cadence fell 10% below the freely chosen cadence for more than 5 s, despite strong verbal encouragement. Pulmonary gas exchange was measured continuously throughout exercise (Cortex MetaLyzer 3B, Leipzig, Germany). $\dot{V}O_{2max}$ and MAP were defined as the highest $\dot{V}O_2$ and power output achieved for a 30 and 60 s period during the test, respectively.

CP and W'. Participants performed a series of 3–5 constant-load tests that were continued until the limit of tolerance between 70% and 100% of MAP, the sequence of which was randomized. These were designed to elicit exhaustion within 2 to 15 min (39). Time to exhaustion (t) was recorded to the nearest second and was taken as either volitional exhaustion or when cadence fell 10% below the freely chosen cadence for more than 5 s, despite strong verbal encouragement. No feedback regarding the power output or times achieved were provided; however, participants were permitted to view pedal cadence throughout. To enhance the accuracy of parameter estimates, when the standard error of CP was >5% and W' >10%, an additional test was performed.

The parameters of the power-duration relationship, CP and W', were calculated using the inverse linear relationship (*Eq. 1*), the linear work-time model (*Eq. 2*), and the hyperbolic relationship (*Eq. 3*). The equation associated with the lowest combined standard error was selected and used for all further analyses.

$$P = W' \times (1/t) + CP \quad (1)$$

$$W = CP \times t + W' \quad (2)$$

$$t = W'/(P - CP) \quad (3)$$

Muscle sampling and analysis. Participants (n = 16) consented to provide muscle biopsy samples (CON, n = 7; BFR, n = 9). Muscle biopsies were obtained at rest from the lateral portion of the vastus lateralis muscle under local anesthesia (1% lidocaine) using the percutaneous needle biopsy technique with suction. Pre- and posttraining samples were obtained through separate incisions 2 cm apart on the same leg. Muscle samples were split into two portions. One portion was immediately embedded in mounting medium (Tissue-Tek OCT Compound, Sakura Finetek Europe, the Netherlands) and immediately frozen in liquid nitrogen-cooled isopentane. The other portion was snap-frozen in liquid nitrogen. All samples were then stored at −80°C until analysis.

Immunohistochemistry. Transverse serial sections (8 μm) were obtained using a cryotome and placed onto poly L-lysine-coated glass slides. Sections were fixed for 10 min in 3.7% formaldehyde at room temperature and blocked with phosphate-buffered saline (PBS) containing 2% bovine serum albumin (BSA) and 5% goat serum for 1 h

at room temperature. Serial muscle sections were then incubated with either primary antibody CD-31 (ab119339, Abcam, Cambridge, UK) diluted 1:100 and MHC II (ab91506, Abcam) diluted 1:1,000 in PBS/2% BSA or MHC I (A4.951, Developmental Studies Hybridoma Bank, Iowa City, IA) diluted 1:500 in PBS/2% BSA for 1 h at room temperature. A separate slide was also incubated in CD-31 as described above and was subsequently incubated with Ki-67 (ab92742, Abcam) diluted 1:250 in PBS/2% BSA overnight at room temperature. Sections were then incubated for 2 h at room temperature with the appropriate secondary antibodies: goat anti-mouse Alexa Fluor 488 (CD-31, MHC I) and goat anti-rabbit Alexa Fluor 594 (MHC II, Ki-67) diluted 1:500 in PBS/2% BSA. Following incubation, coverslips were mounted with Fluoromount aqueous mounting medium (F4680, Sigma-Aldrich, Dorset, UK). Specificity of staining was assessed with no primary antibody negative controls.

Images were captured with a fluorescence microscope (Leica DM2500) at ×20 magnification. Images were taken across the entire cross-sectional area (CSA) of the sample to avoid bias toward smaller fibers. Camera exposure time and gain were adjusted and kept consistent for all images captured for each participant. An average of 117 ± 43 type I and 90 ± 49 type II fibers were analyzed per sample. Capillarity was expressed as capillary to fiber ratio, capillary density, and number of capillary contacts around type I (CC type I) and type II (CC type II) fibers. Ki-67-positive nuclei colocalized within capillaries were expressed per fiber (all Ki-67 data are CON, $n = 6$ and BFR, $n = 9$, with one participant missing because of insufficient tissue sample size). Only transverse fibers were included in the analysis, which was assessed primarily by the presumption of circularity. Any fibers that were clearly oblique or not transverse to the long axis of the fiber were excluded from analysis. CSA of fibers was assessed by manually drawing the perimeter of each muscle fiber with the image analysis software Fiji (ImageJ). Although absolute fiber size may be overestimated because of fiber swelling during the thawing of frozen sections, this should be consistent between all samples. The investigator was blinded to the exercise training status and condition of samples for all analyses.

Western blot analysis. Muscle tissue was homogenized in cold lysis buffer containing PBS/0.2% Triton X-100 and protease and phosphatase inhibitor cocktail (Fisher Scientific, Loughborough, UK). Samples were blitzed using a tissue lyser (Qiagen, UK) twice for 2 min at 20 Hz and centrifuged at 12,000 *g* for 10 min to pellet insoluble material. The supernatant was transferred to a fresh Eppendorf tube, and protein concentrations were determined by Pierce 660 protein assay according to the manufacturer's instructions (Fisher Scientific). Samples were mixed with dH_2O, 4x LDS sample buffer (Invitrogen, Loughborough, UK), and 0.1% β-mercaptoethanol (Sigma-Aldrich) to a concentration of 1.5 μg/μl. Protein (15 μg) was loaded on to 4%–20% TGX polyacrylamide gels (Bio-Rad, Herts, UK) and separated by electrophoresis at 100 V for 80 min. All samples were run in duplicate to establish coefficients of variation. Proteins were transferred onto PVDF membrane at 30 V for 90 min (Bio-Rad) and washed for 5 min in Tris-buffered saline with tween (TBST) before being blocked in 5% blotting-grade milk (Bio-Rad) for 1 h at room temperature. Membranes were washed three times for 5 min in TBST and were incubated overnight with the following primary antibodies: citrate synthase (CS) (ab129095, Abcam), cytochrome c oxidase (COX) subunit II (ab110258, Abcam), and COX IV (ab33985, Abcam) diluted 1:1,000 in 3% blotting-grade milk at 4°C. Membranes were then washed three times for 5 min in TBST and incubated with the appropriate secondary antibody: anti-mouse horseradish peroxidase-conjugated secondary antibody (Dako, Stockport, UK) or anti-rabbit horseradish peroxidase-conjugated secondary antibody (Bio-Rad) diluted 1:10,000 in 3% blotting-grade milk for 1 h at room temperature. Following three 5 min washes in TBST, membranes were incubated with enhanced chemiluminescence substrate (ClarityMax, Bio-Rad) for 5 min. Membranes were visualized using image analysis (ChemiDocTM XRS+, Bio-Rad) and band densities determined using image analysis software (Quality One 1-D analysis software v 4.6.8, Bio-Rad). GAPDH was used as a loading control, and protein content was expressed in arbitrary units relative to GAPDH. All protein content data are CON, $n = 7$ and BFR, $n = 7$, with two participants missing because of insufficient tissue sample size. The coefficients of variation for CS, COX II, and COX IV were 5.1 ± 3.9%, 6.8 ± 5.8%, and 4.3 ± 3.2%, respectively.

Exercise Training

Participants completed a 4-wk supervised SIT program (2 sessions per week) with each session separated by a minimum of 48 h. Participants were encouraged to maintain their regular training regime, with the exception of performing any form of interval training. This was to ensure that a substantial reduction in training volume was avoided. Each training session consisted of repeated 30-s maximal sprints performed on a mechanically braked cycle ergometer (SE-780 50, Monark, Stockholm, Sweden) against a manually applied resistance equivalent to 0.075 kg/kg body mass. The training was progressive, in which all participants performed a total of 4, 5, 6, and 7 maximal 30-s sprints in *weeks 1*, *2*, *3*, and *4*, respectively. Each sprint was separated by a 4.5-min recovery period, during which participants immediately dismounted the cycle ergometer and lay in a semisupine position upon a couch. In BFR, participants were subjected to BFR (applied within 25 s of each sprint). This was achieved by rapidly applying pneumatic pressure cuffs (Hokanson SC12L) as high up as possible on the proximal portion of each thigh, which were inflated (E20 Rapid Cuff Inflator and AG101 Cuff Inflator Air Source, Hokanson, WA) to a pressure of ~120 mmHg for 2 min (this pressure was kept constant throughout the 4-wk training period). The cuffs were then rapidly deflated, and participants remained in the supine position until 30 s before the next sprint where they remounted the ergometer in time for the subsequent sprint, which began precisely 4.5 min after the previous sprint ended. In CON, participants remained in the semisupine position before remounting the ergometer in time for the subsequent sprint. Pre- and posttraining measurements of peak power output and mean power output (MPO) were obtained (Monark software) from the best sprint within the first and last training sessions, respectively. Total work completed throughout the training period was calculated as a sum of the product of the MPO from each sprint and sprint duration.

Statistics

Training data were analyzed using an unpaired *t*-test. Two-factor repeated measures ANOVA with one within factor (time; pre vs. post) and one between factor (condition; CON vs. BFR) was utilized to undertake all subsequent analysis. Where significant interaction effects were observed, Bonferroni-corrected post hoc paired *t*-tests were used to locate differences. Data are presented as mean ± SD. Significance was accepted at $P \leq 0.05$.

RESULTS

Performance measures. There were no differences in physiological and performance measures before training between groups (Table 1). Participants completed 99% of the assigned training sessions without any complications (one participant missed one training session). The total amount of work done throughout the training was not different ($P = 0.75$) between CON (815 ± 88 kJ) and BFR (830 ± 129 kJ).

Physiological and performance variables measured before and after CON and BFR are presented in Table 1. There were significant interactions for absolute and relative $\dot{V}O_{2max}$. Sub-

Table 1. *Physiological and performance variables before and after control and BFR training interventions*

	CON			BFR					
Parameter	Pre	Post	Percentage change	Pre	Post	Percentage change	ANOVA interaction *P* value	ANOVA main effect of time *P* value	ANOVA main effect of condition *P* value
Body mass, kg	74.6 ± 7.7	74.3 ± 8.5	−0.3	76.3 ± 8.3	75.6 ± 7.7	−0.8	0.59	0.24	0.67
$\dot{V}O_{2max}$, l/min	4.59 ± 0.46	4.56 ± 0.48	−0.8	4.70 ± 0.63	4.98 ± 0.72*	5.9	0.02	0.08	0.45
$\dot{V}O_{2max}$, ml·min^{-1}·kg^{-1}	63.2 ± 4.4	63.0 ± 5.9	−0.3	62.2 ± 3.4	65.2 ± 4.4*	4.9	0.04	0.06	0.79
MAP, W	393 ± 47	397 ± 51	1.0	402 ± 51	412 ± 41	2.6	0.44	0.09	0.56
MAP, W/kg	5.3 ± 0.5	5.4 ± 0.6	1.5	5.3 ± 0.4	5.5 ± 0.4	3.5	0.40	0.03	0.81
CP, W	287 ± 39	297 ± 43	3.6	296 ± 40	306 ± 36	3.3	0.93	0.001	0.58
W′, kJ	18.1 ± 5.1	16.2 ± 4.3	−10.4	17.9 ± 6.5	17.4 ± 5.1	−3.0	0.53	0.26	0.80
PPO, W	1,057 ± 174	1,112 ± 179	5.2	1,065 ± 245	1,142 ± 262	7.2	0.62	0.008	0.85
PPO, W/kg	14.3 ± 2.3	15.0 ± 2.2	5.4	13.8 ± 2.7	14.9 ± 2.8	8.3	0.53	0.004	0.80
MPO, W	686 ± 73	692 ± 62	0.8	701 ± 113	722 ± 112	3.1	0.23	0.05	0.60
MPO, W/kg	9.2 ± 0.6	9.3 ± 0.6	1.4	9.1 ± 1.0	9.5 ± 1.1	4.2	0.22	0.01	0.99

Values are mean ± SD. All data are CON, $n = 10$ and BFR, $n = 11$, except for $\dot{V}O_{2max}$ where $n = 8$ and PPO and MPO where $n = 10$. BFR, blood flow restriction; CON, control; CP, critical power; MAP, maximal aerobic power; MPO, mean power output during 30-s sprint; PPO, peak power output during 30-s sprint; $\dot{V}O_{2max}$, maximal oxygen uptake; *W′*, curvature constant. *Significantly different to pretraining (Bonferroni-corrected post hoc paired *t*-test; $P < 0.05$).

sequent post hoc tests revealed that both absolute (Fig. 1*A*) and relative $\dot{V}O_{2max}$ increased following BFR (absolute: $P = 0.02$, relative: $P = 0.01$) but not in CON (absolute: $P = 0.56$, relative: $P = 0.88$). Absolute MAP (Fig. 1*B*) was unchanged with training in either group. Relative MAP increased with training (main effect for time; $P = 0.03$); however, there was no difference between CON and BFR.

The inverse linear relationship produced the lowest combined standard error for CP (CON: pre, 1.8 ± 1.0%; post; 1.1 ± 0.7%; BFR: pre, 1.7 ± 0.8%; post, 1.9 ± 1.1%) and *W′* (CON: pre; 7.4 ± 4.4%; post; 4.9 ± 3.5%; BFR: pre, 8.4 ± 5.7%; post, 9.1 ± 5.9%), and therefore this equation was used to calculate parameter estimates. CP (Fig. 1*C*) increased with training; however, there was no difference between CON

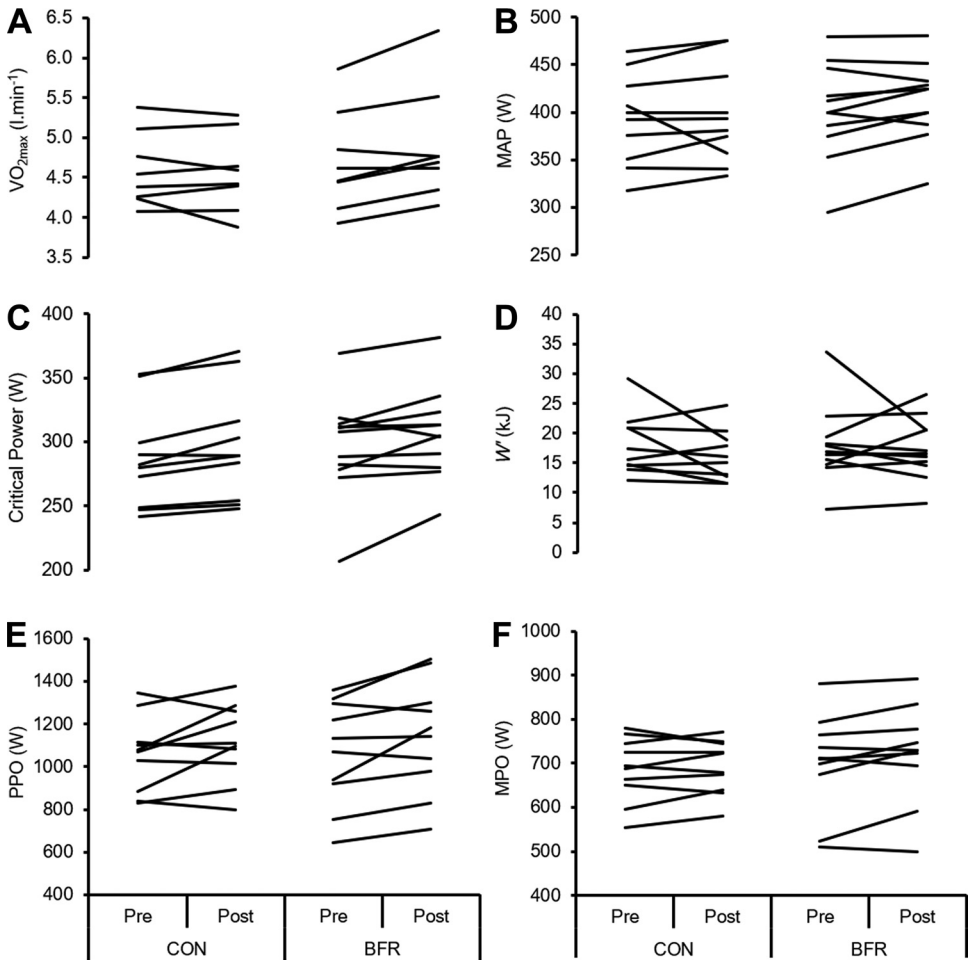

Fig. 1. Individual responses of maximal oxygen uptake ($\dot{V}O_{2max}$) (*A*), maximal aerobic power (MAP) (*B*), critical power (CP) (*C*), curvature constant (*W′*) (*D*), peak power output (PPO) (*E*), and mean power output (MPO) (*F*) before and after control (CON) and blood flow restriction (BFR) training interventions.

and BFR. W' (Fig. 1*D*) was unchanged with training in either group.

Absolute (Fig. 1*E*) and relative peak power output and absolute (Fig. 1*F*) and relative MPO increased with training; however, there were no differences between CON and BFR.

Capillarization and muscle morphology. All measures of capillarization (Fig. 2) were unchanged with training in either group (Table 2). The number of Ki-67 proliferating cells colocalized with capillaries was not significantly changed (interaction; $P = 0.06$) with training in either group (Fig. 3). CSA of type I and type II fibers were unchanged with training in either group (Table 2).

Mitochondrial enzyme protein content. Protein content of CS, COX II, and COX IV (Fig. 4) were all unchanged with training in either group.

DISCUSSION

This study has demonstrated that 4 wk of SIT increased CP in trained individuals; however, the addition of BFR did not enhance this further. Furthermore, SIT with or without BFR did not induce any changes in skeletal muscle capillarity or mitochondrial protein content in our trained population. The study has, however, confirmed the potency of SIT combined with BFR in increasing $\dot{V}O_{2max}$ to a greater extent than SIT alone.

We had hypothesized that the increase in CP following SIT would be further enhanced with BFR. In contrast to this hypothesis, while there was an increase in CP of 3.6% with SIT, there was no greater enhancement with BFR which had a similar magnitude of increase of 3.3%. The improvement in CP is in line with previous research that has demonstrated increases in CP following high-intensity interval training (15, 40). The increases of 10%–15% in the aforementioned studies are notably greater than that of the present study; however, considering our shorter training period and well-trained participants, this is not surprising. Nevertheless, it is important to note that the gains of ~3.5% are greater than the smallest worthwhile change in power for well-trained individuals of 1% (36) and therefore represents a meaningful observation.

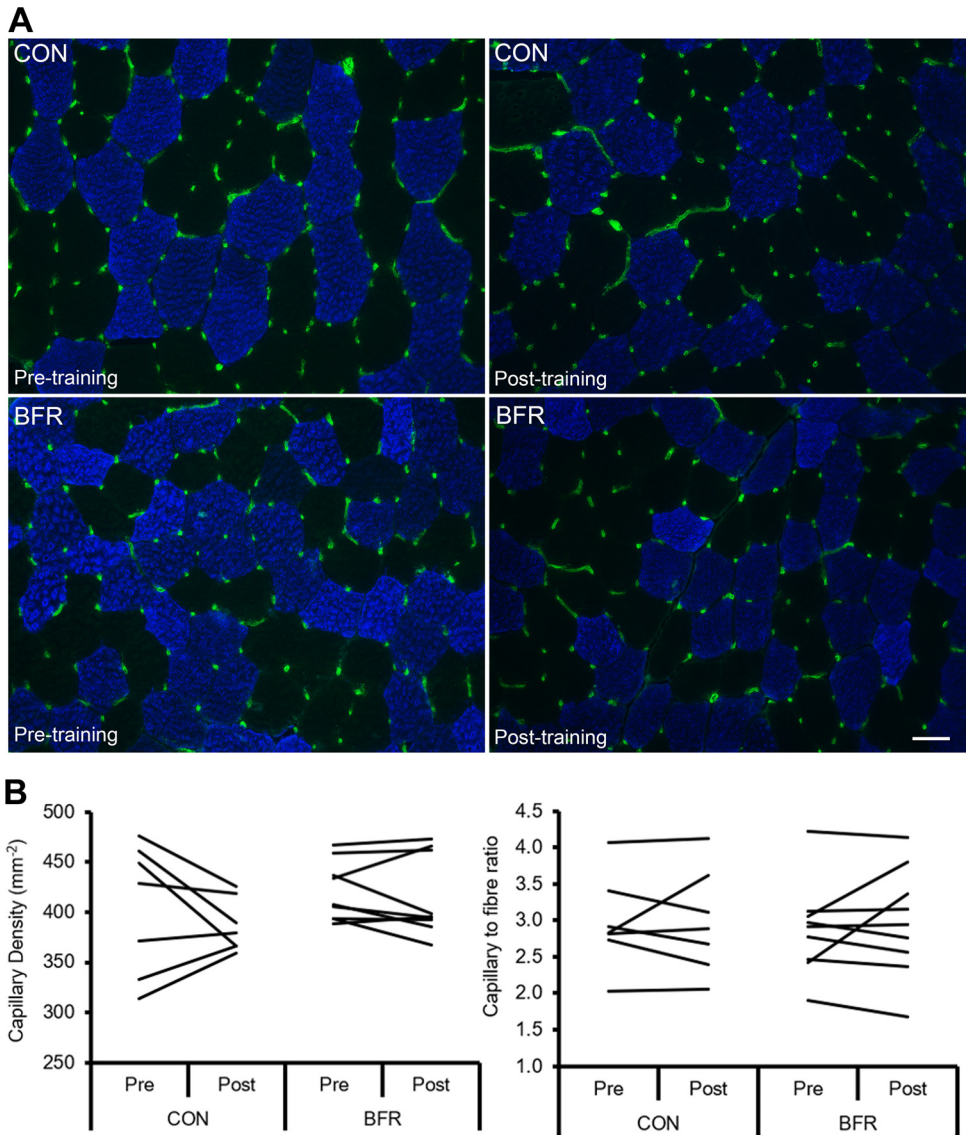

Fig. 2. Muscle capillaries before and after control (CON) and blood flow restriction (BFR) training interventions. *A*: representative images of muscle capillaries stained with CD-31 (green) and type II fibers (blue). Scale bar = 50 μm. *B*: individual responses of capillary density and capillary to fiber ratio. Data are CON, $n = 7$ and BFR, $n = 9$.

Table 2. *Capillarization and muscle morphology before and after control and BFR training interventions*

	CON			BFR					
Parameter	Pre	Post	Percentage change	Pre	Post	Percentage change	ANOVA interaction *P* value	ANOVA main effect of time *P* value	ANOVA main effect of condition *P* value
CD, mm^{-2}	405 ± 65	386 ± 27	−4.6	420 ± 30	415 ± 40	−1.3	0.49	0.22	0.27
C:F	2.97 ± 0.63	2.98 ± 0.71	0.4	2.87 ± 0.64	2.97 ± 0.75	3.5	0.68	0.60	0.88
CC Type I	7.03 ± 1.21	6.92 ± 1.54	−1.6	6.99 ± 1.25	7.19 ± 1.43	2.7	0.44	0.85	0.86
CC Type II	6.45 ± 1.09	6.63 ± 1.40	2.8	6.01 ± 1.06	6.32 ± 1.53	5.1	0.73	0.20	0.56
CSA Type I, μm^{-2}	6,456 ± 1,651	6,379 ± 1,764	−1.2	6,146 ± 1,242	6,375 ± 1472	3.7	0.62	0.81	0.83
CSA Type II, μm^{-2}	6,908 ± 1,463	6,998 ± 1,415	1.3	5,896 ± 963	6,364 ± 1639	7.9	0.47	0.29	0.23
Ki-67/fiber	0.07 ± 0.05	0.06 ± 0.04	−19.7	0.05 ± 0.06	0.10 ± 0.09	103.4	0.06	0.29	0.85

Values are mean ± SD. All data are CON, $n = 7$ and BFR, $n = 9$, except Ki-67/fiber which is CON, $n = 6$ and BFR, $n = 9$. BFR, blood flow restriction; CC Type I, capillary contacts of type I muscle fibers; CC Type II, capillary contacts of type II muscle fibers; CD, capillary density; C:F, capillary-to-fiber ratio; CON, control; CSA Type I, cross sectional area of type I muscle fibers; CSA Type II, cross sectional area of type II muscle fibers; Ki-67/fiber, Ki-67 positive capillaries per fiber.

Given the established relationship between capillarity and high-intensity exercise performance (24), particularly CP (32), together with the potency of the acute angiogenic stimulus our novel training intervention provides (48), it was surprising that we did not observe an increase in any measure of capillarity in either training group. The lack of increase in capillarity could be due to the volume of training undertaken, given that, in comparison to rodents where angiogenesis can occur within a week (53), in humans it is generally considered that angiogenesis manifests later than other training adaptions, typically occurring after 4–5 wk of training (3, 26). While the current training protocol was 4 wk in duration, it involved only eight low-volume training sessions. Although SIT has been shown to increase skeletal muscle capillarity, this was in untrained individuals ($\dot{V}O_{2max}$ of 41.9 ± 1.8 $ml \cdot min^{-1} \cdot kg^{-1}$) and was after 18 sessions over a longer period of 6 wk (11), resulting in a training volume more than double that of the present study. Volume of training seems to play a greater role than intensity in stimulating angiogenesis (17). For example, high-intensity interval training has been shown to produce a lower acute angiogenic response in interstitial fluid than moderate-intensity training (20), and an increase in training intensity at the expense of the volume of training in trained individuals has been demonstrated to reduce skeletal muscle VEGF protein content (18) and stunt increases in capillarization (20). Therefore, in spite of our hypothesis, the eight sessions may still have been an insufficient stimulus to induce an increase in capillarization. This is further exacerbated by the trained nature of our participants in which the baseline capillary to fiber ratio of 2.9 is around double that previously reported in untrained participants of ~1.4 (3, 26).

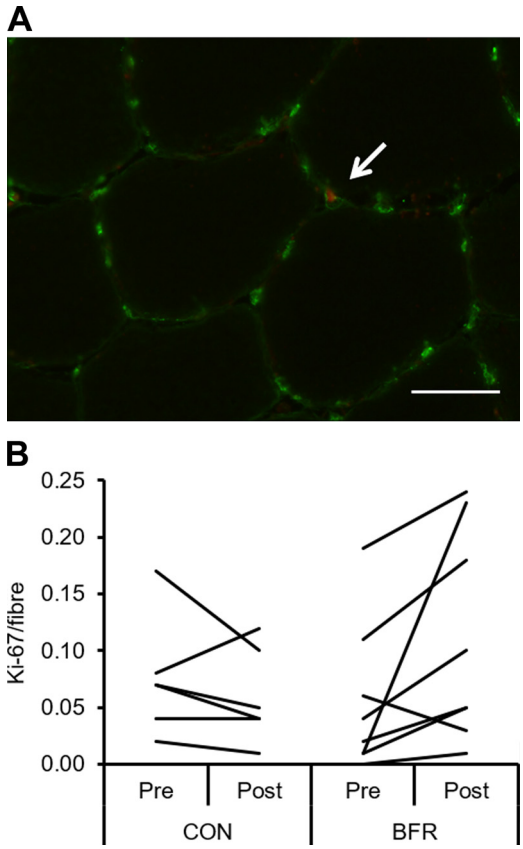

Fig. 3. Presence of proliferating endothelial cells before and after control (CON) and blood flow restriction (BFR) training interventions. *A*: representative image of Ki-67 (red) -positive endothelial cells (green). Scale bar = 50 μm. *B*: individual responses of Ki-67-positive endothelial cells per fiber. Data are CON, $n = 6$ and BFR, $n = 9$. One bar for BFR is hidden as data are pre = 0.00 and post = 0.00.

Nevertheless, the angiogenic potential of the present training intervention was further explored by investigating the presence of proliferating endothelial cells. The antibody Ki-67 detects a proliferation-associated nuclear antigen in which its colocalization within endothelial cells in skeletal muscle allows the identification of proliferating endothelial cells, and thus location of growing capillaries (21, 26), and is therefore a measure that would precede increases in skeletal muscle capillarity. There was no significant change in Ki-67-positive endothelial cells following training in either group, although the interaction effect was $P = 0.06$, and there was an approximate 100% increase in Ki-67-positive endothelial cells following BFR, which was not present after CON. Indeed, there was a greater presence of the number of Ki-67-positive endothelial cells in seven out of nine participants of the BFR group compared with only one participant demonstrating any evidence of increased endothelial cell proliferation in CON, and a calculation of Cohen's d revealed a medium effect size of 0.63. These observations suggest the potential of postexercise BFR to enhance angiogenesis is worth further exploration, and to

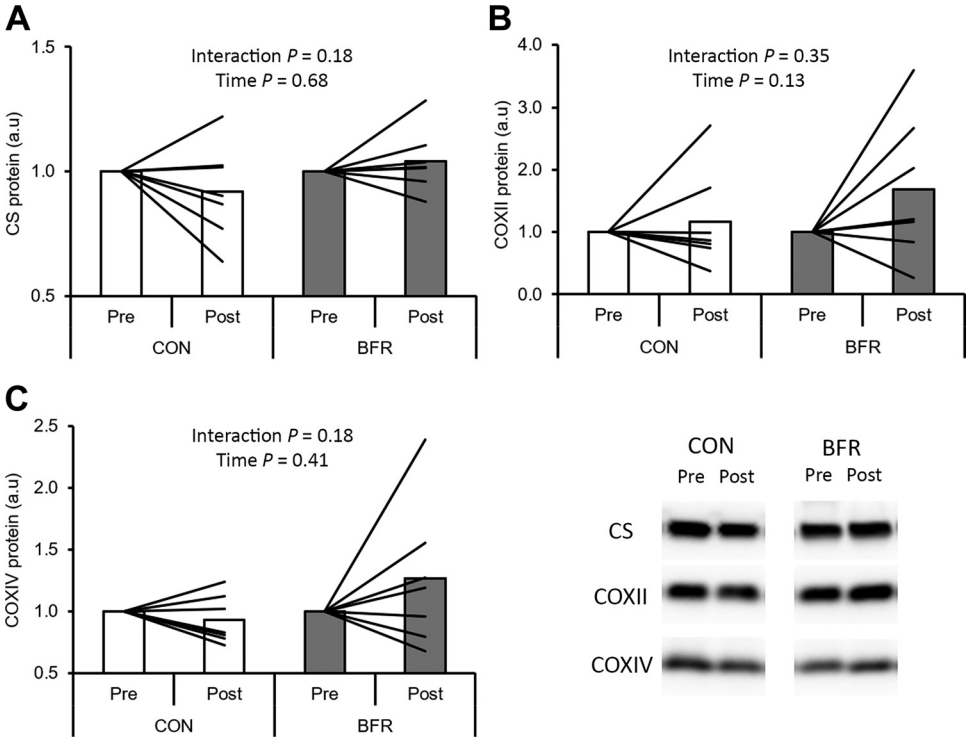

Fig. 4. Content of citrate synthase (CS) (*A*), cytochrome c oxidase (COX) II (*B*), and COX IV (*C*) protein before and after control (CON) and blood flow restriction (BFR) training interventions. Values are expressed as fold changes relative to pretraining values. Bars represent the mean, and lines represent individual responses. Data are CON, $n = 7$ and BFR, $n = 7$.

induce measurable further adaptation in this already well-adapted population may require a greater training period or a higher training volume.

There were no changes in the protein content of mitochondrial enzymes CS, COX II, and COX IV following either training group. This contrasts with previous findings that have consistently demonstrated increases in markers of mitochondrial biogenesis following SIT, including increased activity of CS and COX (8–10, 16), increased protein content of COX II and COX IV (7, 16), and increased maximal mitochondrial respiration (19). These previous investigations have, however, only been undertaken on untrained or recreationally active populations; therefore, it seems likely that the lack of effect in the present study is attributable to our already well-adapted, trained population.

In the absence of any observable angiogenesis and mitochondrial biogenesis, the improvement in CP in both groups could be attributable to multiple factors related to the so-called anaerobic capacity of skeletal muscle. For example, increases in skeletal muscle buffering capacity (54) and monocarboxylate transporter protein (5) have been demonstrated following high-intensity interval training in trained individuals. These adaptations, which would enhance the ability for the removal of fatigue-inducing metabolites and thus be expected to increase the power at which the loss of metabolic homeostasis occurs, could be hypothesized to increase CP. Such adaptations may also be expected to increase W', which, as the second parameter of the power-duration relationship, has classically been considered to represent an anaerobic component (34). W' was, however, unchanged in the present study, as has typically been reported in many high-intensity training studies that have reported an increase in CP (15, 40, 51). It is important to consider the interrelated nature of CP and W', which have regularly been reported to change in opposite directions in response to multiple interventions (38), and, as such, the observed increase in CP may have offset any increases in W'.

Although CP increased in both groups, $\dot{V}O_{2max}$ increased in the BFR group only. This supports our previous work (48) that initially demonstrated the potency of this novel training intervention, with an increase in $\dot{V}O_{2max}$ of ~4.5%, and agrees with further studies that have demonstrated an increase in $\dot{V}O_{2max}$ with the addition of BFR to low-intensity (~40% of $\dot{V}O_{2max}$) exercise (1, 35). Improvements in $\dot{V}O_{2max}$ have been demonstrated to precede increases in skeletal muscle capillaries (3), and while submaximal thresholds are predominantly determined by peripheral mechanisms (28), central components, in particular maximal cardiac output, are considered to be the principal limiting factors of $\dot{V}O_{2max}$, at least within whole body exercise, such as cycling (4, 28). Therefore, on the basis that we have not observed any increase in capillarity, the increase in $\dot{V}O_{2max}$ following BFR is perhaps more likely to be attributable to central adaptations, i.e., increased cardiac output, with SIT alone not presenting a sufficient challenge within our trained population. The addition of BFR during and after exercise poses a significant challenge to the central cardiovascular system through the induction of the exercise pressor reflex (2, 45) that results in an increase in heart rate and systolic blood pressure (6, 25, 41, 42, 46). Central cardiovascular adaptations have previously been reported with BFR exercise where Park et al. (35) demonstrated that 2 wk of walk training combined with BFR increased stroke volume by 21.4%. Although, no assessment of stroke volume was made in the control group of that study, so the effect of exercise alone is unknown; it seems plausible that the pressor reflex-related stimulus of BFR could induce a central adaptive response that contributes to an increase in maximal cardiac output and thus $\dot{V}O_{2max}$.

The present study is not without its limitations. While the use of trained individuals clearly enhances the validity of the application of the study findings to elite training practice, this resulted in a reduced sample size, in particular of participants who consented to muscle biopsies. Although typical of the current literature, the sample size must therefore be considered when interpreting the results, which is likely to have reduced the chances of correctly accepting or rejecting the null hypotheses. This is perhaps reflected in the present study with several parameters that displayed *P* values close to the critical value. An absolute BFR cuff pressure of 120 mmHg was also utilized for all participants. It is known that there is a variation between individuals in the level of BFR imposed by a standard absolute external cuff pressure (23), which will affect the level of muscle oxygenation and muscle metabolite accumulation (29, 47). Indeed, unpublished work in our laboratory using the same standard cuff pressure suggests that the decrease in muscle oxygenation imposed by the postexercise BFR in the present training protocol varies between 11% and 43% (Mitchell EA, Bailey SJ, and Ferguson RA, unpublished observations). It is therefore possible that the physiological signals imposed with the addition of postexercise BFR were not consistent between individuals and may have impacted the extent of any adaptations. Furthermore, the present study only focused on the peripheral adaptations to the current intervention. As discussed above, it seems likely that the observed increase in $\dot{V}O_{2max}$ could be attributable to central adaptations, such as increased cardiac output. The central adaptive responses to BFR exercise clearly require further investigation.

In conclusion, the addition of postexercise BFR did not enhance the increase in CP observed after 4 wk of SIT in trained individuals. SIT with or without BFR did not induce any changes in skeletal muscle capillarity or mitochondrial protein content. The study has, however, confirmed previous findings of the potency of combining postexercise BFR during SIT in enhancing $\dot{V}O_{2max}$ in trained individuals.

ACKNOWLEDGMENTS

The authors thank the participants for commitment and effort. The authors also thank Xavier Frisch, Said Ibeggazene, Rosa Leftwich, Samantha Rees-Clark, Ismael Serrablo-Torrejon, Tom Smith, and Robert Sullivan for technical assistance at various points throughout the experiment.

Present address of C. W. Taylor: Team Sky, National Cycling Centre, Stuart St., Manchester, M11 4DQ, UK.

Present address of M. C. Turner: University Hospitals of Leicester NHS Trust, Infirmary Square, Leicester LE1 5WW, UK.

The monoclonal antibody for MHC I, developed by Blau HM, was obtained from the Developmental Studies Hybridoma Bank, created by the National Institute of Child Health and Human Development of the National Institutes of Health and maintained at The University of Iowa, Department of Biology.

GRANTS

M. C. Turner is supported by the National Institute for Health Research (NIHR) Leicester Biomedical Research Centre. The views expressed are those of the author(s) and not necessarily those of the National Health Service (NHS), the NIHR, or the Department of Health and Social Care.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

E.A.M., C.W.T., and R.A.F. conceived and designed research; E.A.M. and R.A.F. performed experiments; E.A.M. analyzed data; E.A.M., N.R.W.M., M.C.T., and R.A.F. interpreted results of experiments; E.A.M. prepared figures; E.A.M. and R.A.F. drafted manuscript; E.A.M., N.R.W.M., M.C.T., C.W.T., and R.A.F. edited and revised manuscript; E.A.M., N.R.W.M., M.C.T., C.W.T., and R.A.F. approved final version of manuscript.

REFERENCES

1. **Abe T, Fujita S, Nakajima T, Sakamaki M, Ozaki H, Ogasawara R, Sugaya M, Kudo M, Kurano M, Yasuda T, Sato Y, Ohshima H, Mukai C, Ishii N.** Effects of low-intensity cycle training with restricted leg blood flow on thigh muscle volume and $\dot{V}O_{2max}$ in young men. *J Sports Sci Med* 9: 452–458, 2010.
2. **Alam M, Smirk FH.** Observations in man upon a blood pressure raising reflex arising from the voluntary muscles. *J Physiol* 89: 372–383, 1937. doi:10.1113/jphysiol.1937.sp003485.
3. **Andersen P, Henriksson J.** Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise. *J Physiol* 270: 677–690, 1977. doi:10.1113/jphysiol.1977.sp011975.
4. **Bassett DR Jr, Howley ET.** Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc* 32: 70–84, 2000. doi:10.1097/00005768-200001000-00012.
5. **Bickham DC, Bentley DJ, Le Rossignol PF, Cameron-Smith D.** The effects of short-term sprint training on MCT expression in moderately endurance-trained runners. *Eur J Appl Physiol* 96: 636–643, 2006. doi:10.1007/s00421-005-0100-x.
6. **Bull RK, Davies CT, Lind AR, White MJ.** The human pressor response during and following voluntary and evoked isometric contraction with occluded local blood supply. *J Physiol* 411: 63–70, 1989. doi:10.1113/jphysiol.1989.sp017560.
7. **Burgomaster KA, Cermak NM, Phillips SM, Benton CR, Bonen A, Gibala MJ.** Divergent response of metabolite transport proteins in human skeletal muscle after sprint interval training and detraining. *Am J Physiol Regul Integr Comp Physiol* 292: R1970–R1976, 2007. doi:10.1152/ajpregu.00503.2006.
8. **Burgomaster KA, Heigenhauser GJ, Gibala MJ.** Effect of short-term sprint interval training on human skeletal muscle carbohydrate metabolism during exercise and time-trial performance. *J Appl Physiol (1985)* 100: 2041–2047, 2006. doi:10.1152/japplphysiol.01220.2005.
9. **Burgomaster KA, Howarth KR, Phillips SM, Rakobowchuk M, Macdonald MJ, McGee SL, Gibala MJ.** Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. *J Physiol* 586: 151–160, 2008. doi:10.1113/jphysiol.2007.142109.
10. **Burgomaster KA, Hughes SC, Heigenhauser GJ, Bradwell SN, Gibala MJ.** Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. *J Appl Physiol (1985)* 98: 1985–1990, 2005. doi:10.1152/japplphysiol.01095.2004.
11. **Cocks M, Shaw CS, Shepherd SO, Fisher JP, Ranasinghe AM, Barker TA, Tipton KD, Wagenmakers AJ.** Sprint interval and endurance training are equally effective in increasing muscle microvascular density and eNOS content in sedentary males. *J Physiol* 591: 641–656, 2013. doi:10.1113/jphysiol.2012.239566.
12. **Costill DL, Flynn MG, Kirwan JP, Houmard JA, Mitchell JB, Thomas R, Park SH.** Effects of repeated days of intensified training on muscle glycogen and swimming performance. *Med Sci Sports Exerc* 20: 249–254, 1988. doi:10.1249/00005768-198806000-00006.
13. **Daniels JT, Yarbrough RA, Foster C.** Changes in $\dot{V}O_{2max}$ and running performance with training. *Eur J Appl Physiol Occup Physiol* 39: 249–254, 1978. doi:10.1007/BF00421448.
14. **Flueck M.** Myocellular limitations of human performance and their modification through genome-dependent responses at altitude. *Exp Physiol* 95: 451–462, 2010. doi:10.1113/expphysiol.2009.047605.
15. **Gaesser GA, Wilson LA.** Effects of continuous and interval training on the parameters of the power-endurance time relationship for high-intensity exercise. *Int J Sports Med* 9: 417–421, 1988. doi:10.1055/s-2007-1025043.
16. **Gibala MJ, Little JP, van Essen M, Wilkin GP, Burgomaster KA, Safdar A, Raha S, Tarnopolsky MA.** Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance. *J Physiol* 575: 901–911, 2006. doi:10.1113/jphysiol.2006.112094.
17. **Gliemann L.** Training for skeletal muscle capillarization: a Janus-faced role of exercise intensity? *Eur J Appl Physiol* 116: 1443–1444, 2016. doi:10.1007/s00421-016-3419-6.

18. **Gliemann L, Gunnarsson TP, Hellsten Y, Bangsbo J.** 10-20-30 training increases performance and lowers blood pressure and VEGF in runners. *Scand J Med Sci Sports* 25: e479–e489, 2015. doi:10.1111/sms.12356.
19. **Granata C, Oliveira RS, Little JP, Renner K, Bishop DJ.** Training intensity modulates changes in PGC-1α and p53 protein content and mitochondrial respiration, but not markers of mitochondrial content in human skeletal muscle. *FASEB J* 30: 959–970, 2016. doi:10.1096/fj.15-276907.
20. **Hoier B, Passos M, Bangsbo J, Hellsten Y.** Intense intermittent exercise provides weak stimulus for vascular endothelial growth factor secretion and capillary growth in skeletal muscle. *Exp Physiol* 98: 585–597, 2013. doi:10.1113/expphysiol.2012.067967.
21. **Høier B, Rufener N, Bojsen-Møller J, Bangsbo J, Hellsten Y.** The effect of passive movement training on angiogenic factors and capillary growth in human skeletal muscle. *J Physiol* 588: 3833–3845, 2010. doi:10.1113/jphysiol.2010.190439.
22. **Hoppeler H, Howald H, Conley K, Lindstedt SL, Claassen H, Vock P, Weibel ER.** Endurance training in humans: aerobic capacity and structure of skeletal muscle. *J Appl Physiol (1985)* 59: 320–327, 1985. doi:10.1152/jappl.1985.59.2.320.
23. **Hunt JE, Stodart C, Ferguson RA.** The influence of participant characteristics on the relationship between cuff pressure and level of blood flow restriction. *Eur J Appl Physiol* 116: 1421–1432, 2016. doi:10.1007/s00421-016-3399-6.
24. **Iaia FM, Perez-Gomez J, Thomassen M, Nordsborg NB, Hellsten Y, Bangsbo J.** Relationship between performance at different exercise intensities and skeletal muscle characteristics. *J Appl Physiol (1985)* 110: 1555–1563, 2011. doi:10.1152/japplphysiol.00420.2010.
25. **Iida H, Kurano M, Takano H, Kubota N, Morita T, Meguro K, Sato Y, Abe T, Yamazaki Y, Uno K, Takenaka K, Hirose K, Nakajima T.** Hemodynamic and neurohumoral responses to the restriction of femoral blood flow by KAATSU in healthy subjects. *Eur J Appl Physiol* 100: 275–285, 2007. doi:10.1007/s00421-007-0430-y.
26. **Jensen L, Bangsbo J, Hellsten Y.** Effect of high intensity training on capillarization and presence of angiogenic factors in human skeletal muscle. *J Physiol* 557: 571–582, 2004. doi:10.1113/jphysiol.2003.057711.
27. **Jones AM, Vanhatalo A, Burnley M, Morton RH, Poole DC.** Critical power: implications for determination of $\dot{V}O_{2max}$ and exercise tolerance. *Med Sci Sports Exerc* 42: 1876–1890, 2010. doi:10.1249/MSS.0b013e3181d9cf7f.
28. **Joyner MJ, Coyle EF.** Endurance exercise performance: the physiology of champions. *J Physiol* 586: 35–44, 2008. doi:10.1113/jphysiol.2007.143834.
29. **Karabulut M, McCarron J, Abe T, Sato Y, Bemben M.** The effects of different initial restrictive pressures used to reduce blood flow and thigh composition on tissue oxygenation of the quadriceps. *J Sports Sci* 29: 951–958, 2011. doi:10.1080/02640414.2011.572992.
30. **Laursen PB, Jenkins DG.** The scientific basis for high-intensity interval training: optimising training programmes and maximising performance in highly trained endurance athletes. *Sports Med* 32: 53–73, 2002. doi:10.2165/00007256-200232010-00003.
31. **Londeree BR.** Effect of training on lactate/ventilatory thresholds: a meta-analysis. *Med Sci Sports Exerc* 29: 837–843, 1997. doi:10.1097/00005768-199706000-00016.
32. **Mitchell EA, Martin NRW, Bailey SJ, Ferguson RA.** Critical power is positively related to skeletal muscle capillarity and type I muscle fibers in endurance-trained individuals. *J Appl Physiol (1985)* 125: 737–745, 2018. doi:10.1152/japplphysiol.01126.2017.
33. **Monod H, Scherrer J.** The work capacity of a synergic muscular group. *Ergonomics* 8: 329–338, 1965. doi:10.1080/00140136508930810.
34. **Morton RH.** The critical power and related whole-body bioenergetic models. *Eur J Appl Physiol* 96: 339–354, 2006. doi:10.1007/s00421-005-0088-2.
35. **Park S, Kim JK, Choi HM, Kim HG, Beekley MD, Nho H.** Increase in maximal oxygen uptake following 2-week walk training with blood flow occlusion in athletes. *Eur J Appl Physiol* 109: 591–600, 2010. doi:10.1007/s00421-010-1377-y.
36. **Paton CD, Hopkins WG.** Tests of cycling performance. *Sports Med* 31: 489–496, 2001. doi:10.2165/00007256-200131070-00004.
37. **Perry CG, Lally J, Holloway GP, Heigenhauser GJ, Bonen A, Spriet LL.** Repeated transient mRNA bursts precede increases in transcriptional and mitochondrial proteins during training in human skeletal muscle. *J Physiol* 588: 4795–4810, 2010. doi:10.1113/jphysiol.2010.199448.
38. **Poole DC, Burnley M, Vanhatalo A, Rossiter HB, Jones AM.** Critical power: an important fatigue threshold in exercise physiology. *Med Sci Sports Exerc* 48: 2320–2334, 2016. doi:10.1249/MSS.0000000000000939.
39. **Poole DC, Ward SA, Gardner GW, Whipp BJ.** Metabolic and respiratory profile of the upper limit for prolonged exercise in man. *Ergonomics* 31: 1265–1279, 1988. doi:10.1080/00140138808966766.
40. **Poole DC, Ward SA, Whipp BJ.** The effects of training on the metabolic and respiratory profile of high-intensity cycle ergometer exercise. *Eur J Appl Physiol Occup Physiol* 59: 421–429, 1990. doi:10.1007/BF02388623.
41. **Poton R, Polito MD.** Hemodynamic response to resistance exercise with and without blood flow restriction in healthy subjects. *Clin Physiol Funct Imaging* 36: 231–236, 2016. doi:10.1111/cpf.12218.
42. **Renzi CP, Tanaka H, Sugawara J.** Effects of leg blood flow restriction during walking on cardiovascular function. *Med Sci Sports Exerc* 42: 726–732, 2010. doi:10.1249/MSS.0b013e3181bdb454.
43. **Romanul FC.** Distribution of capillaries in relation to oxidative metabolism of skeletal muscle fibres. *Nature* 201: 307–308, 1964. doi:10.1038/201307a0.
44. **Saltin B, Henriksson J, Nygaard E, Andersen P, Jansson E.** Fiber types and metabolic potentials of skeletal muscles in sedentary man and endurance runners. *Ann N Y Acad Sci* 301: 3–29, 1977. doi:10.1111/j.1749-6632.1977.tb38182.x.
45. **Spranger MD, Krishnan AC, Levy PD, O'Leary DS, Smith SA.** Blood flow restriction training and the exercise pressor reflex: a call for concern. *Am J Physiol Heart Circ Physiol* 309: H1440–H1452, 2015. doi:10.1152/ajpheart.00208.2015.
46. **Staunton CA, May AK, Brandner CR, Warmington SA.** Haemodynamics of aerobic and resistance blood flow restriction exercise in young and older adults. *Eur J Appl Physiol* 115: 2293–2302, 2015. doi:10.1007/s00421-015-3213-x.
47. **Takarada Y, Takazawa H, Sato Y, Takebayashi S, Tanaka Y, Ishii N.** Effects of resistance exercise combined with moderate vascular occlusion on muscular function in humans. *J Appl Physiol (1985)* 88: 2097–2106, 2000. doi:10.1152/jappl.2000.88.6.2097.
48. **Taylor CW, Ingham SA, Ferguson RA.** Acute and chronic effect of sprint interval training combined with postexercise blood-flow restriction in trained individuals. *Exp Physiol* 101: 143–154, 2016. doi:10.1113/EP085293.
49. **Thomas K, Goodall S, Stone M, Howatson G, St Clair Gibson A, Ansley L.** Central and peripheral fatigue in male cyclists after 4-, 20-, and 40-km time trials. *Med Sci Sports Exerc* 47: 537–546, 2015. doi:10.1249/MSS.0000000000000448.
50. **Vanhatalo A, Black MI, DiMenna FJ, Blackwell JR, Schmidt JF, Thompson C, Wylie LJ, Mohr M, Bangsbo J, Krustrup P, Jones AM.** The mechanistic bases of the power-time relationship: muscle metabolic responses and relationships to muscle fibre type. *J Physiol* 594: 4407–4423, 2016. doi:10.1113/JP271879.
51. **Vanhatalo A, Doust JH, Burnley M.** A 3-min all-out cycling test is sensitive to a change in critical power. *Med Sci Sports Exerc* 40: 1693–1699, 2008. doi:10.1249/MSS.0b013e318177871a.
52. **Vanhatalo A, Jones AM, Burnley M.** Application of critical power in sport. *Int J Sports Physiol Perform* 6: 128–136, 2011. doi:10.1123/ijspp.6.1.128.
53. **Waters RE, Rotevatn S, Li P, Annex BH, Yan Z.** Voluntary running induces fiber type-specific angiogenesis in mouse skeletal muscle. *Am J Physiol Cell Physiol* 287: C1342–C1348, 2004. doi:10.1152/ajpcell.00247.2004.
54. **Weston AR, Myburgh KH, Lindsay FH, Dennis SC, Noakes TD, Hawley JA.** Skeletal muscle buffering capacity and endurance performance after high-intensity interval training by well-trained cyclists. *Eur J Appl Physiol Occup Physiol* 75: 7–13, 1996. doi:10.1007/s004210050119.