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

Running head: Forearm responses to hypoxic training

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ABSTRACT

Intermittent exposure to hypoxia can lead to improved endurance performance. Currently, it is unclear whether peripheral adaptions 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 ($m\dot{V}O_2$), 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% FiO_2 ~3250m altitude) and normoxic conditions. mBF, $m\dot{V}O_2$ and perfusion were assessed pre, post 4-weeks training, and following 4-weeks detraining. Hierarchical linear modelling found that $m\dot{V}O_2$ increased at a significantly faster rate (58%) with hypoxic training (0.09 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ per week); perfusion increased at a significantly (69%) faster rate with hypoxic training (3.72 μM per week). mBF did not significantly change for the normoxic condition, but there was a significant increase of 0.38 $ml \cdot min^{-1} \cdot 100ml^{-1}$ per week (95% CI: 0.35, 0.40) for the hypoxic condition. During 4-weeks detraining, $m\dot{V}O_2$ 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 the mechanisms are less well understood. Currently, it is not clear whether peripheral adaptions 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 m$\dot{V}O_2$. 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 (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, m$\dot{V}O_2$ and muscle perfusion in the flexor digitorum profundus (FDP) and 2) a slowed decline in mBF, m$\dot{V}O_2$ 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 ± SD demographic and anthropometric characteristics.

	Mean	± SD
Age (years)	20.7	1.1
Mass (kg)	84.0	14.2
Height (cm)	177.2	7.6
BMI ($kg \cdot m^2$)	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_2 (~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, m$\dot{V}O_2$ 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 (O_2Hb) 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 O_2Hb 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 (Artinins 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, Artinins 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^{o} 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 m$\dot{V}O_2$, 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 m$\dot{V}O_2$ 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, m$\dot{V}O_2$, 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, $m\dot{V}O_2$ 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 ($\mu M \cdot s^{-1}$) and converted into millilitres blood per minute per 100 milliletres tissue ($ml \cdot min^{-1} \cdot 100ml^{-1}$) using the following formula;

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

An average Hb concentration of 8.5 mmol/L was used whilst the molecular weight of Hb was assumed to be 64.458 $g \cdot mol^{-1}$. The molecular ratio between Hb and O_2 (1:4) was also taken into account. Using NIRS, changes in tHb were used to indicate changes in perfusion (blood volume μM) (Stone, Fryer, Ryan, & Stoner, 2016) at rest and at 25% MVIC over a 20 s period.

Muscle oxygen consumption ($m\dot{V}O_2$)

Measurements of $m\dot{V}O_2$ 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 ($\mu mol \cdot s^{-1}$) and converted to millilitres O_2 per minute per 100 gram tissue ($mlO_2 \cdot min^{-1} \cdot 100g^{-1}$) using the following formula;

$$m\dot{V}O_2 = Abs(((\Delta HHb \times 60)/(10 \times 1.04)) \times 4) \times 22.4/1000 \text{ in } mlO_2 \cdot min^{-1}100g^{-1}$$

where each Hb molecule blinds four 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 $kg \cdot L^{-1}$ 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

$$Y_{si} = \pi_{0i} + \pi_{1i} *(Time_{si}) + \pi_{2i} *(Time *Hypoxia_{si}) + \pi_{3i} *(Base_{si}) + e_{si} \quad (6)$$

where π_{0i} (intercept) represents the outcome of interest for person i when $Time_{si}$ = week 1, Time = time (week), and π_{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 mVO_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 mVO_2, perfusion and mBF for exercise (pre and post) and 4 weeks recovery.

		$m\dot{V}O_2$		Perfusion		mBF	
		$mlO_2 \cdot min^{-1} \cdot 100g^{-1}$		µM		$ml \cdot min^{-1} \cdot 100ml^{-1}$	
	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^{+}	*(0.311-0.778)*	127^{+}	*(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, mVO_2 = muscle oxygen consumption; mBF = muscle blood flow; $^{+}$ = normoxic post is significantly ($p < 0.05$) greater than pre (baseline); * = hypoxic condition is significantly ($p < 0.05$) greater than normoxic condition.

mVO_2 ($mlO_2 \cdot min^{-1} \cdot 100g^{-1}$)

The final model predicted that $m\dot{V}O_2$ significantly increased by 0.090 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ 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 ($ml \cdot min^{-1} \cdot 100ml^{-1}$)

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 $ml \cdot min^{-1} \cdot 100ml^{-1}$ per week

Table 3 Hierarchical linear model for $m\dot{V}O_2$, perfusion and mBF

		$m\dot{V}O_2$ (mlO_2/min/100)			Perfusion (μM)			mBF (ml· min^{-1}·100ml^{-1})			
		Est.	*SE*	p	Est.	*SE*	p	Est.	*SE*	p	
Exercise											
Intercept	β_{00}	0.197	*0.066*	0.018	111.9	*3.225*	<0.001	3.140	*0.504*	<0.001	Initial
Time (wk)	β_{10}	0.090	*0.022*	0.004	3.722	*1.203*	0.015	-0.084	*-0.084*	0.677	Δ per/wk
Hypoxia	β_{20}	0.052	*0.013*	0.001	2.568	*0.920*	0.013	0.460	*0.167*	0.014	Additional Δ
Base	β_{30}	0.373	*0.530*	0.491	0.205	*0.101*	0.060	0.671	*0.251*	0.017	Person-centered
Recovery											
Intercept	β_{00}	0.938	*0.158*	<0.001	135.4	*6.780*	<0.001	3.982	*0.730*	<0.001	Initial
Time (wk)	β_{10}	-0.092	*0.017*	<0.001	-2.266	*0.807*	0.023	-0.144	*0.112*	0.235	Δ per/wk
Hypoxia	β_{20}	0.002	*0.006*	0.794	0.446	*0.338*	0.192	-0.006	*0.044*	0.893	Additional Δ
Base	β_{30}	0.467	*0.564*	0.411	0.376	*0.081*	<0.001	0.882	*0.153*	<0.001	Person-centered

β_{00} = intercept (baseline); β_{10}-30 = slope (change); mVO2 = 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 $m\dot{V}O_2$ 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) $m\dot{V}O_2$ 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, m$\dot{V}O_2$ 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 $\dot{V}O_2$ or brachial artery blood flow. However, research has previously demonstrated that pulmonary $\dot{V}O_2$ 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, m$\dot{V}O_2$, 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 m$\dot{V}O_2$, muscle perfusion, and mBF. Although a full mechanistic explanation is beyond the scope of this paper, mitochondrial biogenesis, increased cappilarization, and compensatory dilation, respectively, may have contributed. Increased mitochondrial biogenesis may have contributed to the increased m$\dot{V}O_2$ 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_2 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, $m\dot{V}O_2$ and muscle perfusion responses following training in hypoxia. As such, given that recent studies have shown that the delivery and consumption of 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, $m\dot{V}O_2$ 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 $m\dot{V}O_2$, 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% FiO_2 ~3250m altitude) conditions, followed by 4 weeks de-training. Findings suggest that 4-weeks intermittent training significantly increased $m\dot{V}O_2$, 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 O_2 delivery and utilization. Future investigation is required to determine whether hemodynamic peripheral adaptions 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.

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Supplemental Data

Supplemental Table 1 hierarchical linear models for $m\dot{V}O_2$ during exercise

		M1 Uncond.		M2 + Hypoxia		M3 + Base	
		Est.	*p*	Est.	p	Est.	*p*
Fixed Effects							
Intercept	β_{00}	0.200	0.028	0.199	0.021	0.197	0.018
Time	β_{10}	0.116	<0.01	0.089	0.003	0.090	0.004
Hypoxia	β_{20}			0.052	0.001	0.052	0.001
Base	β_{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_{within}			36.02	34.64
$R_{between}$			-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; β_{00} = intercept (baseline); β_{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 $m\dot{V}O_2$ during recovery

		M1 Uncond.		M2 + Hypoxia		M3 + Base	
		Est.	*p*	Est.	*p*	Est.	*p*
Fixed Effects							
Intercept	β_{00}	0.933	<0.001	0.933	<0.001	0.938	<0.001
Time	β_{10}	-0.090	<0.001	-0.092	<0.001	-0.092	<0.001
Hypoxia	β_{20}			0.004	0.463	0.002	0.794
Base	β_{30}					0.467	0.411
Random Variance							
Level 1	*E*	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
R_{within}					-0.60		-0.96
$R_{between}$					0.03		-8.54

M = model; β_{00} = intercept (baseline); β_{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.	*p*	Est.	*p*	Est.	*p*
Fixed Effects							
Intercept	β_{00}	110.6	<0.001	110.6	<0.001	111.9	<0.001
Time	β_{10}	5.538	0.002	4.071	0.011	3.722	0.015
Hypoxia	β_{20}			2.933	0.007	2.568	0.013
Base	β_{30}					0.205	0.060
Random Variance							
Level 1	*E*	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_{within}					24.45		32.81
$R_{between}$					-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; β_{00} = intercept (baseline); β_{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	β_{00}	138.2	<0.001	138.2	<0.001	135.4	<0.001
Time	β_{10}	-2.509	0.018	-2.828	0.015	-2.266	0.023
Hypoxia	β_{20}			0.639	0.093	0.446	0.192
Base	β_{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_{within}					2.66		22.02
$R_{between}$					-3.62		2.26

M = model; β_{00} = intercept (baseline); β_{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	β_{00}	2.821	0.001	2.820	<0.001	3.140	<0.001
Time	β_{10}	0.274	0.218	0.044	0.841	-0.084	0.677

Hypoxia	β_{20}			0.460	0.022	0.460	0.014
Base	β_{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_{within}					17.00		16.51
$R_{between}$					-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; β_{00} = intercept (baseline); β_{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	β_{00}	4.591	<0.001	4.591	<0.001	3.982	<0.001
Time	β_{10}	-0.249	0.104	-0.263	0.093	-0.144	0.235
Hypoxia	β_{20}			0.029	0.562	-0.006	<0.001
Base	β_{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_{within}					-0.83		26.25
$R_{between}$					0.81		46.94

M = model; β_{00} = intercept (baseline); β_{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.