



This is a peer-reviewed, post-print (final draft post-refereeing) version of the following published document, This is an Accepted Manuscript of an article published by Taylor & Francis in Journal of Sports Sciences on 17 December 2018, available online:  
<https://www.tandfonline.com/doi/abs/10.1080/02640414.2018.1554177?journalCode=rjsp20>  
and is licensed under All Rights Reserved license:

**Fryer, Simon M ORCID logoORCID: <https://orcid.org/0000-0003-0376-0104>, Stone, Keeron J ORCID logoORCID: <https://orcid.org/0000-0001-6572-7874>, Dickson, Tabitha, Wilhelmsen, Andrew, Cowen, Daniel, Faulkner, James, Lambrick, Danielle M and Stoner, Lee (2019) The effects of 4 weeks normobaric hypoxia training on microvascular responses in the forearm flexor. Journal of Sports Sciences, 37 (11). pp. 1235-1241. doi:10.1080/02640414.2018.1554177**

Official URL: <https://www.tandfonline.com/doi/abs/10.1080/02640414.2018.1554177?journalCode=rjsp20>  
DOI: <http://dx.doi.org/10.1080/02640414.2018.1554177>  
EPrint URI: <https://eprints.glos.ac.uk/id/eprint/6239>

#### **Disclaimer**

The University of Gloucestershire has obtained warranties from all depositors as to their title in the material deposited and as to their right to deposit such material.

The University of Gloucestershire makes no representation or warranties of commercial utility, title, or fitness for a particular purpose or any other warranty, express or implied in respect of any material deposited.

The University of Gloucestershire makes no representation that the use of the materials will not infringe any patent, copyright, trademark or other property or proprietary rights.

The University of Gloucestershire accepts no liability for any infringement of intellectual property rights in any material deposited but will remove such material from public view pending investigation in the event of an allegation of any such infringement.

PLEASE SCROLL DOWN FOR TEXT.

# **The effects of 4 weeks normobaric hypoxia training on microvascular responses in the forearm flexor.**

**Running head:** Forearm responses to hypoxic training

**Type:** Original Investigation

**Keywords:** Hypoxic training, hypoxemia, perfusion, near-infrared spectroscopy

**Authors:** <sup>1</sup>Fryer, S., <sup>1</sup>Stone, K., <sup>1</sup>Dickson, T., <sup>2</sup>Wilhelmsen, A., <sup>1</sup> Cowen, D., <sup>3</sup> Faulkner, J., <sup>4</sup>Lambrick, D. <sup>5</sup> & Stoner, L.

## **Corresponding author details**

<sup>1</sup> Dr Simon Fryer  
University of Gloucestershire  
School of Sport and Exercise,  
Faculty of Applied Sciences,  
Oxstalls Campus,  
Gloucester,  
GL2 9HW.  
[dr.s.fryer@gmail.com](mailto:dr.s.fryer@gmail.com)  
Tel: +44 (0) 1242 715221

## **Additional Contributing Authors**

<sup>1</sup> Mr Keeron Stone  
University of Gloucestershire  
School of Sport and Exercise,  
Faculty of Applied Sciences,  
Oxstalls Campus,  
Gloucester,  
GL2 9HW.  
[Kstone1@glos.ac.uk](mailto:Kstone1@glos.ac.uk)  
Tel: +44 (0) 1242 715192

<sup>1</sup> Dr Tabitha Dickson  
University of Gloucestershire  
School of Sport and Exercise,  
Faculty of Applied Sciences,  
Oxstalls Campus,  
Gloucester,  
GL2 9HW.

[tdickson@glos.ac.uk](mailto:tdickson@glos.ac.uk)

Tel: +44 (0) 1242 714625

<sup>2</sup>Mr Andrew Wilhelmsen  
University of Nottingham,  
Faculty of Medicine and Health Sciences,  
School of Life Sciences,  
Metabolic and Molecular Physiology Research Group,  
Email: [andrewwilhelmsen@gmail.com](mailto:andrewwilhelmsen@gmail.com)  
Tel: + 44 (0) 7848 899240

<sup>1</sup> Mr Daniel Cowen  
University of Gloucestershire  
School of Sport and Exercise,  
Faculty of Applied Sciences,  
Oxstalls Campus,  
Gloucester,  
GL2 9HW.  
[dcowen@glos.ac.uk](mailto:dcowen@glos.ac.uk)  
Tel: +44 (0) 1242 715214

<sup>3</sup>Dr James.Faulkner  
University of Winchester  
Faculty of Business, Law and Sport  
Sparkford Road,  
Winchester,  
SO22 4NR  
Email [James.Faulkner@winchester.ac.uk](mailto:James.Faulkner@winchester.ac.uk)  
Tel: +44 (0)1962 624932

<sup>4</sup>Dr Danielle Lambrick  
University of Southampton  
Faculty of Health Sciences  
Highfield  
Southampton  
SO17 1BJ  
**Tel:** (023) 8059 5916  
Email: [D.M.Lambrick@soton.ac.uk](mailto:D.M.Lambrick@soton.ac.uk)

<sup>5</sup>Dr Lee Stoner  
University of North Carolina  
Department of Sport and Exercise,  
306 Woollen,  
Chapel Hill,  
NC 27599  
USA  
Email: [dr.l.stoner@gmail.com](mailto:dr.l.stoner@gmail.com)  
Tel: +1.919.962.0534

## ABSTRACT

Intermittent exposure to hypoxia can lead to improved endurance performance. Currently, it is unclear whether peripheral adaptations play a role in improving oxygen delivery and utilization following both training and detraining. This study aimed to characterize skeletal muscle blood flow (mBF), oxygen consumption ( $\dot{m}\text{VO}_2$ ), and perfusion adaptations to i) 4-weeks handgrip training in hypoxic and normoxic conditions, and ii) following 4-weeks detraining. Using a randomised crossover design, 9 males completed 30-min handgrip training four times a week in hypoxic (14%  $\text{FiO}_2$  ~3250m altitude) and normoxic conditions. mBF,  $\dot{m}\text{VO}_2$  and perfusion were assessed pre, post 4-weeks training, and following 4-weeks detraining. Hierarchical linear modelling found that  $\dot{m}\text{VO}_2$  increased at a significantly faster rate (58%) with hypoxic training ( $0.09 \text{ mlO}_2 \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$  per week); perfusion increased at a significantly (69%) faster rate with hypoxic training ( $3.72 \mu\text{M}$  per week). mBF did not significantly change for the normoxic condition, but there was a significant increase of  $0.38 \text{ ml} \cdot \text{min}^{-1} \cdot 100\text{ml}^{-1}$  per week (95% CI: 0.35, 0.40) for the hypoxic condition. During 4-weeks detraining,  $\dot{m}\text{VO}_2$  and perfusion significantly declined at similar rates for both conditions, whereas mBF decreased significantly faster following hypoxic training. Four weeks hypoxic training increases the delivery and utilisation of oxygen in the periphery.

**Key words:** haemodynamic, microvascular adaptation, near infrared spectroscopy, handgrip exercise

## INTRODUCTION

Acute exposure to hypoxia leads to a diminished endurance performance in part because of an impaired aerobic metabolism (Casey & Joyner, 2011; Hamlin, Marshall, Hellemans, Ainslie, & Anglem, 2010). The impairment in the aerobic metabolism results from a decreased partial

pressure of oxygen ( $O_2$ ), and the subsequent reduction in  $O_2$  delivery to active skeletal muscle (Marshall et al., 2008; Noakes, Peltonen, & Rusko, 2001). However, both chronic and intermittent exposure to hypoxia has been shown to moderate this impairment and enhance both aerobic and anaerobic performance (Gore, Clark, & Saunders, 2007; Hamlin et al., 2010; Meeuwsen, Hendriksen, & Holewijn, 2001). Improvements in endurance performance have been attributed to a variety of central and peripheral adaptations such as an increased: pulmonary  $\dot{V}O_2$  (DeLorey, Shaw, Shoemaker, Kowalchuk, & Paterson, 2004), compensatory dilation (Roach, Koskolou, Calbet, & Saltin, 1999), capillary oxygen saturation (Hamlin et al., 2010) and erythropoietin-induced elevation in haematocrit, resulting in improved  $O_2$  carrying capacity (Bailey & Davies, 1997; Neubauer, 2001). Whilst there is evidence that both central and peripheral adaptations play a role in improving performance (Katayama et al., 2001; Noakes et al., 2001; Terrados, Jansson, Sylven, & Kaijser, 1990), many of the peripheral mechanisms are less well understood. Currently, it is not clear whether peripheral adaptations play a role in improving  $O_2$  delivery and utilization in the muscle, and if they do, how long the effects last when training ceases (Katayama, Matsuo, Ishida, Mori, & Miyamura, 2003). More specifically, skeletal muscle blood flow (mBF), muscle oxygen consumption ( $m\dot{V}O_2$ ), and muscle perfusion responses to prolonged intermittent training and de-training in hypoxic conditions have not yet been characterized.

Although peripheral responses are not as well understood (Marshall et al., 2008), extensive research has investigated macro-vascular responses to both intermittent hypoxic exposure and training (DeLorey et al., 2004; Hughson & Kowalchuk, 1995; MacDonald, Tarnopolsky, & Hughson, 2000; Rowell, Saltin, Kiens, & Christensen, 1986). For example, in response to submaximal exercise (leg extension) in hypoxia, limb blood flow has been suggested to increase in order to compensate for the reduction in  $O_2$  availability (Rowell et al., 1986).

However, divergent research has suggested that there is either no change in limb blood flow following hypoxic training, (MacDonald et al., 2000) or that the response is slowed (Hughson & Kowalchuk, 1995; Springer, Barstow, Wasserman, & Cooper, 1991). Authors of these conflicting findings attributed their responses to potential adaptations in mBF and  $\dot{m}\text{VO}_2$ . However, these responses were not quantified; and the peripheral responses seen directly in the muscle is not well documented.

Therefore, the aim of this investigation was to determine the forearm flexor responses to 4 weeks training in normoxic and hypoxic (14% fraction of inspired oxygen ( $\text{FiO}_2$ ) ~3250m altitude) conditions, followed by 4 weeks de-training. Specifically, we hypothesised that compared to normoxic training, training in hypoxia would be associated with: 1) a greater increase in mBF,  $\dot{m}\text{VO}_2$  and muscle perfusion in the flexor digitorum profundus (FDP) and 2) a slowed decline in mBF,  $\dot{m}\text{VO}_2$  and perfusion following 4-weeks of detraining.

## **MATERIALS AND METHODS**

### **Participants**

Following institutional ethical approval, which conformed to the declaration of Helsinki and the standards of the Journal, 9 healthy non-wrist or flexor trained males who were free from injury were recruited. All participants provided written informed consent prior to any data collection. If a participant conducted regular exercise, which may have stressed the forearm flexors such as tennis or rock climbing, they were excluded from the study. Participant anthropometric and demographic data is presented in Table 1. All participants were physically active and took part in sports that predominantly used the lower limbs such as football, running and cycling, and thus were they were not hand or wrist flexor trained. Further, participants were not suffering from metabolic diseases, nor taking medication known to have any vascular

actions.

**Table 1** participants mean  $\pm$  SD demographic and anthropometric characteristics.

	Mean	$\pm$ SD
Age (years)	20.7	1.1
Mass (kg)	84.0	14.2
Height (cm)	177.2	7.6
BMI (kg·m <sup>2</sup> )	26.6	2.8
Forearm Girth (cm)	29.5	1.9
Flexor Skin Fold (mm)	4.2	0.9

### Experimental Design

Utilizing a randomized cross-over design, participants completed a 4-week handgrip training intervention followed by 4-weeks of detraining, in both normoxic and normobaric hypoxic conditions. Handgrip training was chosen as the small muscle mass does not require a large central hemodynamic response compared to running or cycling which could confound the findings. Outcome measures were assessed before and immediately after the 4 week training period and then at the end of every week during the 4 week detraining phase to determine a post washout period. This resulted in 108 data points per outcome measure. Training in normoxia was completed in an environmentally controlled exercise physiology laboratory, with conditions regulated at 18°C and 50% humidity. Training in hypoxia was completed in a normobaric environmental chamber (9388 Athlete Training Room, Sanyo Gallenkamp PLC, Loughborough, UK); with conditions regulated at 14% FiO<sub>2</sub> (~3250m altitude), 18°C and 50% humidity which has previously been shown to enhance performance Dufour et al. (2006).

## **Training Programme Procedures**

The training programme closely matched the frequency and volume used by Terrados et al. (1990) who conducted isolated leg exercises for 30 min 3-4 times a week for a 4 week period. For each hypoxic and normoxic training, every participant attended 4 x 30 min sessions per week (16 total training sessions; a total of 8 hours training per condition); a 98% training attendance was recorded). Each session comprised of 30-min intermittent rhythmic isometric hand-grip exercise at 30% of maximal voluntary isometric contraction (MVIC) using a digital handgrip dynamometer (model 12-0286, Smedley Digital Hand Dynamometer, Stoelting Co, Wood Dale, Ill). After completion of each 30 min training session, a 60 s 'sprint' at maximal contraction intensity and frequency was performed to elicit a heightened metabolic fatigue of the local musculature (Dudley, Abraham, & Terjung, 1982; Ryan, Southern, Brizendine, & McCully, 2013). Only the non-dominant arm was subjected to the training protocol, minimising the influence of any existing training status. Based on the findings of Ryan et al. (2013) and due to the nature of the endurance training protocol, no change in MVIC was expected. Nevertheless, principles of progressive overload and auto-regulation were applied through tracking MVIC on the first training day of each week. Concentric contraction frequency increased linearly across time, commencing at 0.3 Hz in week one (~540 repetitions per session) reaching 1.2 Hz in week four (~2160 repetitions per session). In order to maintain the contraction frequency, all participant used a metronome. Once a training phase was completed, the 4 week detraining period started. Following the 4 week detraining, an additional 4 week washout period was used to ensure outcome variables had returned to baseline. Participants were also encouraged to avoid any additional wrist-flexor training for the duration of the study period.

## **Experimental Procedures**



### *Near-infrared spectroscopy (NIRS)*

Continuous-wave NIRS in conjunction with a Hokanson rapid inflation device was used to determine forearm mBF,  $\dot{m}\text{VO}_2$  and a measure of muscle perfusion in the FDP. Continuous-wave NIRS relies upon the relative transparency of tissue to infrared light and the oxygen dependent absorption characteristics of haemoglobin (Hb) to determine oxy-haemoglobin ( $\text{O}_2\text{Hb}$ ) and deoxy-haemoglobin (HHb), the sum of which is total haemoglobin (tHb). Application of the modified Beer-Lambert Law allows for absolute concentration changes in chromophores to be determined (Delpy et al., 1988; Hillman et al., 2001). It is important to note that NIRS measures the relative concentration of  $\text{O}_2\text{Hb}$  and oxy-myoglobin as well as HHb deoxy-myoglobin. As such it cannot distinguish between myoglobin and Hb chromophores. For clarity, the combination of Hb and myoglobin will be referred to as Hb in this paper.

The NIRS system used in the present study consisted of an Artinis Portalite optode (Artinis Medical Systems BV, Zetten, The Netherlands), comprised of three light-emitting diodes, positioned 30 mm, 35 mm and 40 mm from a single receiver, which transmitted infrared light at two wavelengths (760 nm and 850 nm). A differential path-length factor of 4.0 was used to correct for photon scattering within the tissue for the calculation of absolute concentration changes (Duncan et al., 1996; Ferrari, Wei, Carraresi, De Blasi, & Zaccanti, 1992). Data were sampled at 25Hz using software inherent to the NIRS (OxySoft, Artinis Medical Systems BV, Zetten, The Netherlands) and stored for offline analysis. The optode was fixed to the skin close to the belly of the FDP with bi-adhesive tape and covered with an opaque cloth to prevent signal contamination by ambient light. The FDP was located using anthropometric markers and palpation in accordance with Fryer et al. (2015). To ascertain the effect of subcutaneous tissue thickness on NIRS measures, forearm skinfold thickness at the FDP was determined using Harpenden skinfold callipers (Baty International, West Sussex, England) during a

familiarization visit, one week prior to commencement of training. For all participants, the FDP skinfold was less than the 6.4 mm which has previously been reported to affect NIRS signal quality (M. Van Beekvelt, Borghuis, Van Engelen, Wevers, & Colier, 2001), and as such the effect of adipose tissue on NIRS signal was thought to be negligible.

### *Experimental Protocol*

Participants were familiarised with all experimental procedures prior to the start of data collection. For the haemodynamic assessments that were performed pre and post the intervention, participants reported to the exercise physiology laboratory between the hours of 7 a.m. and 10 a.m. All participants were overnight fasted, consuming water only, and refrained from caffeine, alcohol and physical activity for 12 h prior to each assessment. The laboratory was quiet and dimly lit. Participants were asked to lie supine in a comfortable position with the non-dominant hand rested on the hand dynamometer. As the participant was resting in a supine position for some time, the forearm was placed at an upward angle of 30° to avoid venous pooling. Further, the forearm was supported at the wrist and above the elbow to ensure unrestricted circulation in the forearm as suggested by (Van Beekvelt, Van Engelen, Wevers, & Colier, 2002). A rapid inflation cuff specially designed to inflate in < 1 s was fitted to the bicep, proximal to the NIRS optode. Following NIRS instrumentation, participants rested for at least 20 min. In accordance with the protocol suggested by Lucero et al. (2017); to assess mBF and  $\dot{m}\text{VO}_2$ , three consecutive 10 s venous (60 mmHg) and arterial (220 mmHg) occlusions were performed, each separated by a deflation period of 50 s where exercise was conducted; tHb (blood volume, an indicator of perfusion) was monitored throughout. Using 10 s occlusions for determination of mBF and  $\dot{m}\text{VO}_2$  has previously been shown to produce high – very high reliability values during both rest and exercise (ICC's 0.82 – 0.96) (Lucero et al., 2017). Following the resting mBF,  $\dot{m}\text{VO}_2$ , and perfusion measures, participants performed 9 min of

rhythmic handgrip exercise at 25% of MVIC at a contraction frequency of 0.3 Hz; using visual digital feedback, participants maintained MVIC accuracy within 5% from the targeted 25% MVIC. After minute 3 of the exercise bout, the 3 x arterial and 3 x venous occlusions (10 s), and the measure of perfusion (20s) which were completed at rest, were repeated at 25% MVC in line with the protocol suggested by Lucero et al. (2017). To permit a stable NIRS signal, participants briefly paused exercising during each 10 s inflation period. A schematic of the occlusion protocol for determination of mBF,  $\dot{m}V\text{O}_2$  and perfusion are presented in Figure 1.

----- Insert Figure 1 Near Here-----

#### *Muscle blood flow and perfusion*

Forearm mBF was derived from NIRS using the venous occlusion method, by evaluating the rate of increase in tHb (De Blasi, Almenrader, Aurisicchio, & Ferrari, 1997; M. C. Van Beekvelt, Colier, Wevers, & Van Engelen, 2001). Absolute concentration changes of tHb were expressed in micromolars per second ( $\mu\text{M}\cdot\text{s}^{-1}$ ) and converted into millilitres blood per minute per 100 millilitres tissue ( $\text{ml}\cdot\text{min}^{-1}\cdot 100\text{ml}^{-1}$ ) using the following formula;

$$\text{mBF} = \text{Abs}(((\Delta\text{tHb} \times 60)/([\text{Hb}] \times 1000)/4)) \times 1000/10 \text{ in } \text{ml}\cdot\text{min}^{-1}\cdot 100\text{ml}^{-1}$$

An average Hb concentration of 8.5 mmol/L was used whilst the molecular weight of Hb was assumed to be 64.458  $\text{g}\cdot\text{mol}^{-1}$ . The molecular ratio between Hb and  $\text{O}_2$  (1:4) was also taken into account. Using NIRS, changes in tHb were used to indicate changes in perfusion (blood volume  $\mu\text{M}$ ) (Stone, Fryer, Ryan, & Stoner, 2016) at rest and at 25% MVIC over a 20 s period.

#### *Muscle oxygen consumption ( $\dot{m}V\text{O}_2$ )*

Measurements of  $\dot{m}V\text{O}_2$  were derived from NIRS using the arterial occlusion method, by

evaluating the rate of increase in HHb (De Blasi et al., 1997). Concentration changes of HHb were expressed in micromolars per second ( $\mu\text{mol}\cdot\text{s}^{-1}$ ) and converted to millilitres  $\text{O}_2$  per minute per 100 gram tissue ( $\text{mlO}_2\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ) using the following formula;

$$\text{m}\dot{\text{V}}\text{O}_2 = \text{Abs}(((\Delta\text{HHb} \times 60)/(10 \times 1.04)) \times 4) \times 22.4/1000 \text{ in } \text{mlO}_2\cdot\text{min}^{-1}100\text{g}^{-1}$$

where each Hb molecule binds four  $\text{O}_2$  molecules and it was assumed that the molar volume of gas is 22.4 L under standard temperature pressure dry (STPD) conditions (M. C. Van Beekvelt et al., 2001). A value of  $1.04 \text{ kg}\cdot\text{L}^{-1}$  was used for muscle density (Vierordt, 1906).

#### *Flexor and extensor locations*

To locate the FDP a line was drawn on the anterior side of the forearm from the medial epicondyle of the humerus to the base of the carpus (lunate) proximal to the ring finger. The NIRS optode was placed 33% distal to the epicondyle of the humerus. It is acknowledged that due to the complexity of the forearm muscular anatomy and inter-individual differences in muscle locations, the NIRS optodes may, in part, overlay muscles adjacent to the target muscles.

#### *Maximal voluntary isometric contraction*

To determine each participants exercise training target and to track any changes during the training protocol, MVIC's were determined prior to each 4 week training period and at the start of each training week using a digital recording hand dynamometer (Smedley Digital Hand Dynamometer, S Dynamometer, Stoelting Co, Wood Dale, Ill). Three MVIC's were performed in a supine position separated by 30 s. The average value of the three contractions was recorded.

#### *Statistical analysis*

The primary study outcomes (perfusion, mBF, m $\dot{V}O_2$ ) were analyzed using hierarchical linear modeling (HLM) with the HLM6 (Scientific Software International, Inc., Lincolnwood, Illinois) statistical package. Statistical significance was defined as  $p < 0.05$ . For each outcome, separate analyses were run to determine the effect of hypoxia on the per week change with exercise, and the effect of hypoxia on the per week detraining response. Three models were run for each analysis. Model one specified time (change per week), and was used to estimate measurement reliability - defined as the intraclass correlation coefficient between intercept and slope random variance components (Raudenbush & Bryk, 2002). Poor reliability (below 0.10) would render the data incapable of identifying relationships between variables for the given population sample size (Raudenbush & Bryk, 2002). Model two specified hypoxia (difference between condition) as a dummy-coded variable (normoxia = 0, hypoxia = 1). Model three specified the baseline value for the respective time point as a group-centered covariate, to control for baseline differences between conditions (hypoxia vs. normoxia) for the given time point. The final within-subject (level 1) and between-subject (level 2) models were specified:

#### Level-1 Model

$$Y_{si} = \pi_{0i} + \pi_{1i} * (\text{Time}_{si}) + \pi_{2i} * (\text{Time} * \text{Hypoxia}_{si}) + \pi_{3i} * (\text{Base}_{si}) + e_{si} \quad (6)$$

where  $\pi_{0i}$  (intercept) represents the outcome of interest for person  $i$  when  $\text{Time}_{si} = \text{week } 1$ ,  $\text{Time} = \text{time (week)}$ , and  $\pi_{1i}$  (slope) represents change in the outcome per week. Hypoxia represents a dummy coded variable to identify normoxia (0) and hypoxia (1), and base represents group centered baseline. The intercept and slopes specified at level 1 become outcomes at level 2.

#### Level-2 Model

(7)

$$\pi_{0i} = \beta_{00} + r_{0i}$$

$$\pi_{1i} = \beta_{10} + r_{1i}$$

$$\pi_{2i} = \beta_{20}$$

$$\pi_{3i} = \beta_{30}$$

where  $r_{0i}$  and  $r_{1i}$  are the unique increments associated with individual  $i$ , indicating that the individual intercepts and slopes were allowed to randomly vary.

## RESULTS

Raw data for  $m\dot{V}O_2$ , perfusion and  $mBF$  for exercise (pre and post) and 4 weeks detraining is presented in Table 2. For each outcome, three HLM models were specified, and the final model is shown in Table 3. The full models are shown in their entirety in Supplemental Tables 1-6; the full models include (i) an unconditional model (M1), to which predictors are subsequently added, and the variance explained can be calculated; (ii) the specification of hypoxia (M2), to determine whether the outcome is significantly affected by the hypoxic condition; and (iii) a model which includes baseline (M3), to covary for differences in baseline values between visits for a given subject. For simplicity, only the final models are reported in Table 3, and discussed below.

**Table 2** mean raw data, 95% LCI and UCI  $m\dot{V}O_2$ , perfusion and  $mBF$  for exercise (pre and post) and 4 weeks recovery.

		$m\dot{V}O_2$		Perfusion		$mBF$	
		$mlO_2 \cdot min^{-1} \cdot 100g^{-1}$		$\mu M$		$ml \cdot min^{-1} \cdot 100ml^{-1}$	
	Week	Mean	(95% UCI-LCI)	Mean	(95% UCI-LCI)	Mean	(95% UCI-LCI)
Normoxia	Pre	0.339	(0.161-0.517)	114	(108-120)	3.49	(2.61-4.37)
	Post	0.544 <sup>+</sup>	(0.311-0.778)	127 <sup>+</sup>	(116-138)	2.84	(1.79-3.90)
	Post 1	0.382	(0.223-0.541)	118	(111-126)	2.53	(1.69-3.37)

	Post 2	0.326	(0.183-0.468)	118	(112-125)	3.10	(2.41-3.78)
	Post 3	0.297	(0.156-0.437)	121	(111-132)	3.36	(2.17-4.55)
	Post 4	0.294	(0.148-0.441)	119	(107-131)	2.70	(2.02-3.39)
Hypoxia	Pre	0.291	(0.143-0.440)	118	(109-128)	2.70	(2.18-3.22)
	Post	0.779*	(0.529-1.028)	139*	(129-148)	4.99*	(3.28-6.71)
	Post 1	0.476	(0.387-0.565)	124	(114-134)	3.55	(2.83-4.28)
	Post 2	0.268	(0.144-0.392)	122	(115-128)	2.56	(1.92-3.20)
	Post 3	0.280	(0.153-0.407)	123	(111-134)	2.70	(1.96-3.45)
	Post 4	0.266	(0.141-0.390)	121	(111-131)	2.66	(1.82-3.49)

*LCI* = lower confidence interval; *UCI* = upper confidence interval,  $m\dot{V}O_2$  = muscle oxygen consumption; *mBF* = muscle blood flow; <sup>+</sup> = normoxic post is significantly ( $p < 0.05$ ) greater than pre (baseline); \* = hypoxic condition is significantly ( $p < 0.05$ ) greater than normoxic condition.

#### *mVO<sub>2</sub> (mlO<sub>2</sub>·min<sup>-1</sup>·100g<sup>-1</sup>)*

The final model predicted that  $m\dot{V}O_2$  significantly increased by 0.090 mlO<sub>2</sub>·min<sup>-1</sup>·100g<sup>-1</sup> each week (95% CI: 0.082, 0.098), and that the rate of change was 58% faster (0.052, 95% CI: 0.050, 0.054) for hypoxia. Post exercise  $m\dot{V}O_2$  significantly declined by 0.029 per week, and there was no effect of hypoxia.

#### *Perfusion (μM)*

The final model predicted that perfusion significantly increased by 3.72 μM each week (95% CI: 3.69, 3.75), and that the rate of change was 69% faster (2.57, 95% CI: 2.54, 2.59) in hypoxia. The measures of perfusion significantly declined by 2.27 μM per week following cessation of the 4-week training intervention (weeks post 1 – 4), and there was no effect of hypoxia.

#### *mBF (ml·min<sup>-1</sup>·100ml<sup>-1</sup>)*

The final model predicted that *mBF* did not significant change for the normoxic condition, but for the hypoxic condition, there was a significant increase of 0.38 ml·min<sup>-1</sup>·100ml<sup>-1</sup> per week

**Table 3** Hierarchical linear model for  $\dot{m}V\text{O}_2$ , perfusion and mBF

		$\dot{m}V\text{O}_2$ ( $\text{mlO}_2/\text{min}/100$ )			Perfusion ( $\mu\text{M}$ )			mBF ( $\text{ml} \cdot \text{min}^{-1} \cdot 100\text{ml}^{-1}$ )			
		Est.	SE	p	Est.	SE	p	Est.	SE	p	
Exercise											
Intercept	$\beta_0$	0.19	0.06	0.018	111.	3.22	<0.00	3.14	0.50	<0.00	Initial
	$0$	7	6		9	5	1	0	4	1	
Time	$\beta_1$	0.09	0.02	0.004	3.72	1.20	0.015	-	-		$\Delta$ per/wk
(wk)	$0$	0	2		2	3		0.08	0.08	0.677	
								4	4		
Hypoxia	$\beta_2$	0.05	0.01	0.001	2.56	0.92	0.013	0.46	0.16	0.014	Additional $\Delta$
	$0$	2	3		8	0		0	7		
Base	$\beta_3$	0.37	0.53	0.491	0.20	0.10	0.060	0.67	0.25	0.017	Person-centered
	$0$	3	0		5	1		1	1		
Recovery											
Intercept	$\beta_0$	0.93	0.15	<0.00	135.	6.78	<0.00	3.98	0.73	<0.00	Initial
	$0$	8	8	1	4	0	1	2	0	1	
Time	$\beta_1$	-	0.01	<0.00	-	0.80	0.023	-	0.11		$\Delta$ per/wk
(wk)	$0$	0.09	0.01	1	2.26	0.7		0.14	0.11	0.235	
		2	7		6			4	2		
Hypoxia	$\beta_2$	0.00	0.00	0.794	0.44	0.33	0.192	-	0.04	0.893	Additional $\Delta$
	$0$	2	6		6	8		0.00	0.04		
								6	4		
Base	$\beta_3$	0.46	0.56	0.411	0.37	0.08	<0.00	0.88	0.15	<0.00	Person-centered
	$0$	7	4		6	1	1	2	3	1	

$\beta_{00}$  = intercept (baseline);  $\beta_{10-30}$  = slope (change);  $\dot{m}V\text{O}_2$  = muscle oxygen consumption; mBF = muscle blood flow

## DISCUSSION

In response to hypoxia there is an acute impairment in endurance performance, followed by an improvement after chronic training has occurred (Hughson & Kowalchuk, 1995; MacDonald et al., 2000; Springer et al., 1991). The contribution of peripheral hemodynamic factors such as mBF and  $\dot{m}V\text{O}_2$  to the chronic training response has not been characterized. The current findings demonstrate that, following 30min hand grip dynamometry training 4 times a week for a 4-week period in both normoxia and hypoxia: 1) mBF increased for the hypoxic but not the normoxic condition, 2)  $\dot{m}V\text{O}_2$  increased for both conditions, but 58% faster for hypoxia,



and, 3) perfusion increased for both conditions, but 69% faster for the hypoxic training. Further, following 4 weeks of detraining, while mBF and perfusion decreased at similar rates across conditions,  $\dot{m}\text{VO}_2$  decreased at a significantly faster rate following hypoxic training.

### *Study Limitations and Strengths*

Several limitations should be addressed in order to better contextualize the findings. First, we did not measure haematocrit throughout the intervention. However, as the exercise stress was targeting a small muscle group (forearm flexors), it is unlikely to have provoked alterations in haematocrit concentrations. Second, our preliminary findings are limited to young healthy untrained males. Last, we did not assess whole body  $\dot{\text{V}}\text{O}_2$  or brachial artery blood flow. However, research has previously demonstrated that pulmonary  $\dot{\text{V}}\text{O}_2$  consumption and limb blood flow are increased with hypoxic training (Hughson & Kowalchuk, 1995; MacDonald et al., 2000; Springer et al., 1991). The major strength of this study is that it is the first to investigate the hemodynamic peripheral adaptations (mBF,  $\dot{m}\text{VO}_2$ , and muscle perfusion) to prolonged (4 weeks) hypoxic training. Further, this study utilized a model that would enable the investigation of peripheral adaptations, while limiting the influence of extraneous factors, including contributions from the cardiorespiratory and endocrine systems.

### *Comparison with Previous Studies*

Findings from the current study suggest that, compared to normoxic training, 4 weeks hypoxic training results in significantly greater increases in  $\dot{m}\text{VO}_2$ , muscle perfusion, and mBF. Although a full mechanistic explanation is beyond the scope of this paper, mitochondrial biogenesis, increased capillarization, and compensatory dilation, respectively, may have contributed. Increased mitochondrial biogenesis may have contributed to the increased  $\dot{m}\text{VO}_2$  following hypoxic training. The current study required participants to end each training session

with a 60 s maximal handgrip ‘sprint’, which has previously been used as a model to stimulate mitochondrial biogenesis (Ryan et al., 2013). An increase in the number of mitochondria in skeletal muscle fibres would have enhanced the capacity to utilize O<sub>2</sub> as a function of aerobic metabolism.

Previously it has been shown that in response to hypoxic training, using the live low train high method, an increased capillary density and the capillary to fibre ratio is seen (M Vogt et al., 2001), as well as gene expression (Michael Vogt & Hoppeler, 2010). Although it is not currently known what extent, this has on protein concentration, which is ultimately responsible for structural or functional phenotypes (Michael Vogt & Hoppeler, 2010). In the context of the current study, increased capillarization may have contributed to the increased perfusion, assuming that our indicator of perfusion represents blood on the arterial side, which is likely when considering that the muscle pump would expel blood on the venous side. Further, structural adaptations such as an increased capillary density (assessed using biopsy) have previously been reported following hypoxic training (Terrados et al., 1990).

Previously, it has been reported that conduit artery (femoral) blood flow increases, but not significantly in response to hypoxic training (MacDonald et al., 2000). Using NIRS, the current study was able to measure skeletal muscle microvascular flow (mBF), and found that mBF was increased following hypoxic, but not normoxic training. One possible explanation could be compensatory dilation. Compensatory dilation involves an additional increase in the vasodilator properties in the muscle and vasculature, which has been suggested to occur when the body is exposed to hypoxia (Joyner & Casey, 2014). The exact pathway regulating this additional dilation has not been characterized, but it is suggested that in HGD, B-adrenergic receptors, adenosine, and nitric oxide are all potentially associated (Joyner & Casey, 2014). In

the context of the current study, compensatory dilation may have allowed for blood flow to be increased to metabolically active skeletal muscle cells without stressing the cardiac system. Further research is warranted, using the current exercise model, albeit with simultaneous monitoring of cardiac, conduit and microvascular function.

### *Implications*

It may be that the nature of the isometric contraction in hypoxia has an effect of mBF,  $\dot{m}\text{VO}_2$  and muscle perfusion responses following training in hypoxia. As such, given that recent studies have shown that the delivery and consumption of  $\text{O}_2$  in the FDP are significant determinants of performance in rock climbers (Fryer et al., 2015); this may be a potential method to improving physiological competence and performance. Consequently, improvements seen in the current study may have performance benefits for specific group of athletes such as rock climbers or tennis players, as well as other sporting populations which place a large demand on the forearm flexors. Although the current study showed that the training effect was short lived for mBF,  $\dot{m}\text{VO}_2$  and perfusion, it may be enough to enhance performance during one-off competitions such as the Olympics or World Cup events. As such, future studies should look to determine: 1) the optimal time at which hypoxic training should start and finish, in order to maximise potential benefits, 2) whether the improvements reported correspond with a significant increase in a performance task, and 3) the physiological mechanisms responsible.

In addition to sports performance, the data presented may be of use in a clinical setting. Whilst we did not measure the metabolic response, an increase in mBF has previously been attributed to an increase in glucose disposal in patients with type II diabetes (Baron, Laakso, Brechtel, & Edelman, 1991; Clark et al., 2003; Holten et al., 2004). As the current study shows that hypoxic

training further enhanced mBF, the use of intermittent hypoxic training could prove to be a novel tool in further enhancing vascular health. Future studies should look to determine whether there are similar increases in  $\dot{m}\text{VO}_2$ , mBF and perfusion in a diabetic/metabolic syndrome population.

### *Conclusion*

The aim of this investigation was to determine forearm flexor haemodynamic responses to 4 weeks training in normoxic and hypoxic (14%  $\text{FiO}_2$  ~3250m altitude) conditions, followed by 4 weeks de-training. Findings suggest that 4-weeks intermittent training significantly increased  $\dot{m}\text{VO}_2$ , mBF (hypoxia only), and muscle perfusion, and that the rate of change is greater when the training is conducted under hypoxic conditions. Collectively, these findings suggest that, in response to chronic hypoxic training, peripheral hemodynamic adaptations enhance  $\text{O}_2$  delivery and utilization. Future investigation is required to determine whether hemodynamic peripheral adaptations directly contribute to enhanced endurance performance following hypoxic training, as well as to identify the mechanisms responsible for hemodynamic peripheral adaptations.

### *Conflicts of Interest*

No authors have any conflicts of interest; i.e. there are no professional relationships with anyone that may benefit from research presented. The results from the present study are original, presented clearly, honestly, and without fraud, fabrication, or plagiarism.

The corresponding author, on behalf of all authors, takes full responsibility for the manuscript.

## REFERENCES

- Bailey, D. M., & Davies, B. (1997). Physiological implications of altitude training for endurance performance at sea level: a review. *British Journal of Sports Medicine*, 31(3), 183-190.
- Baron, A. D., Laakso, M., Brechtel, G., & Edelman, S. V. (1991). Mechanism of insulin resistance in insulin-dependent diabetes mellitus: a major role for reduced skeletal muscle blood flow. *The Journal of Clinical Endocrinology & Metabolism*, 73(3), 637-643.
- Casey, D. P., & Joyner, M. J. (2011). Local control of skeletal muscle blood flow during exercise: influence of available oxygen. *Journal of Applied Physiology*, 111(6), 1527-1538.
- Clark, M. G., Wallis, M. G., Barrett, E. J., Vincent, M. A., Richards, S. M., Clerk, L. H., & Rattigan, S. (2003). Blood flow and muscle metabolism: a focus on insulin action. *American Journal of Physiology Endocrinology and Metabolism*, 284(2), E241-E258.
- De Blasi, R. A., Almenrader, N., Aurisicchio, P., & Ferrari, M. (1997). Comparison of two methods of measuring forearm oxygen consumption (VO<sub>2</sub>) by near infrared spectroscopy. *Journal of Biomedical Optics*, 2(2), 171-175.
- DeLorey, D. S., Shaw, C. N., Shoemaker, J. K., Kowalchuk, J. M., & Paterson, D. H. (2004). The effect of hypoxia on pulmonary O<sub>2</sub> uptake, leg blood flow and muscle deoxygenation during single - leg knee - extension exercise. *Experimental Physiology*, 89(3), 293-302.
- Delpy, D. T., Cope, M., van der Zee, P., Arridge, S., Wray, S., & Wyatt, J. (1988). Estimation of optical pathlength through tissue from direct time of flight measurement. *Physics in Medicine and Biology*, 33(12), 1433.
- Dudley, G. A., Abraham, W. M., & Terjung, R. L. (1982). Influence of exercise intensity and duration on biochemical adaptations in skeletal muscle. *Journal of Applied Physiology*, 53(4), 844-850.
- Dufour, S. P., Ponsot, E., Zoll, J., Doutreleau, S., Lonsdorfer-Wolf, E., Geny, B., . . . Billat, V. (2006). Exercise training in normobaric hypoxia in endurance runners. I. Improvement in aerobic performance capacity. *Journal of Applied Physiology*, 100(4), 1238-1248.
- Duncan, A., Meek, J. H., Clemence, M., Elwell, C. E., Fallon, P., Tyszczuk, L., . . . Delpy, D. T. (1996). Measurement of cranial optical path length as a function of age using phase resolved near infrared spectroscopy. *Pediatric Research*, 39(5), 889-894.
- Ferrari, M., Wei, Q., Carraresi, L., De Blasi, R. A., & Zaccanti, G. (1992). Time-resolved spectroscopy of the human forearm. *Journal of Photochemistry and Photobiology B: Biology*, 16(2), 141-153.
- Fryer, S., Stoner, L., Lucero, A., Witter, T., Scarrott, C., Dickson, T., . . . Draper, N. (2015). Haemodynamic kinetics and intermittent finger flexor performance in rock climbers. *International Journal of Sports Medicine*, 36(2), 137-142.
- Gore, C. J., Clark, S. A., & Saunders, P. U. (2007). Nonhematological mechanisms of improved sea-level performance after hypoxic exposure. *Medicine & Science in Sports & Exercise*, 39(9), 1600-1609.
- Hamlin, M., Marshall, H., Hellemans, J., Ainslie, P., & Anglem, N. (2010). Effect of intermittent hypoxic training on 20 km time trial and 30 s anaerobic performance. *Scandinavian Journal of Medicine & Science in Sports*, 20(4), 651-661.
- Hillman, E. M., Hebden, J. C., Schweiger, M., Dehghani, H., Schmidt, F. E., Delpy, D. T., & Arridge, S. R. (2001). Time resolved optical tomography of the human forearm. *Physics in Medicine and Biology*, 46(4), 1117.

- Holten, M. K., Zacho, M., Gaster, M., Juel, C., Wojtaszewski, J. F., & Dela, F. (2004). Strength training increases insulin-mediated glucose uptake, GLUT4 content, and insulin signaling in skeletal muscle in patients with type 2 diabetes. *Diabetes*, 53(2), 294-305.
- Hughson, R. L., & Kowalchuk, J. M. (1995). Kinetics of oxygen uptake for submaximal exercise in hyperoxia, normoxia, and hypoxia. *Canadian Journal of Applied Physiology*, 20(2), 198-210.
- Joyner, M. J., & Casey, D. P. (2014). Muscle blood flow, hypoxia, and hypoperfusion. *Journal of Applied Physiology*, 116(7), 852-857.
- Katayama, K., Matsuo, H., Ishida, K., Mori, S., & Miyamura, M. (2003). Intermittent hypoxia improves endurance performance and submaximal exercise efficiency. *High Altitude Medicine & Biology*, 4(3), 291-304.
- Katayama, K., Sato, Y., Morotome, Y., Shima, N., Ishida, K., Mori, S., & Miyamura, M. (2001). Intermittent hypoxia increases ventilation and SaO<sub>2</sub> during hypoxic exercise and hypoxic chemosensitivity. *Journal of Applied Physiology*, 90(4), 1431-1440.
- Lucero, A., Gifty, A., Lawrence, W., Neway, B., Credeur, D. P., Faulkner, J., . . . Stoner, L. (2017). Reliability of Muscle Blood Flow and Oxygen Consumption During Exercise Using Near-Infrared Spectroscopy. *Experimental Physiology*.
- MacDonald, M., Tarnopolsky, M., & Hughson, R. (2000). Effect of hyperoxia and hypoxia on leg blood flow and pulmonary and leg oxygen uptake at the onset of kicking exercise. *Canadian Journal of Physiology and Pharmacology*, 78, 67-74.
- Marshall, H. C., Hamlin, M. J., Hellemans, J., Murrell, C., Beattie, N., Hellemans, I., . . . Ainslie, P. N. (2008). Effects of intermittent hypoxia on SaO<sub>2</sub>, cerebral and muscle oxygenation during maximal exercise in athletes with exercise-induced hypoxemia. *European Journal of Applied Physiology*, 104(2), 383-393.
- Meeuwssen, T., Hendriksen, I. J., & Holewijn, M. (2001). Training-induced increases in sea-level performance are enhanced by acute intermittent hypobaric hypoxia. *European Journal of Applied Physiology*, 84(4), 283-290.
- Neubauer, J. A. (2001). Invited review: Physiological and pathophysiological responses to intermittent hypoxia. *Journal of Applied Physiology*, 90(4), 1593-1599.
- Noakes, T. D., Peltonen, J. E., & Rusko, H. K. (2001). Evidence that a central governor regulates exercise performance during acute hypoxia and hyperoxia. *Journal of Experimental Biology*, 204(18), 3225-3234.
- Raudenbush, S. W., & Bryk, A. S. (2002). *Hierarchical linear models: Applications and data analysis methods* (Vol. 1): Sage.
- Roach, R. C., Koskolou, M. D., Calbet, J. A., & Saltin, B. (1999). Arterial O<sub>2</sub> content and tension in regulation of cardiac output and leg blood flow during exercise in humans. *American Journal of Physiology-Heart and Circulatory Physiology*, 276(2), H438-H445.
- Rowell, B., Saltin, B., Kiens, B., & Christensen, N. (1986). Is peak quadriceps blood flow in humans even higher during exercise with hypoxemia? *American Journal of Physiology Heart and Circulatory Physiology*, 251(5), H1038-H1044.
- Ryan, T. E., Southern, W. M., Brizendine, J. T., & McCully, K. K. (2013). Activity-induced changes in skeletal muscle metabolism measured with optical spectroscopy. *Medicine and science in sports and exercise*, 45(12).
- Springer, C., Barstow, T. J., Wasserman, K., & Cooper, D. M. (1991). Oxygen uptake and heart rate responses during hypoxic exercise in children and adults. *Medicine and science in sports and exercise*, 23(1), 71-79.
- Stone, K. J., Fryer, S. M., Ryan, T., & Stoner, L. (2016). The validity and reliability of continuous-wave near-infrared spectroscopy for the assessment of leg blood volume

- during an orthostatic challenge. *Atherosclerosis*, 251, 234-239.
- Terrados, N., Jansson, E., Sylven, C., & Kaijser, L. (1990). Is hypoxia a stimulus for synthesis of oxidative enzymes and myoglobin? *Journal of Applied Physiology*, 68(6), 2369-2372.
- Van Beekvelt, Van Engelen, B. G. M., Wevers, R. A., & Colier, W. N. J. M. (2002). In vivo quantitative near-infrared spectroscopy in skeletal muscle during incremental isometric handgrip exercise. *Clinical Physiology and Functional Imaging*, 22(3), 210-217.
- Van Beekvelt, M., Borghuis, M., Van Engelen, B., Wevers, R., & Colier, W. (2001). Adipose tissue thickness affects in vivo quantitative near-IR spectroscopy in human skeletal muscle. *Clinical Science*, 101(1), 21-28.
- Van Beekvelt, M. C., Colier, W., Wevers, R. A., & Van Engelen, B. G. (2001). Performance of near-infrared spectroscopy in measuring local  $\text{O}_2$  consumption and blood flow in skeletal muscle. *Journal of Applied Physiology*, 90(2), 511-519.
- Vierordt, H. (1906). *Anatomische, physiologische und physikalische Daten und Tabellen zum Gebrauche für Mediziner*: Fischer.
- Vogt, M., & Hoppeler, H. (2010). Is hypoxia training good for muscles and exercise performance? *Progress in Cardiovascular Diseases*, 52(6), 525-533.
- Vogt, M., Puntchart, A., Geiser, J., Zuleger, C., Billeter, R., & Hoppeler, H. (2001). Molecular adaptations in human skeletal muscle to endurance training under simulated hypoxic conditions. *Journal of Applied Physiology*, 91(1), 173-182.

## Supplemental Data

Supplemental Table 1 hierarchical linear models for  $\text{m}\dot{\text{V}}\text{O}_2$  during exercise

		M1 Uncond.		M2 + Hypoxia		M3 + Base	
		Est.	p	Est.	p	Est.	p
Fixed Effects							
Intercept	$\beta_{00}$	0.200	0.028	0.199	0.021	0.197	0.018
Time	$\beta_{10}$	0.116	<0.01	0.089	0.003	0.090	0.004
Hypoxia	$\beta_{20}$			0.052	0.001	0.052	0.001
Base	$\beta_{30}$					0.373	0.491
Random Variance							
Level 1	$E$	0.021	0.047	0.013	0.045	0.014	0.023
Intercept	$U_{00}$	0.030	0.257	0.031	0.002	0.027	0.007

Slope	$U_{10}$	0.002	0.002	0.003
		%exp		%exp
$R_{\text{within}}$		36.02		34.64
$R_{\text{between}}$		-2.18		9.42
Reliability		Est.	Est.	Est.
Intercept	$U_{00}$	0.60	0.71	0.67
Slope	$U_{10}$	0.46	0.58	0.62

M = model;  $\beta_{00}$  = intercept (baseline);  $\beta_{10-30}$  = slope (change); *Est* = *estimated*; *E* = level 1 variance (measures within subject); *U* = level 2 variance (between subjects); %exp = *percentage variance explained*

Supplemental Table 2 hierarchical linear models for  $\dot{m}\text{VO}_2$  during recovery

		M1 Uncond.		M2 + Hypoxia		M3 + Base	
		Est.	<i>p</i>	Est.	<i>p</i>	Est.	<i>p</i>
Fixed Effects							
Intercept	$\beta_{00}$	0.933	<0.001	0.933	<0.001	0.938	<0.001
Time	$\beta_{10}$	-0.090	<0.001	-0.092	<0.001	-0.092	<0.001
Hypoxia	$\beta_{20}$			0.004	0.463	0.002	0.794
Base	$\beta_{30}$					0.467	0.411
Random Variance							
Level 1	<i>E</i>	0.022		0.022		0.022	
Intercept	$U_{00}$	0.168	<0.001	0.168	<0.001	0.183	<0.001
Slope	$U_{10}$	0.001	0.086	0.001	0.088	0.001	0.045
		%exp		%exp		%exp	
$R_{\text{within}}$		-0.60		-0.96		-0.96	
$R_{\text{between}}$		0.03		-8.54		-8.54	



M = model;  $\beta_{00}$  = intercept (baseline);  $\beta_{10-30}$  = slope (change); *Est* = *estimated*; *E* = level 1 variance (measures within subject); *U* = level 2 variance (between subjects); %exp = *percentage variance explained*

Supplemental Table 3 hierarchical linear models for perfusion during exercise

		M1 Uncond.		M2 + Hypoxia		M3 + Base	
		Est.	<i>p</i>	Est.	<i>p</i>	Est.	<i>p</i>
Fixed Effects							
Intercept	$\beta_{00}$	110.6	<0.001	110.6	<0.001	111.9	<0.001
Time	$\beta_{10}$	5.538	0.002	4.071	0.011	3.722	0.015
Hypoxia	$\beta_{20}$			2.933	0.007	2.568	0.013
Base	$\beta_{30}$					0.205	0.060
Random Variance							
Level 1	<i>E</i>	92.59	>0.500	69.95	>0.500	62.21	0.303
Intercept	$U_{00}$	29.37	>0.500	30.84	>0.500	31.01	0.275
Slope	$U_{10}$	3.712		3.901		4.006	
					%exp	%exp	
R <sub>within</sub>					24.45	32.81	
R <sub>between</sub>					-5.01	-5.82	
Reliability		Est.		Est.		Est.	
Intercept	$U_{00}$	0.25		0.32		0.35	
Slope	$U_{10}$	0.27		0.33		0.37	

M = model;  $\beta_{00}$  = intercept (baseline);  $\beta_{10-30}$  = slope (change); *Est* = *estimated*; *E* = level 1 variance (measures within subject); *U* = level 2 variance (between subjects); %exp = *percentage variance*

Supplemental Table 4 hierarchical linear models for perfusion during recovery

		M1 Uncond.		M2 + Hypoxia		M3 + Base	
		Est.	p	Est.	p	Est.	p
Fixed Effects							
Intercept	$\beta_{00}$	138.2	<0.001	138.2	<0.001	135.4	<0.001
Time	$\beta_{10}$	-2.509	0.018	-2.828	0.015	-2.266	0.023
Hypoxia	$\beta_{20}$			0.639	0.093	0.446	0.192
Base	$\beta_{30}$					0.376	<0.001
Random Variance							
Level 1	$E$	123.4		120.1		96.19	
Intercept	$U_{00}$	232.6	0.043	240.9	0.037	227.8	0.028
Slope	$U_{10}$	1.066	0.310	1.288	0.291	0.666	0.411
					%exp	%exp	
$R_{\text{within}}$					2.66	22.02	
$R_{\text{between}}$					-3.62	2.26	

M = model;  $\beta_{00}$  = intercept (baseline);  $\beta_{10-30}$  = slope (change); *Est* = *estimated*; *E* = level 1 variance (measures within subject); *U* = level 2 variance (between subjects); %exp = *percentage variance explained*

Supplemental Table 5 hierarchical linear models for mBF during exercise

		M1 Uncond.		M2 + Hypoxia		M3 + Base	
		Est.	p	Est.	p	Est.	p
Fixed Effects							
Intercept	$\beta_{00}$	2.821	0.001	2.820	<0.001	3.140	<0.001
Time	$\beta_{10}$	0.274	0.218	0.044	0.841	-0.084	0.677

Hypoxia	$\beta_{20}$			0.460	0.022	0.460	0.014
Base	$\beta_{30}$					0.671	0.017
Random Variance							
Level 1	$E$	3.085	>0.500	2.560	>0.500	2.138	>0.500
Intercept	$U_{00}$	0.019	>0.500	0.024	>0.500	0.143	>0.500
Slope	$U_{10}$	0.037		0.047		0.019	
					%exp	%exp	
$R_{\text{within}}$					17.00	16.51	
$R_{\text{between}}$					-27.34	-192.16	
Reliability		Est.		Est.		Est.	
Intercept	$U_{00}$	0.01		0.01		0.07	
Slope	$U_{10}$	0.10		0.14		0.07	

M = model;  $\beta_{00}$  = intercept (baseline);  $\beta_{10-30}$  = slope (change); *Est* = *estimated*; *E* = level 1 variance (measures within subject); *U* = level 2 variance (between subjects); %exp = *percentage variance explained*

Supplemental Table 6 hierarchical linear models for mBF during recovery

		M1 Uncond.		M2 + Hypoxia		M3 + Base	
		Est.	$p$	Est.	$p$	Est.	$p$
Fixed Effects							
Intercept	$\beta_{00}$	4.591	<0.001	4.591	<0.001	3.982	<0.001
Time	$\beta_{10}$	-0.249	0.104	-0.263	0.093	-0.144	0.235
Hypoxia	$\beta_{20}$			0.029	0.562	-0.006	<0.001
Base	$\beta_{30}$					0.882	0.893
Random Variance							
Level 1	$E$	2.170		2.188		1.600	

Intercept	$U_{00}$	3.110	0.121	3.084	0.125	1.655	0.218
Slope	$U_{10}$	0.057	0.279	0.056	0.285	0.025	0.420
					%exp	%exp	
$R_{\text{within}}$					-0.83	26.25	
$R_{\text{between}}$					0.81	46.94	

M = model;  $\beta_{00}$  = intercept (baseline);  $\beta_{10-30}$  = slope (change); *Est* = *estimated*; *E* = level 1 variance (measures within subject); *U* = level 2 variance (between subjects); %exp = *percentage variance explained*

### Figure 1 Legend

Schematic representation of the timing protocol for determination of muscle perfusion, muscle blood flow [venous occlusion (VO)], and muscle oxygen consumption [arterial occlusion (AO)]. This protocol was conducted during each baseline assessment, and post weeks 1, 2, 3 and 4 following hypoxic and normoxic training.

Figure 1 abbreviations. Min = minutes; s = seconds; AO = arterial occlusion; VO = venous occlusions; HGD = handgrip dynamometry.

