

# The effects of muscle blood flow restriction during running training on measures of aerobic capacity and run time to exhaustion

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## Abstract

**Purpose** Training with blood flow restriction (BFR) is known to enhance muscle mass and strength during resistance training activities. However, little is known about the BFR effects during aerobic training. This investigation examines the effects of running training performed with or without BFR on physiology and performance.

**Method** Sixteen subjects (age  $24.9 \pm 6.9$  years, height  $172.9 \pm 7.8$  cm, weight  $75.1 \pm 13.8$  kg) were assigned to a BFR or control (CON) group for eight sessions of training. Before and after training, subjects completed an incremental test to determine peak running velocity (PRV) maximal oxygen uptake ( $\dot{V}O_{2\max}$ ) and running economy (RE), followed by a time to exhaustion run (TTE) performed at PRV. Training for both groups consisted of progressively increasing volumes of 30-s repetitions completed at 80% of PRV.

**Results** The BFR and CON groups reported gains ( $6.3 \pm 3.5$  vs  $4.0 \pm 3.3\%$ ) in  $\dot{V}O_{2\max}$  following training with only trivial ( $ES = 0.18$ ) differences between groups. Similarly, PRV and incremental test time increased in both training groups with a small ( $ES \sim 0.3$ ) additional enhancement in favour of the BFR group. Running economy improved in the BFR group but not in CON ( $ES = 0.4$ ). TTE also increased in

both BFR ( $27 \pm 9\%$ ) and CON groups ( $17 \pm 6\%$ ) with a small ( $ES = 0.31$ ) additional benefit in favour of the BFR group.

**Conclusions** Using BFR during training appears to confer small but potentially worthwhile improvements in RE, PRV and TTE measures. The improvements following BFR training are likely due to muscular rather than cardiovascular function.

**Keywords** Blood flow restriction · Vascular occlusion · Aerobic exercise · Running training · KAATSU

## Abbreviations

|                    |                        |
|--------------------|------------------------|
| ANOVA              | Analysis of variance   |
| BFR                | Blood flow restriction |
| CI                 | Confidence interval    |
| CON                | Control                |
| EMG                | Electromyography       |
| ES                 | Effect size            |
| HR                 | Heart rate             |
| PRV                | Peak running velocity  |
| RE                 | Running economy        |
| TTE                | Time to exhaustion     |
| $\dot{V}O_{2\max}$ | Maximum oxygen uptake  |

## Introduction

Performing exercise sessions with accompanying blood flow restriction (BFR) is an increasingly popular method of applying greater physiological stress whilst training at low exercise intensities. Blood flow restriction training involves reducing arterial blood flow to muscles, whilst occluding venous return (Pope et al. 2013). As a consequence of training with BFR, intramuscular oxygen delivery may be reduced and a decreased rate of metabolite clearance

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observed. It is proposed that performing exercise with BFR, therefore, creates a more stressful muscular environment and that this may provide a greater stimulus for physical adaptation (Suga et al. 2009). Some observed effects of exercise with BFR from previous studies include increases in exercise heart rate, enhanced levels of muscle recruitment, increases in systemic hormones and elevated adrenal hormone concentrations (for review of mechanisms, see Loenneke and Pujol 2009).

The majority of previous investigations into the effects of BFR have focused on resistance training activities with untrained or moderately trained subjects. Furthermore, the main focus of BFR research has been on improving muscular hypertrophy and strength using training intensities as low as 20–30% of subjects' one repetition maximum. An early study by Abe et al. (2005) reported significantly greater gains in lower body muscle mass and strength following low-intensity strength training utilising BFR compared to a non-BFR training group. Similarly, Yasuda et al. (2011) reported significant increases in upper body muscle mass following 6 weeks of low-load bench press training in a blood flow restricted condition. One of the few studies utilising competitive athletes (Cook et al. 2014) utilised a high-load strength training regime with semi-professional rugby players, and reported significantly larger gains in strength and sprinting speed following BFR training than when subjects performed identical training without BFR.

While the effects of BFR on strength and hypertrophy are well documented (Loenneke et al. 2012), there are fewer studies examining the effects of BFR on measures associated with aerobic activities, and in those that do exist the results are often unclear or conflicting. A study by Park et al. (2010) examined the effects of BFR after 2 weeks of daily walking sessions in either an occluded or non-occluded state with collegiate basketball players. The results indicated significant increases in maximum oxygen consumption ( $\dot{V}O_{2\max}$ ) in the BFR group only following training. In a similar investigation, Abe et al. (2010) examined the effects of 8 weeks of occluded cycling exercise on  $\dot{V}O_{2\max}$  and anaerobic capacity. The BFR intervention involved 15 min of low intensity cycling (40%  $\dot{V}O_{2\max}$ ), while the non-BFR cohort completed 45 min at the same intensity. The findings showed a ~6% increase in maximal oxygen uptake and a ~15% increase in time to exhaustion in the BFR group, whilst little change occurred in the control group, despite their considerably greater training volume. Similarly, a recent comprehensive study comparing several different occluded and non-occluded training regimes, (de Oliveira et al. 2016) reported that low-intensity training with occlusion led to significantly larger gains in aerobic capacity than the same training without BFR and indeed the increases observed were similar to those caused by much higher intensity interval training. However, in contrast to other studies, Keramidis et al.

(2012) reported no significant improvements in  $\dot{V}O_{2\max}$  following 6 weeks of high-intensity training (~90%  $\dot{V}O_{2\max}$ ) between a BFR and non-BFR training group, despite both groups experiencing significant increases in peak power and cycle time to exhaustion at 150% peak power.

The mixed findings in the BFR literature may in part be attributed to the diverse range of training methods and exercise intensities used and also the different occlusion procedures utilised. Previous studies have used various methods to induce BFR including blood pressure cuffs (Laurentino et al. 2008), pneumatic tourniquet (Wernbom et al. 2006, 2009) and even specialist KAATSU devices (Abe et al. 2005) which may possibly induce differing levels of occlusion. Furthermore, all these occlusion methods commonly require specialist equipment or are otherwise impractical for regular training particular during certain sporting activities (e.g. running). In an attempt to overcome some of the complications associated with implementing BFR during training, several studies have utilised a more practical occlusion method using simple elasticated muscle wraps. In a series of studies, Loenneke et al. (2011) found that  $\dot{V}O_{2\max}$  and energy expenditure were significantly greater when performing walking exercise with BFR using elastic straps than without. Similarly, the same authors report that training with practical BFR increases muscle activation and growth without increasing muscle damage (Wilson et al. 2013).

Given the diverse range of findings in previous studies and also the lack of studies examining the effects of BFR on aerobic characteristics, the aim of this current investigation was to compare the effects of 4-week of running training, with or without a practical BFR methodology, on aerobic capacity and other measures associated with running performance.

## Methods

### Subjects

Sixteen subjects (10 males and 6 females) volunteered to participate in the study, which was approved by the institutions research ethics committee. Subjects were informed of all potential risks involved in the study and gave their written informed consent. All subjects were classified as healthy and regularly participated in a range of recreational sporting and physical activities for approximately 2–4 h per week. As a condition of participation, all subjects were required to be familiar with treadmill running and agree to not begin any other new exercise regime during the study period. The subjects mean ( $\pm$  SD) age, height and weight were  $25 \pm 7$  years,  $173 \pm 8$  cm and  $75 \pm 14$  kg, respectively. Prior to testing subjects completed a medical screening questionnaire to

ensure they were free from significant risk factors and all were deemed suitable for participation. During the study period, the subjects were requested to maintain their normal diet and regular physical activity regime.

### Experimental design

The study was a matched-pair parallel group design. Each participant initially reported to the laboratory to complete a series of baseline tests and become familiar with the training intervention to be used in the study. Following baseline testing, participants were pair matched (based on their sex and test performance), and randomly allocated into either a control (CON,  $n=8$ ) or blood flow restriction group (BFR,  $n=8$ ). Both groups then completed 4 weeks (eight sessions) of supervised treadmill running training before repeating the test protocols. Prior to each testing and training session, subjects were instructed to avoid any strenuous physical activity for 24 h. Subjects were also instructed to refrain from eating and to avoid caffeine-containing beverages or supplements 2 h before a session commenced. Throughout all sessions, cooling was provided via a 30 cm pedestal fan, and the ambient temperature of the laboratory was controlled at  $\sim 20^\circ\text{C}$  with a relative humidity of  $\sim 50$ – $60\%$ . All experimental group training sessions were performed under the direct supervision of the same investigator (SMA).

### Exercise testing

Prior to and following the training intervention subjects completed a series of tests on a motorised treadmill (Technogym, Germany) with a gradient fixed at 1% in order to better simulate on-road conditions (Jones and Doust 1996). Prior to testing subjects completed a standardised warm-up consisting of 5 min of treadmill running commencing at  $5\text{-km h}^{-1}$  and increasing by  $1\text{-km h}^{-1}$  each minute. Following the run, subjects completed a series of dynamic stretches for two sets of 15 repetitions; leg swings (front to back and across the body), lunge, body weight squat, walking knee to chest and calf raises. On completion of the warm-up subjects completed a maximal incremental test commencing at  $5\text{-km h}^{-1}$  and increasing by  $1\text{-km h}^{-1}$  each minute until subjects reached volitional exhaustion. Peak running velocity (PRV) was determined as the highest running speed completed for a full 1-min duration plus the appropriate proportion of any partially completed stage. During the incremental test, respiratory gases were continuously measured with a metabolic cart (Metalyser 3B, Cortex, Leipzig, Germany) calibrated in accordance with the manufacturer's instruction using Alpha gas standards. The maximum oxygen uptake ( $\dot{V}\text{O}_{2\text{max}}$ ) was determined as the highest 30 s oxygen uptake value recorded during the incremental test. In addition, we determined a measure of running economy (RE) using the

Oxygen response recorded breath by breath during the incremental test. A linear portion of the Oxygen response slope (that occurred below a respiratory exchange ratio of 1.0) was identified for each individual and the angle of the slope calculated by dividing the change in oxygen consumption by the change in running speed over the identified portion. The change in speed used was a minimum of  $3\text{ km h}^{-1}$  and typically occurred between 7 and  $12\text{ km h}^{-1}$  dependent on each individual's running ability.

Following the incremental test, subjects completed 15 min of passive recovery before commencing a high-intensity time to exhaustion (TTE) run. For the TTE, subjects were required to run for as long as possible at their previously determined incremental test PRV. Both pre- and post-training, TTE runs were performed at the same PRV achieved during the pre-testing session. During the TTE test, subjects were blinded to any external feedback (run time, heart rate etc.) that may have influenced the run duration. Time in the TTE was recorded to the nearest second using a stopwatch. One participant in the BFR group was unable to complete the post-testing TTE test due to ankle pain and, therefore, the data were omitted from the final TTE analysis.

### Training intervention

On completion of pre-testing, subjects were pair-matched, based on their sex and  $\dot{V}\text{O}_{2\text{max}}$ , before being randomly assigned into either the BFR or CON group. The subjects in both groups then completed 4 weeks of identical training (with the exception that the BFR group trained using a practical occlusion procedure) on the same treadmill used during experimental testing. Training was carried out twice weekly, at the same time of day for each subject and with at least 2 days of rest in between sessions. Prior to commencing each training session, subjects completed the same warm-up procedure as described for the incremental test. Throughout all training, the treadmill speed was maintained at 80% of each individual PRV. The training sessions required subjects to complete repeated bouts of 30-s efforts interspersed with 30-s of passive rest (during which they straddled the treadmill belt). The initial training session consisted of 2 sets of 5 repetitions (total 10 repetitions), thereafter the total volume of training was increased progressively by 2 min (equivalent to 2 repetitions) per session until the final session of 3 sets of 8 repetitions (total 24 repetitions). Between each exercise set, subjects in both training groups rested for 150 s, primarily to allow a brief ( $\sim 60\text{ s}$ ) break from wearing the wraps in the BFR cohort, and to allow adequate time for re-wrapping. Participants also wore a recording heart rate monitor (Polar A300, Finland), during each training session which was subsequently downloaded and analysed using Polarflow software (Polar electro Oy, Kempele, Finland). The mean heart rate achieved across all completed exercise sets (not

including the in between sets rest periods) was used in the final data analysis.

## Occlusion method

During the training sessions, the BFR group only undertook a practical occlusion method based on the protocol described by Wilson et al. (2013). Prior to the first training session, subjects in the BFR group were familiarised with the wrap technique and pressure scale as described by Wilson et al. (2013). Elastic wraps (Get Strength Heavy Duty 75 mm, Waiuku, New Zealand) were placed proximally on the subjects' thighs immediately inferior to the inguinal fold and wrap pressure was increased by the experimenter until subjects reported a perceived pressure of 7 out of 10 (moderate but pain-free pressure). For consistency, the same researcher wrapped each subject, and supervised all training sessions during the study.

## Data analysis

Simple group statistics are shown as means  $\pm$  between-subject standard deviations unless otherwise stated. The mean effects of training and their 95% confidence limits were estimated using a magnitude based approach via made for purpose spreadsheets (Hopkins 2006). The spreadsheets utilise the unequal-variances *t* statistic to compare change scores for measured variable both within and between each groups from pre- to post-tests. The spreadsheets also provide the effect size (ES) statistic for observed changes within and between groups. As we do not know how a change in our measured variables will affect actual running performance, we chose 0.2 standard deviation units (change in mean divided by the between subject SD) as the smallest worthwhile change. The effect size was further interpreted

using the established thresholds of 0.2, 0.5, and 0.8 for small, moderate, and large effects, respectively, in accordance with the recommendations of Cohen (1988). Effect size values  $<0.2$  were deemed trivial differences. Uncertainties of the observed mean effects were expressed with  $\pm 95\%$  confidence intervals. In addition, analyses for between-group changes pre- to post-testing were performed by a repeated-measures analysis of variance (ANOVA) with factors for time and group. Differences in mean training heart rate between groups were assessed using a one-way ANOVA. All significance-based analyses were performed using SPSS statistical software, version 20 for Windows (SPSS Inc., Chicago, IL, USA) with alpha set a priori at 0.05.

## Results

The pre- and post-values for all measured variables along with the absolute magnitude of change that occurred within the BFR and CON groups after training are shown in Table 1. The BFR group reported small (ES 0.2–0.5) increases in  $\dot{V}O_{2\max}$ , VE, RE, PRV and incremental run time, and a large (ES = 0.85) increase in TTE from pre- to post-tests. In comparison, the CON group reported small and moderate increases in  $\dot{V}O_{2\max}$  and TTE (ES = 0.37 and 0.62, respectively) only, over the training period. Overall the ANOVA analysis reported that there were no significant ( $p < 0.05$ ) between-group differences due to training type (BFR or CON) on any measured variable. Despite reporting no significant differences in the change scores between groups, the effect size analysis indicated small (ES 0.24–0.40) additional enhancements in all measured variables, except  $\dot{V}O_{2\max}$ , in favour of the BFR group.

During the experimental training sessions, all subjects exercised at 80% of their PRV achieved in the pre-training

**Table 1** The mean ( $\pm$ SD) values for all measured variables (pre and post training) for the BFR and CON groups, and the observed changes (mean  $\pm$  95% CI) within groups

|                                                             | BFR group            |                       |                              | CON group            |                       |                              | BFR–CON group difference |                |                    |
|-------------------------------------------------------------|----------------------|-----------------------|------------------------------|----------------------|-----------------------|------------------------------|--------------------------|----------------|--------------------|
|                                                             | Pre mean ( $\pm$ SD) | Post mean ( $\pm$ SD) | Group change ( $\pm$ 95% CI) | Pre mean ( $\pm$ SD) | Post mean ( $\pm$ SD) | Group change ( $\pm$ 95% CI) | Mean ( $\pm$ 95% CI)     | <i>p</i> value | ES ( $\pm$ 95% CI) |
| <b>Physiological variables</b>                              |                      |                       |                              |                      |                       |                              |                          |                |                    |
| $\dot{V}O_{2\max}$ (ml kg <sup>-1</sup> min <sup>-1</sup> ) | 46.1 (7.0)           | 49.0 (7.1)            | 2.9 (2.0)                    | 46.6 (4.7)           | 48.4 (3.6)            | 1.8 (1.8)                    | 1.1 (2.4)                | 0.33           | 0.18 (0.43)        |
| VE (L min <sup>-1</sup> )                                   | 133 (32)             | 142 (33)              | 8.7 (10.1)                   | 123 (31)             | 124 (34)              | 1.6 (10.0)                   | 7.1 (16.2)               | 0.36           | 0.24 (0.48)        |
| RE (ml kg <sup>-1</sup> kmh <sup>-1</sup> )                 | 4.5 (1.0)            | 4.2 (1.1)             | − 0.3 (0.5)                  | 4.7 (1.3)            | 4.8 (1.1)             | 0.1 (0.4)                    | − 0.4 (0.6)              | 0.60           | 0.40 (0.52)        |
| <b>Functional characteristics</b>                           |                      |                       |                              |                      |                       |                              |                          |                |                    |
| TTE (s)                                                     | 151 (40)             | 190 (40)              | 39 (17)                      | 167 (39)             | 195 (42)              | 28 (11)                      | 11 (19)                  | 0.22           | 0.31 (0.50)        |
| PRV (km h <sup>-1</sup> )                                   | 13.8 (1.2)           | 14.3 (1.3)            | 0.6 (0.4)                    | 14.1 (1.0)           | 14.3 (1.3)            | 0.2 (0.6)                    | 0.4 (0.7)                | 0.25           | 0.34 (0.62)        |
| Increment time (s)                                          | 535 (63)             | 568 (62)              | 33 (26)                      | 559 (51)             | 570 (65)              | 11 (36)                      | 22 (41)                  | 0.26           | 0.40 (0.68)        |

The between-group differences (BFR–CON) are reported as means ( $\pm$  95% CI) along with the observed *p* value and corresponding effect size for the difference between groups

incremental test. The 80% PRV intensity equated to 82.6 and 83.1% of the BFR and CON groups  $\dot{V}O_{2\max}$ , respectively. Despite training at a similar relative intensity, analysis of the mean training heart rate data across all training sessions indicated that the BFR group trained at a significantly ( $p=0.04$ ) higher percentage of their maximum heart rate than the CON group ( $75 \pm 6$  vs  $69 \pm 5\%$ , respectively) equivalent to a large observed effect size ( $ES=1.05$ ).

## Discussion

The primary aim of this current research was to assess the effectiveness of a 4-week running training programme that includes the use of a practical blood occlusion (BFR) protocol. The key finding was that the use of BFR appears to increase exercise heart rate and lead to small additional enhancements in several physiological and functional measures in comparison to performing the same training without BFR.

Our findings indicate that training with BFR appears to induce an additional stress on the cardiovascular system as evidenced by the significantly higher training heart rates (reported as a % of maximum HR) in the BFR group compared to the CON group. An increase in exercise heart when training with BFR is consistent with previous BFR findings (Loenneke et al. 2011) and is proposed to be due to a reduction in venous return which limits ventricular stroke volume and, therefore, the heart rate increases to compensate. Alongside the increased exercise heart rate, our findings indicate similar ( $ES \sim 0.4$ ) increases in  $\dot{V}O_{2\max}$  in both the BFR and CON training groups after the training period. There was a trend towards a greater increase in  $\dot{V}O_{2\max}$  in the BFR group (6.4%) than the CON group (4.0%) though the difference between groups was not significant ( $p=0.33$ ) and estimated as a trivial ( $ES=0.18$ ) effect. The increase in  $\dot{V}O_{2\max}$  seen in the BFR group is identical to that previously reported by Abe et al. (2010) and similar to the 5.6% increase reported by de Olivera (2016) after repeated sessions of BFR low-intensity cycle training. In contrast to our study, these previous studies reported no increase in  $\dot{V}O_{2\max}$  in their control groups. A probable explanation for the difference between our control subjects and those in the Abe and de Olivera studies is that both our study groups trained at a substantially higher exercise ( $\sim 80$  vs  $\sim 40\%$   $\dot{V}O_{2\max}$ ) intensity than they used. It is, therefore, perhaps unsurprising that our CON group also increased their  $\dot{V}O_{2\max}$  given the intensity of exercise they were required to perform during training. Overall then it appears that performing high-intensity training with the addition of BFR provides little additional benefit in  $\dot{V}O_{2\max}$  (despite causing increases in heart rate) when exercise intensity is already high enough to substantially tax the cardio-vascular system.

While  $\dot{V}O_{2\max}$  increased in both our subject groups, peak running velocity and the accompanying incremental test time increased by a larger amount in the BFR group compared to CON. A possible explanation for the greater increases in PRV and incremental run time in the BFR group may be related to the gains in running economy observed in the BFR group. Our results indicate that the submaximal oxygen cost (slope of the  $O_2$  running speed relationship) reduced by  $\sim 7\%$  in the BFR group, while the CON group remained relatively unchanged. The improvement in running economy would potentially allow an athlete to achieve a higher maximal running speed for the same relative oxygen consumption. It is likely that the improvement in RE witnessed in the BFR group occurred as a result of local muscular adaptations rather than cardio-vascular changes; specifically, we hypothesise that an increase in muscle strength led to the improved running economy. Previous studies by Abe et al. (2005, 2006) and more recently de Oliveira et al. (2016) have reported significant increases in muscle cross-sectional area, and strength following several weeks of BFR training. Similarly, studies utilising other non-occluded strength training techniques have consistently reported enhancements in aerobic economy (Paton and Hopkins 2004) and concurrent improvements in endurance performance following strength training. Therefore, it is plausible that the economy improvements we found are a direct result of enhanced muscle strength which may potentially be mediated by an increase in systemic hormone concentrations (Beaven et al. 2008; Cook et al. 2014). Further evidence for an enhanced muscle strength following BFR training comes from Yusada et al. (2009) who has previously reported that the integrated muscle electromyography (EMG) signal (an indication of muscle recruitment strength) is substantially greater when subjects perform training at the same intensity with occlusion compared to without. Unfortunately, we possessed no objective way to measure changes in muscle mass or EMG activity, and did not perform any specific isolated strength assessment and cannot, therefore, verify this hypothesis directly.

In addition to the improvement in PRV and running economy in the BFR trained group, we also observed substantial increases in high-intensity run TTE. Once again while both experimental groups substantially increased their post-training TTE, the BFR trained group experienced a small ( $ES=0.31$ ) additional enhancement of  $\sim 8\%$  over the CON group in our study. As the intensity of the TTE test was essentially greater than  $\dot{V}O_{2\max}$ , a large portion of the test would rely on anaerobic capabilities and the ability to withstand local muscle fatigue. It is possible that the increases in strength (particularly in type 1 muscle fibres) previously proposed also lead to the increase in TTE by delaying type 2 muscle fibre recruitment and, thereby, the production of fatiguing metabolites such as lactate. Indeed this hypothesis is further supported by de

Oliviera et al. (2016) who reported that training with BFR leads to a significant decrease in the rate of lactate appearance during incremental exercise. Alternatively, it is also possible that an increased ability to withstand local metabolic disturbances caused by increased levels of muscle and blood lactate experienced during the BFR training led to the TTE increase. As training with BFR reduces local muscle  $O_2$  availability, this may have led to an earlier recruitment of type 2 muscle fibres during training and a corresponding increase in lactate production (Takano et al. 2005). Therefore, it is possible that the BFR group experienced increases in muscle buffering capacity due to their longer exposure to higher lactate levels during the BFR training.

While it appears that training with BFR offers several potential performance benefits, we acknowledge that there are several limitations of the current study. Firstly, the sample size is small and variations in the individual response to training may limit the clarity of observed changes; this is particularly the case when traditional hypotheses-based significance analysis is used. Hence, our reason to include a magnitude-based assessment (along with confidence levels for the observed changes) as this is less reliant on sample size to provide meaningful results. Secondly while previous studies have validated the practical BFR protocol utilised, we had no objective measure of the occlusion level achieved in our study. It is possible, therefore, that we did not achieve consistency of occlusion in each individual over every training session. However, we made attempt to limit potential variations in occlusion levels by ensuring that all wrapping procedures were clearly explained and performed by the same experienced researcher during the study. Finally, we did not blind the subjects to the occlusion protocol, so it is possible that some placebo effect occurred in the BFR group. However, given both groups utilised the same general training procedure and experienced substantial gains in most measured variables we are confident that the placebo effect played little part in the final study outcomes.

In conclusion, the present study builds upon previous training research using BFR and, to our knowledge, is the first to utilise a running protocol in combination with a high relative training intensity. Our findings indicate that training with BFR during high intensity sessions may provide additional beneficial adaptations in physiological characteristics which in turn may lead to practically worthwhile gains in measures related to running performance. These gains observed after BFR training appear to be predominately due to peripheral adaptations mostly occurring at the muscular level, possibly via an increase in muscle strength or an enhanced ability to resist fatigue inducing metabolites. Future studies with larger sample sizes are required to verify the utility of BFR during high-intensity exercise and elucidate the potential performance adaptation mechanisms.

## Compliance with ethical standards

**Conflict of interest** The authors declare they have no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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