Acute and chronic effect of sprint interval training combined with postexercise blood-flow restriction in trained individuals

Conor W. Taylor\textsuperscript{1,2}, Stephen A. Ingham\textsuperscript{2} and Richard A. Ferguson\textsuperscript{1}

\textsuperscript{1}School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, UK
\textsuperscript{2}English Institute of Sport, Performance Centre, Loughborough University, Loughborough, UK

New Findings

- What is the central question of this study?
  Does the combination of sprint interval training with postexercise blood-flow restriction enhance maximal aerobic physiology and performance in trained individuals?

- What is the main finding and its importance?
  We demonstrate the potency of combining blood-flow restriction with sprint interval training in increasing maximal oxygen uptake in trained individuals; however, this did not translate to an enhanced exercise performance. We also show that blood-flow restriction combined with sprint interval training enhanced postexercise hypoxia-inducible factor-1\(\alpha\) mRNA expression, suggesting the possibility for greater hypoxia-mediated adaptations, such as enhanced capillary growth, with this intervention.

This investigation assessed the efficacy of sprint interval training (SIT) combined with postexercise blood-flow restriction as a novel approach to enhance maximal aerobic physiology and performance. In study 1, a between-groups design was used to determine whether 4 weeks (2 days per week) of SIT (repeated 30 s maximal sprint cycling) combined with postexercise blood-flow restriction (BFR) enhanced maximal oxygen uptake (\(\dot{V}_O_2_{\text{max}}\)) and 15 km cycling time-trial performance (15 km TT) compared with SIT alone (CON) in trained individuals. The \(\dot{V}_O_2_{\text{max}}\) increased after BFR by 4.5\% (\(P = 0.01\)) but was unchanged after CON. There was no difference in 15 km TT performance after CON or BFR. In study 2, using a repeated-measures design, participants performed an acute bout of either BFR or CON. Muscle biopsies were taken before and after exercise to examine the activation of signalling pathways regulating angiogenesis and mitochondrial biogenesis. Phosphorylation of p38MAPK\(^{\text{Thr180/Tyr182}}\) increased by a similar extent after CON and BFR. There was no difference in the magnitude of increase in PGC-1\(\alpha\), VEGF and VEGFR-2 mRNA expression between protocols; however, HIF-1\(\alpha\) mRNA expression increased (\(P = 0.04\)) at 3 h only after BFR. We have demonstrated the potency of combining BFR with SIT in increasing \(\dot{V}_O_2_{\text{max}}\) in trained individuals, but this did not translate to an enhanced exercise performance. Sprint interval training alone did not induce any observable adaptation. Although the mechanisms are not fully understood, we present preliminary evidence that BFR leads to enhanced HIF-1\(\alpha\)-mediated cell signalling.

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Corresponding author R. A. Ferguson, School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, Leicestershire LE11 3TU, UK. Email: r.a.ferguson@lboro.ac.uk

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Introduction

Increasing the volume of submaximal endurance training does not appear to promote improvements in exercise capacity or maximal oxygen consumption ($\dot{V}_O_2_{\text{max}}$) in well-trained endurance athletes (Hickson et al. 1981; Denis et al. 1982; Costill et al. 1988). In contrast, trained cyclists who supplement their aerobic base training with high-intensity interval training (HIT) demonstrate improvements in endurance capabilities (Lindsay et al. 1996; Westgarth-Taylor et al. 1997; Weston et al. 1997) and, as such, HIT (e.g. involving longer duration intervals of approximately 1–8 min at submaximal exercise intensities) regularly features in many athletes’ structured training programmes. Another example of HIT (sometimes called sprint interval training; SIT) comprises four to six 30 s ‘all-out’ maximal efforts interspersed by ~4 min passive recovery. Recent evidence demonstrates that this type of training is an effective strategy to induce rapid increases in oxidative capacity and associated endurance capacity in healthy active and sedentary individuals (Burgomaster et al. 2005, 2006, 2008; Gibala et al. 2006); however, the efficacy of this type of low-volume sprint interval training model has yet to be established in trained individuals.

Despite the observed benefits of different types of HIT in promoting improvements in exercise capacity and $\dot{V}_O_2_{\text{max}}$ in trained individuals, there is increasing scope to identify and adopt new low-volume training methods that result in greater adaptive responses. One potential strategy to enhance endurance adaptation would be to combine exercise with blood-flow restriction (BFR). For example, one-legged endurance exercise [45 min at ~50% one-legged peak oxygen uptake ($\dot{V}_O_2_{\text{peak}}$) for 4 weeks, four times per week] performed with moderate blood-flow restriction (~20%) has been demonstrated to increase exercise capacity and $\dot{V}_O_2_{\text{max}}$ above that performed with a normal blood supply (Sundberg et al. 1993). More recently, BFR using blood-pressure cuffs combined with low-resistance training has become a popular approach for enhancing adaptations in both skeletal muscle (Burgomaster et al. 2003; Fujita et al. 2007) and the peripheral vasculature, in particular within the conduit arteries and capillary bed (Hunt et al. 2013).

Both HIT and SIT have been shown to enhance skeletal muscle capillary growth (Jensen et al. 2004; Cocks et al. 2013, respectively), a critical adaptation to enhance oxygen and substrate delivery. Exercise-induced capillary growth (angiogenesis) is a highly complex process, mediated by signal-transduction pathways involving several transcription and angiogenic growth factors, including hypoxia-inducible factor-1α (HIF-1α), peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) and the primary capillary growth factor vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 (Chinsomboon et al. 2009; Geng et al. 2010). These molecular pathways are activated in response to different physiological signals (e.g. hypoxia, shear stress, mechanical and metabolic stress) that are specific to the nature of the exercise stimulus (Egginton, 2009). The addition of BFR, for example, is likely to increase tissue hypoxia, thereby activating HIF-1α-mediated signalling to induce transcription of target genes involved in angiogenesis and energy metabolism (Taylor, 2008).

There were two aims to the present investigation. Firstly, we assessed the potency of combining SIT (repeated 30 s maximal sprint cycling) with BFR in enhancing maximal aerobic physiology and performance in trained participants who already possess enhanced physiological performance capabilities. We hypothesized that BFR combined with SIT would result in a greater increase in $\dot{V}_O_2_{\text{max}}$ and 15 km time-trial (TT) performance than SIT alone. Secondly, we explored the potential mechanisms of adaptation by measuring the expression of key signalling proteins and genes involved in skeletal muscle remodelling that contribute to enhanced aerobic physiological capabilities (i.e. angiogenesis and mitochondrial biogenesis).

Methods

Ethical approval

All experimental procedures were approved by the Loughborough University Ethics Advisory 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.

Participants

A total of 28 healthy trained men who were cycling 120 ± 66 km per week volunteered to participate in the two studies (two of these participants took part in both studies). All completed a medical questionnaire and biopsy screening document prior to participation to mitigate for maximal exercise and biopsy contraindications. Participants did not have a history of neuromuscular, haematological or musculoskeletal abnormalities and were not using pharmacological treatments during the study period.

Experimental design

Study 1: training study. Using a between-groups design, participants performed 4 weeks of SIT either combined with postexercise BFR or SIT alone (CON). Twenty participants took part in this study. There was no significant difference ($P > 0.05$) in pretraining age
Blood-flow-restricted sprint interval training

Table 1. Physiological and performance variables before and after control (CON) and blood-flow restriction (BFR) training interventions (study 1)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CON</th>
<th>Pre</th>
<th>Post</th>
<th>Percentage change</th>
<th>Pre</th>
<th>Post</th>
<th>Percentage change</th>
<th>ANOVA interaction, P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (kg)</td>
<td>75.5 ± 8.4</td>
<td>75.7 ± 7.9</td>
<td>0.3</td>
<td>74.1 ± 7.4</td>
<td>74.0 ± 7.6</td>
<td>0.3</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>VO₂ max (l min⁻¹)</td>
<td>4.6 ± 0.3</td>
<td>4.6 ± 0.4</td>
<td>0.7</td>
<td>4.5 ± 0.4</td>
<td>4.7 ± 0.4*</td>
<td>4.5</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>VO₂ max (ml min⁻¹ kg⁻¹)</td>
<td>61.8 ± 4.7</td>
<td>61.1 ± 4.9</td>
<td>1.1</td>
<td>61.2 ± 4.0</td>
<td>64.1 ± 4.1*</td>
<td>4.7</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>MAP (W)</td>
<td>373 ± 31</td>
<td>374 ± 37</td>
<td>0.2</td>
<td>377 ± 37</td>
<td>392 ± 38</td>
<td>3.8</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>MAP (W kg⁻¹)</td>
<td>5.0 ± 0.4</td>
<td>5.0 ± 0.4</td>
<td>0.2</td>
<td>5.1 ± 0.3</td>
<td>5.3 ± 0.5</td>
<td>4.4</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>PPO (W)</td>
<td>1065 ± 214</td>
<td>1137 ± 259</td>
<td>6.8</td>
<td>938 ± 168</td>
<td>998 ± 203</td>
<td>6.4</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>PPO (W kg⁻¹)</td>
<td>14.0 ± 1.9</td>
<td>15.0 ± 2.9</td>
<td>7.0</td>
<td>12.6 ± 1.5</td>
<td>13.5 ± 2.3</td>
<td>7.1</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>MPO (W)</td>
<td>709 ± 99</td>
<td>711 ± 102</td>
<td>0.2</td>
<td>676 ± 97</td>
<td>696 ± 102</td>
<td>2.9</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>MPO (W kg⁻¹)</td>
<td>9.4 ± 0.8</td>
<td>9.4 ± 0.9</td>
<td>0.3</td>
<td>9.1 ± 0.7</td>
<td>9.4 ± 0.9</td>
<td>3.3</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>15 km TT (s)</td>
<td>1363 ± 50</td>
<td>1361 ± 59</td>
<td>0.1</td>
<td>1347 ± 81</td>
<td>1339 ± 76</td>
<td>0.6</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>Average TT power (W)</td>
<td>280 ± 26</td>
<td>288 ± 29</td>
<td>2.8</td>
<td>282 ± 39</td>
<td>285 ± 37</td>
<td>1.1</td>
<td>0.53</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. Abbreviations: MAP, maximal aerobic power; MPO, mean power output during 30 s sprint; PPO, peak power output during 30 s sprint; TT, time trial; and VO₂ max, maximal oxygen uptake. *P < 0.05, compared with Pre (Bonferroni-corrected post hoc t test). All data are n = 10, except for average TT power, which is n = 7 in each group.

(27 ± 7 versus 26 ± 5 years), height (181 ± 9 versus 178 ± 8 cm), body mass (75.5 ± 8.4 versus 74.1 ± 7.4 kg) and all other baseline outcome measures (Table 1) between the two intervention groups, respectively (CON, n = 10 versus BFR, n = 10). Physiological (VO₂ max) and performance (15 km cycling time-trial) assessments were made before and after training.

Study 2: acute study. Using a repeated-measures design, participants performed an acute bout of CIT either combined with postexercise BFR or CIT alone (CON). Eight participants (age, 32 ± 7 years; height, 180 ± 10 cm; body mass, 75.3 ± 9.1 kg; VO₂ max, 4.3 ± 0.4 l min⁻¹) took part in this study. Muscle biopsies were obtained before exercise, immediately postexercise and 3 h postexercise.

Experimental protocols

Study 1: training study. Participants were initially familiarized with the testing and training procedures during preliminary visits, before being randomly assigned to either the CON or the BFR training intervention. Pretraining outcome measures were assessed on two occasions separated by at least 2 days. Post-training outcome measures were assessed in the same order, each separated by 2 days within 1 week. Laboratory conditions during pre- and post-training measurements remained constant (19–21°C and 40–50% humidity), and all measurements were conducted at the same time of day for each participant. Ergometer saddle and handlebar dimensions recorded for each participant during preliminary testing were standardized for all post-training measures. Participants recorded dietary intake and physical activity performed during the 24 h prior to each of their pretraining tests and replicated these dietary and activity patterns for the 24 h prior to all post-training tests. Participants were instructed to refrain from strenuous exercise 24 h prior to all testing visits and from ingesting alcohol and caffeine during the 48 h preceding testing.

Pre- and post-training outcome measures. All pre- and post-training measures were conducted on an SRM cycle ergometer (Schroberer Rad McBtechink, Weldorf, Germany), calibrated according to the manufacturer's guidelines. Participants performed an incremental test to exhaustion to establish VO₂ max and maximal aerobic power (MAP). Participants began cycling at a freely chosen, constant pedal cadence for 5 min at 120 W, after which power increased by 20 W every 60 s. Pulmonary gas exchange was measured breath by breath throughout exercise (Oxycon Pro; Carefusion, Hoechberg, Germany). The VO₂ max and MAP were defined as the highest VO₂ and power output achieved for a 30 and 60 s period during the test, respectively.

The cycling time trial (15 km TT) involved completing 15 km as quickly as possible. Except for total distance covered, no visual or verbal technical or motivational feedback was given. In order to resemble a time trial on the road, the ergometer was placed into ‘hyperbolic mode’, enabling the participants manually to select their own gear, pedal cadence and thus power output throughout. Power output was recorded (2 Hz sample rate) and averaged throughout the entire duration of the 15 km TT performance trials. Participants consumed
water ad libitum during their familiarization trial, with the volume and timings of consumption recorded and replicated during subsequent trials. Prior to commencing training, participants completed a total of three 15 km TT efforts, each separated by a week, to establish familiarity. Reproducibility for the 15 km TT (i.e. the coefficient of variance, which expresses the typical error as a percentage; Hopkins, 2000), when performed at the same time of day and having consumed an identical diet for the 24 h preceding each trial, was determined between the second and third TT efforts and was 4.9%. The third TT effort was reported as the pretraining outcome measure.

Exercise training. Participants completed a 4 week supervised SIT programme (two sessions per week). Each training session consisted of repeated 30 s maximal sprint cycling bouts 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)^{-1}. The training was progressive, whereby all participants performed a total of four, five, six and seven maximal 30 s cycling bouts in weeks 1, 2, 3 and 4, respectively, with each bout separated by a 4.5 min recovery period. In CON, participants remained seated and stationary on the ergometer and refrained from pedalling during recovery between each sprint. At times, participants would get out of the saddle and stretch their legs and pedal backwards, but this was minimized. In the BFR conditions, participants immediately dismounted from the ergometer after each sprint and lay supine on a couch, upon which they were subjected to lower limb blood-flow restriction (within 15 s of each sprint). This was achieved by rapidly applying a pneumatic pressure cuff (SC12L; Hokanson, Bellevue, WA, USA) as high up as possible on the proximal portion of each thigh, inflated (E20 Rapid Cuff Inflator and AG101 Cuff Inflator Air Source; Hokanson, Bellevue, WA, USA) to a pressure of 130 mmHg for 2 min. After 2 min, the cuff was rapidly deflated and the participants remained in a supine position for a further 2 min. The participants subsequently remounted the ergometer in time for the subsequent sprint, which began precisely 4.5 min after the previous sprint ended. The cuff pressure of 130 mmHg was used following preliminary work that demonstrated this was the highest cuff pressure that could be tolerated in combination with the present sprint-training protocol. This pressure was kept constant throughout the 4 week training period. Participants were instructed to continue normal dietary practices throughout the training period and were instructed to complete daily training diaries to ensure that non-prescribed training remained constant throughout the entire study. Participants performed no other interval training during the study period and were instructed to replicate all other non-prescribed weekly training to ensure that the SIT was the only training change experienced. Pretraining measurements of peak power output (PPO) and mean power output (MPO) were obtained from the first training session after a standardized warm-up. Post-training PPO and MPO were taken from the best of two maximal sprints performed separately during the week of post-training outcome measurements.

Study 2: acute study. Preliminary testing. Participants reported to the laboratory on three separate occasions prior to their first experimental trial. On their first visit, they performed an incremental test to exhaustion, as outlined for study 1, to establish their \( V_{\text{O}_2 \text{max}} \). They also performed a series of randomized maximal sprints (<12 s) on an SRM cycle ergometer at multiple fixed cadences (90, 100, 110, 120 and 130 r.p.m.), which was achieved by placing the ergometer into ‘isokinetic mode’. These sprints were performed to determine each participant’s optimal cadence for PPO, which was subsequently used as the fixed cadence at which each participant performed their experimental trials. On two subsequent visits, participants were familiarized with the experimental protocols.

Experimental protocol. Participants attended the laboratory in the morning (∼08.00 h) of each experimental trial following an overnight fast. On arrival, participants rested supine for 20 min whilst muscle biopsy sites were prepared, before a resting biopsy sample was obtained. Participants then performed a standardized warm-up, consisting of cycling at 120 W for 5 min. Immediately after the warm-up, they performed four 30 s ‘all-out’ sprints at the previously determined fixed pedal cadence, with each sprint separated by 4.5 min of recovery. In CON, participants remained seated and stationary on the ergometer and were helped onto an adjacent couch, where a muscle biopsy was taken within 1 min. Participants then rested passively before having a final muscle biopsy 3 h postexercise. Muscle biopsies were obtained at these time points because mRNA expression for the genes of interest measured has previously been shown to peak 1–6 h after intense exercise (Bartlett et al. 2012; Hoier et al. 2012, 2013a,b) and postexercise phosphorylation of p38MAPK\( ^{\text{Thr}180/\text{Tyr182}} \) has previously been demonstrated to peak and return to baseline within this time course (Gibala et al. 2009; Bartlett et al. 2012).

Participants recorded all food consumed and physical activity during the 24 h prior to their first experimental trial and were instructed to replicate these dietary and activity patterns prior to their second experimental trial, which were separated by 14–21 days. They were also
instructed to refrain from ingesting alcohol and caffeine during the 48 h preceding each trial. Whilst the consumption of food was prohibited at all times during each experimental trial, water was consumed ad libitum. Laboratory conditions remained constant (19–21°C and 40–50% humidity) for both experimental trials.

**Muscle sampling and analysis.** Muscle biopsies were obtained from the medial portion of the vastus lateralis muscle under local anaesthesia (1% lidocaine) using the micro-biopsy technique (Acecut 11 gauge Biopsy Needle; TSK, Tochigi-Ken, Japan). All muscle samples were obtained through separate incisions >2 cm apart, with two samples (~30 mg each) taken from each incision at each time point. Muscle samples were immediately frozen and stored at −80°C until further analyses.

Western blotting. Approximately 20 mg of frozen muscle was ground to powder under liquid nitrogen using a laboratory-grade pestle and mortar before being homogenized in 120 µl of ice-cold lysis buffer [25 mM Tris–HCl (pH 7.4), 50 mM NaF, 100 mM NaCl, 5 mM EGTA, 1 mM EDTA, 10 mM sodium pyrophosphatase, 1 mM Na2VO4, 0.27 µM sucrose, 1% Triton X-100 and 0.1% 2-mercaptoethanol] supplemented with a Pierce™ Protease Inhibitor Tablet (Thermo Scientific, Basingstoke, UK). Homogenates were centrifuged at 13,500g for 10 min at 4°C and the supernatants collected. The protein content of the supernatent was determined using a Pierce™ 660 Protein Assay (Thermo Scientific, Basingstoke, UK). Each sample was solubilized for 5 min at 100°C with an equal volume of sample buffer containing 1 M Tris–HCl (pH 6.8), 8% glycerol, 10% sodium dodeyl sulfate, 0.4% 2-β-mercaptoethanol and 0.05% Bromophenol Blue. For each blot, a negative control was loaded along with 10 µg of each sample and then separated (~2 h at ~100 V) in Tris–glycine running buffer using self-cast 4% stacking and 10% separating polyacrylamide gels. Gels were transferred wet onto nitrocellulose membranes for 1 h at 35 mA in a 100 V) in Tris–glycine running buffer using self-cast 4% stacking and 10% separating polyacrylamide gels. Gels were transferred wet onto nitrocellulose membranes for 1 h at 35 mA in a 1 x transfer buffer (0.3% Tris base, 1.4% glycine and 20% methanol). Membranes were then blocked for 1 h at room temperature in Tris-buffered saline [TBST; 0.19 M Tris (pH 7.6), 1.3 M NaCl and 0.1% Tween-20] with 5% non-fat blocking grade milk. Membranes were washed three times, each time for 5 min, in TBST before being incubated overnight at 4°C with anti-phospho p38 MAPK (Thr180/Tyr182) and anti-total p38 MAPK antibody (Cell Signalling, Danvers, MA, USA), at a concentration of 1:1000 in 1 x TBST. The next morning, membranes were again washed three times, for 5 min each time, in TBST and subsequently incubated with anti-species horseradish peroxidase-conjugated secondary antibody (Bio-Rad, Hemel Hempstead, UK) 1 h at room temperature. After a further three 5 min washes in TBST, membranes were saturated in chemiluminescence (SuperSignal; Thermo Fisher Scientific, Rockford, IL, USA) for 5 min prior to exposure. Membranes were visualized using image analysis (ChemiDoc™ XRS+; Bio-Rad, Hemel Hempstead, UK) and band densities determined (Quality One 1-D analysis software v 4.6.8; Bio-Rad, Hemel Hempstead, UK). Samples from each participant for both exercise protocols were run on the same gel, and all gels were run in duplicate to verify responses. Pre-exercise values of phosphorylation relative to total for each participant were normalized to one, with postexercise and 3 h postexercise values subsequently expressed as the fold-change relative to pre-exercise values.

Real-time RT-PCR. One-step quantitative RT-PCR was used to determine skeletal muscle mRNA levels of genes of interest. Primer sequences (Table 2) were designed by Sigma-Aldrich (Sigma-Aldrich Co. Ltd, Haverhill, UK), ideally with 40–60% GC content and spanning exon–exon boundaries. Primer specificity was determined by performing BLAST and melt curve analysis at the end of each PCR run. Total RNA was isolated from muscle biopsies (~30 mg) using a pestle and mortar and TRIzol® reagent (Life Technologies/Invitrogen, Paisley, UK), according to the manufacturer’s protocol. Sample RNA concentration (232 ± 73 ng µl⁻¹) and purity (260/280: 1.9 ± 0.1) was confirmed using spectrophotometry (NanoDrop) before being stored at −80°C for future use. Twenty-microlitre PCRs were made up as follows in a 96-well plate: 70 ng of RNA in 9.5 µl of nuclease-free water, 0.2 µl of Quantifast Reverse Transcriptase mix (Qiagen, Crawley, UK), 0.15 µl of both forward and reverse primers at 100 µM concentrations, and 10 µl of SYBR green mix (Qiagen, Crawley, UK). All reactions were performed in triplicate. Once PCR plates were prepared, they were transferred to the mx3005p qPCR cycler (Stratagene MX3005P; Agilent Technologies, Wokingham, UK), which was programmed to perform the following steps: 50°C for 10 min (reverse transcription), followed by a 5 min hold at 95°C, and then 40 cycles at 95°C for 10 s and 60°C for 30 s. Fluorescence was detected at the end of each cycle, and expression levels were determined using the 2⁻ΔΔCt method using RNA polymerase II (RP2) as the reference gene. Postexercise values are reported as a fold-change relative to pre-exercise values.

**Statistics**

In study 1, baseline outcome measures and training data were analysed using Student’s unpaired t tests. Subsequent analysis was performed with two-factor repeated-measures ANOVA, with one within factor (time: pre versus post) and one between factor (group: CON versus BFR). In study 2, protein phosphorylation and mRNA data were analysed using a two-way repeated-measures ANOVA. Where significant main
Table 2. Primers used in quantitative real-time PCR analysis

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer sequence</th>
<th>Genbank accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF forward</td>
<td>CTGCTCTACCTCCACCAT</td>
<td>NM_001171630</td>
</tr>
<tr>
<td>VEGF reverse</td>
<td>ATGAACTCACCCCTTCGTT</td>
<td>NM_000459</td>
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<tr>
<td>VEGFR-2 forward</td>
<td>ACGAGAATCTAGTCATAT</td>
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<tr>
<td>VEGFR-2 reverse</td>
<td>TTACTTCTGGTTCTTCACAG</td>
<td>NM_00013261</td>
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<tr>
<td>eNOS forward</td>
<td>AGCAGGAATCAGTCATCTA</td>
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<td>TTACTTCTGGTTCTTCACAG</td>
<td>NM_00013261</td>
</tr>
<tr>
<td>MMP-9 forward</td>
<td>GGCACCTCTATGGTCCTC</td>
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<td>MMP-9 reverse</td>
<td>AGTAGTGGCCGTAGAAGG</td>
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<td>Ang2 forward</td>
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<td>HIF-1α forward</td>
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<tr>
<td>RPII forward</td>
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<tr>
<td>RPII reverse</td>
<td>GGTGGAATCATATTGGAACAT</td>
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</tr>
</tbody>
</table>

Abbreviations: Ang2, angiopoietin-2; eNOS, endothelial nitric oxide synthase; HIF-1α, hypoxic inducible factor-1α; MMP-9, membrane metalloproteinase-9; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; RPII, RNA polymerase II; VEGF, vascular endothelial growth factor; and VEGFR-2, vascular endothelial growth factor receptor-2.

Effects were observed, Bonferroni-corrected post hoc t-tests were used to locate differences. Data are presented as means ± SD unless stated otherwise. Significance was accepted at $P < 0.05$.

Results

Study 1: training study

Participants completed 100% of the assigned training sessions without any complications. Prior to training, there was no difference in any variable between the two training groups (Table 1). The average power output developed over the eight training sessions was greater (Student’s unpaired t test, $P < 0.01$) in CON (642 ± 10 W) compared with BFR (618 ± 11 W). Consequently, the total work done throughout the training was greater (Student’s unpaired t test, $P < 0.01$) in CON (106 ± 22 kJ) compared with BFR (102 ± 21 kJ).

Physiological and performance variables measured before and after CON and BFR are presented in Table 1. Absolute and relative $\dot{V}_{O_2\text{max}}$ (Fig. 1) increased after BFR but were unchanged after CON (interaction; both $P = 0.01$). There was a trend for absolute and relative MAP (Fig. 1) to increase after BFR, with no changes after CON; however, these did not reach statistical significance (interaction; $P = 0.11$ and $P = 0.09$, respectively). Absolute and relative sprint PPO increased (main effect for time; both $P = 0.02$) but there was no difference between training groups. There were no changes in absolute or relative sprint MPO after CON and BFR. The 15 km TT performance time (Fig. 1) and average power output during the TT were unchanged after CON and BFR.

Study 2: acute study

The optimal cadence for PPO was 122 ± 13 r.p.m. There was no difference (Student’s unpaired t test, $P = 0.91$) in PPO achieved during BFR and CON (1147 ± 171 versus 1149 ± 179 W, respectively). Total work done was similar (Student’s unpaired t test, $P = 0.11$) between protocols (BFR, 67.1 ± 9.8 kJ versus CON, 68.3 ± 10.4 kJ).

p38MAPK activation. Phosphorylation of p38MAPK Thr180/Tyr182 increased (main effect for time; $P = 0.02$) 3.2- and 4.1-fold immediately following CON and BFR, respectively, before returning to baseline at 3 h postexercise (Fig. 2). The magnitude of phosphorylation immediately postexercise was not different between protocols (interaction; $P = 0.52$).

Angiogenic mRNA expression. Messenger RNA expression data are presented in Fig. 3. PGC-1α, VEGF and VEGFR-2 increased (main effects for time; all $P = 0.01$) at 3 h in both CON and BFR. There was, however, no difference in the magnitude of fold-change for any of these genes between protocols. HIF-1α mRNA expression increased (interaction; $P = 0.04$) at 3 h only after BFR. There was no change in eNOS (interaction; $P = 0.25$), MMP-9 (interaction; $P = 0.71$) or Ang2 mRNA expression (interaction; $P = 0.52$) in response to either protocol.

Discussion

This study has demonstrated the potency of combining blood-flow restriction with SIT in increasing $\dot{V}_{O_2\text{max}}$ in trained individuals. The mechanisms responsible for this
adaptive response are unclear; however, the present study presents preliminary evidence to suggest that enhanced skeletal muscle remodelling, and in particular capillary density, could occur with BFR compared with SIT alone through enhanced hypoxia-induced cell signalling, given the greater expression of the angiogenic growth factor HIF-1α.

In the present study, $\dot{V}O_{2\text{max}}$ increased by ~4.5% in response to SIT with BFR, compared with 0.7% in CON. The potency of combining BFR in enhancing oxygen uptake and the adaptive response to other forms of exercise has been demonstrated previously. For example, one-legged cycle training, whereby blood flow was restricted during exercise by ~20% through the use of a pressure chamber (Sundberg & Kaijser, 1992), has previously been demonstrated to promote a greater increase in one-legged $\dot{V}O_{2\text{peak}}$ over that performed with normal blood supply (Sundberg et al. 1993). Of course, the use of such pressure chambers is limited by accessibility and ease of use, whereas the use of blood-pressure cuffs placed on the proximal portion of the legs and a rapid inflation system seems effective in providing a fixed pressure for a set duration. The feasibility of combining BFR with whole-body exercise, specifically SIT, has also been demonstrated. This is of particular relevance to well-trained cyclists, for whom there is a requirement to maintain an appropriate load or training intensity as part of a structured training programme. In pilot work, combining SIT and BFR concurrently proved impractical, and even at moderate cuff pressures (100 mmHg) this combination was not tolerable. Either way, this type of training represents a novel way to enhance the adaptive responses in well-trained individuals.

There was no improvement in $\dot{V}O_{2\text{max}}$ with SIT alone in our group of trained individuals (CON). The effects of SIT on $\dot{V}O_{2\text{max}}$ in less-trained individuals is inconsistent, with some studies demonstrating increases in response to training (Burgomaster et al. 2006, 2008; Cocks et al. 2013) and others not (Burgomaster et al. 2005). Indeed, previous investigations into the effects of HIT (that involve longer-duration intervals at submaximal intensities rather than ‘all-out’ maximal sprints) have demonstrated positive adaptive responses in indices of aerobic performance (e.g. $\dot{V}O_{2\text{max}}$, lactate threshold) in well-trained cyclists with pretraining $\dot{V}O_{2\text{max}}$ values of ~65 ml min$^{-1}$ kg$^{-1}$ (Laursen & Jenkins, 2002; Laursen et al. 2005). Differences in the $\dot{V}O_{2\text{max}}$ response to training between the aforementioned studies could be attributable to a number of factors, such as differences in the duration, intensity and frequency of the interval training stimulus, as well as the total training volume prescribed.

![Figure 1](image_url)
The main determinants of $\dot{V}O_2\text{max}$ are complex. Although it is generally accepted that the vascular bed is not a limiting factor for oxygen transport during one-legged knee-extensor exercise (Andersen & Saltin, 1985), the performance of cycling exercise in which a larger muscle mass is recruited stresses both central and peripheral limitations in oxygen delivery and uptake (Poole & Richardson, 1997). As well as the important role that the capillary network has on increasing oxygen delivery and reducing diffusion gradients, it also facilitates greater removal of metabolic end-products, which sustains exercise tolerance (Joyner & Coyle, 2008). Moreover, an increased capillary density specific to type II muscle fibres will improve performance and recovery from maximal and ‘all-out’ exercise (Tesch & Wright, 1983), during which these fibres are preferentially recruited (Greenhaff et al. 1994). As previous work involving BFR has demonstrated this model to be an effective stimulus for enhancing capillary growth (Esbjörnsson et al. 1993), we sought to examine the potency of this type of exercise for enhancing capillary growth by measuring the acute activation and transcription of signalling proteins and genes involved in mediating exercise-induced angiogenesis. The expression of PGC-1α, VEGF and VEGFR-2 mRNA increased to a similar extent in both exercise conditions, suggesting that SIT has the potential in its own right for inducing capillary growth, as has been demonstrated previously in sedentary individuals (Cocks et al. 2013). Of note, there was a greater HIF-1α mRNA expression in the early recovery after BFR, but not CON. Hypoxia-inducible factor-1α is seen as a key regulator of the tissue to hypoxia and thus metabolic stress (Semenza, 2006; Semenza et al. 2006), during which it is stabilized and functions in regulating angiogenesis (Lee et al. 2004). Indeed, a reduction in oxygen tension results in an accumulation of HIF-1α (the oxygen-sensing subunit) protein and translocation into the nucleus for targeted activation of VEGF in human skeletal muscle (Ameln et al. 2005). Acute exercise results in an increase in skeletal muscle HIF-1α mRNA expression provided that the exercise intensity is sufficient and/or the tissue is exposed to a hypoxic stimulus (Vogt et al. 2001; Zoll et al. 2006). Thus, the greater HIF-1α mRNA

![Figure 2. Phosphorylation of p38MAPK Thr180/Tyr182 expressed relative to total p38MAPK immediately before (Pre), immediately after (Post) and 3 h after the CON and BFR protocols. Each subject's Pre values have been normalized to one (hence no error bars are shown for this time point) such that Post and 3 h values are subsequently expressed as the fold-change relative to Pre values. Values are means ± SEM (n = 8). *P < 0.05, compared with Pre (Bonferroni-corrected post hoc t test).](image-url)
expression after BFR is likely to reflect a greater reduction in the intracellular partial pressure of oxygen in the muscle during the restriction periods and an increased potential for activation of downstream HIF-1α-dependent pathways. Whilst this tentatively suggests that BFR provides an intensified stimulus for hypoxia-mediated angiogenesis, further work is clearly required to support this hypothesis. In particular, the inclusion of muscle protein analysis and immunohistochemical techniques to evaluate direct changes in capillary density is necessary.

Maximal oxygen uptake has a clear importance for exercise capacity, as does the performance $\dot{V}_O_2$ and power/velocity at lactate threshold, which is also directly influenced by muscle capillary density (Joyner & Coyle, 2008). These measurements, however, are not necessarily direct assessments of performance per se. We chose to assess exercise performance with a 15 km self-paced TT. Despite the improvements in $\dot{V}_O_2$ max with BFR, this did not translate to an improved TT performance, which did not improve with SIT alone either. Whilst this is perhaps surprising given that HIT has previously been demonstrated to improve TT performance in well-trained athletes (Laursen et al. 2005), the TT distance needs to be considered when evaluating performance improvements. For example, performance during self-paced exercise is predominantly limited by central or peripheral factors, with a greater degree of peripheral fatigue evident after shorter, high-intensity (~6 min) TTs and increased contribution of central fatigue after longer, lower-intensity

![Graphs showing messenger RNA expression of different genes](image)

**Figure 3.** Messenger RNA expression of PGC-1α (A), VEGF (B), VEGFR-2 (C), HIF-1α (D), MMP-9 (E), eNOS (F) and Ang2 (G) immediately before (Pre), immediately after (Post) and 3 h after the CON (open bars) and BFR protocols (filled bars). Gene expression is normalized to RPII mRNA expressed relative to Pre. For definitions of abbreviations, see footnote to Table 2. Values are means ± SEM ($n$ = 8). *P < 0.05, compared with Pre (Bonferroni-corrected post hoc t test).
TTs (>30 min; Thomas et al. 2015). It is also plausible that our 15 km TT may have lacked sensitivity to reflect an increased $\dot{V}_{O_2 \text{max}}$ at least partly. Nevertheless, we feel it is important that future studies continue to include TT tests of performance to provide better information regarding the application of training interventions to athletic populations. There were also slight trends towards significance in some of the performance outcome measures (MAP and MPO; Table 1), although the observed statistical powers for each outcome measure were rather moderate (MAP, $\sim 0.4$ and MPO, $\sim 0.3$). It is feasible that with greater participant numbers these measurements would reach statistical significance. Nevertheless, gains in the region of 2.9–4.4% in these outcome measures in the BFR group, compared with more marginal changes of 0.2–0.3% in the CON group, are more meaningful for athletic performance coaches and scientists rather than achieving a traditional statistical significance.

The present study is not without limitations. The lack of muscle biopsies before and after training, for the assessment of capillarization, limits the development of firmer conclusions that such a training stimulus manifests itself in such skeletal muscle remodelling. Whilst there are limitations in predicting chronic adaptations from acute gene-expression data, previous studies have demonstrated that the acute angiogenic response can help to inform the magnitude of capillary growth with training (Hellsten et al. 2008; Høier et al. 2010, 2012). Temporal factors relating to the timing and number of biopsies need to be considered when making inferences based on transient molecular responses to acute exercise interventions. Although the half-lives of some of the RNAs of respective genes of interest are very short, e.g. HIF-1α is $\sim 5$ min (Berra et al. 2001) and VEGF is $<1$ h, albeit more prolonged in hypoxia (Shima et al. 1995), the fact that it has been shown previously that mRNA expression for these genes peaks between 1 and 6 h after intense exercise (Høier et al. 2012, 2013a, 2013b; Bartlett et al. 2012) suggests that the timing of our postexercise biopsies was appropriate. Finally, the present study did not involve quantification of the percentage of blood-flow restriction induced by the cuff pressure of 130 mmHg. This chosen occlusion pressure, resulting from preliminary work in our laboratory, was the highest cuff pressure that could be tolerated in combination with SIT. Previous work in our laboratory has demonstrated that this cuff pressure around the mid-thigh restricts resting blood flow to the popliteal artery by $\sim 76\%$ (JEA Hunt, C Stodart, RA Ferguson, unpublished data).

In conclusion, we have demonstrated the potency of combining BFR with SIT in increasing $\dot{V}_{O_2 \text{max}}$ in trained athletes, although this did not translate to an enhanced exercise performance. Sprint interval training alone did not induce any observable adaptation. Although the potential mechanisms are not fully understood, we present evidence of an enhanced HIF-1α-mediated cell signalling compared with SIT alone.

References


**Additional information**

**Competing interests**

None declared.

**Author contributions**

All authors contributed to the conception and design of the experiment, interpretation of data and writing of the manuscript. C.T. and R.F. contributed to the collection and analysis of data. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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