The Acute Physiological, Physical and Perceptual Responses to Intermittent Hypoxic Resistance Training

A thesis submitted for the degree

Doctor of Philosophy

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Statement of Originality

The thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository**, subject to the provisions of the Copyright Act 1968.

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Acknowledgement of Collaboration

I hereby certify that the work embodied in this thesis has been done in collaboration with other researchers, or carried out in other institutions. I have included as part of the thesis a statement clearly outlining the extent of collaboration, with whom and under what auspices.

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We, Ben Dascombe, Katie Slattery and Dean Sculley, attest that the research completed within this thesis by the candidate Brendan Scott, was completed in collaboration with the following organisation:

- New South Wales Institute of Sport, Sydney

Supervisor: Ben Dascombe (PhD)	21 / 08 / 2015 Date Signed
Supervisor: Katie Slattery (PhD)	21 / 08 / 2015 Date Signed
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Acknowledgement of Authorship

I hereby certify that the work embodied in this thesis contains a published paper/s/scholarly work of which I am a joint author. I have included as part of the thesis a written statement, endorsed by my supervisor, attesting to my contribution to the joint publication/s/scholarly work.

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We, Ben Dascombe, Katie Slattery and Dean Sculley, attest that Research Higher Degree candidate Brendan Scott was a contributor to the conception, design, writing and revision of the previously mentioned publications.

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List of Publications Arising From This Thesis

Peer-reviewed articles:

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Scott, B. R., Loenneke, J. P., Slattery, K. M., & Dascombe, B. J. (2015). Exercise with blood flow restriction: an updated evidence-based approach for enhanced muscular development. *Sports Medicine*, 45 (3), 313-325.

Scott, B. R., Slattery, K. M., & Dascombe, B. J. (2015). Intermittent hypoxic resistance training: is metabolic stress the key moderator? *Medical Hypotheses*, 84, 145-149.

Scott, B. R., Slattery, K. M., Sculley, D. V., Hodson, J. A., & Dascombe, B. J. (2015). Physical performance during high-intensity resistance exercise in normoxic and hypoxic conditions. *Journal of Strength and Conditioning Research,* 29(3), 807-815.

Scott, B. R., Slattery, K. M., & Dascombe, B. J. (2014). Intermittent hypoxic resistance training: does it provide added benefit? *Frontiers in Physiology*, 5, 397.

Scott, B. R., Slattery, K. M., Sculley, D. V., & Dascombe, B. J. (2014). Hypoxia and resistance exercise: a comparison of localized and systemic methods. *Sports Medicine*, 44(8), 1037-1054.

Scott, B. R. (2014). Using blood flow restriction strategies to manage training stress for athletes. *Journal of Australian Strength and Conditioning*, 22(6), 84-90.

Scott, B. R., Dascombe, B. J., Delaney, J. A., Elsworthy, N., Lockie, R. G., Sculley, D. V., & Slattery, K. M. (2014). The validity and reliability of a customized rigid supportive harness during Smith Machine back squat exercise. *Journal of Strength and Conditioning Research,* 28(3), 636-642.

Scott, B. R., Slattery, K. M., Sculley, D. V., Lockie, R. G., & Dascombe, B. J. (2014). Reliability of telemetric electromyography and near-infrared spectroscopy during high-intensity resistance exercise. *Journal of Electromyography and Kinesiology,* 24(5), 722-730.

Scott, B. R., Duthie, G. M., Thornton, H. R., & Dascombe, B. J. (Under Review). Training monitoring for resistance exercise: theory and applications. *Sports Medicine.*

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- Winner of the ASCA Student Poster Award

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List of Abbreviations

~	Approximately
>	Greater than
≥	Greater than or equal to
<	Less than
≤	Less than or equal to
↑	Increase
↓	Decrease
↔	No change
?	Equivocal or unclear findings
±	Plus/minus
%	Percent
°	Degree/s
$°\cdot s^{-1}$	Degrees per second
°C	Degrees Celsius
µL	Microlitre
η^2	eta squared
Δ	Delta
1RM	1-Repetition maximum
10RM	10-Repetition maximum
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AU	Arbitrary units
BF	Biceps femoris
BFR	Blood flow restriction
BLa^-	Blood lactate
$[BLa^-]$	Blood lactate concentration

bpm	Beats per minute
CI	Confidence intervals
cm	Centimetre
CMJ	Countermovement jump
CR-10	Category-Ratio 10 scale
CSA	Cross-sectional area
CV	Coefficient of variation
EMG	Electromyography
ES	Effect size
F	*F* statistic
F_IO_2	Fraction of inspired oxygen
GH	Growth hormone
GM	Gluteus maximus
h	Hour/s
H^+	Hydrogen ion
HbO_2	Oxyhaemoglobin
HbO_{2min}	Relative minimum oxyhaemoglobin value
$[HbO_2]$	Oxyhaemoglobin concentration
HBS	Harness back squat
HH	High-level hypoxia
HHb	Deoxyhaemoglobin
HHb_{max}	Relative maximum deoxyhaemoglobin value
[HHb]	Deoxyhaemoglobin concentration
HIF-1α	Hypoxia-inducible factor-1α
HR	Heart rate
Hz	Hertz
ICC	Intra-class correlation coefficient
iEMG	Integrated electromyography

IGF-1	Insulin-like growth factor-1
IHRT	Intermittent hypoxic resistance training
kg	Kilogram/s
m	Metre/s
MAPK	Mitogen-activated protein kinase
MDF	Median frequency of the electromyography signal
MH	Moderate-level hypoxia
min	Minute/s
mm	Millimetre/s
mmHg	Millimetres of mercury
$mmol \cdot L^{-1}$	Millimole per litre
MPS	Muscle protein synthesis
mRNA	Messenger ribonucleic acid
$m \cdot s^{-1}$	Metres per second
mTOR	Mammalian target of rapamycin
MTP	Mid-thigh pull
MVC	Maximum voluntary contraction
MVC_3	Maximum voluntary contraction for 3 seconds
MVC_{30}	Maximum voluntary contraction for 30 seconds
n	Number
N	Newtons
NIRS	Near-infrared spectroscopy
nm	nanometer
NORM	Normoxia
O_2	Oxygen
p	Alpha
PCr	Phosphocreatine
pH	Potential hydrogen

P_i	Inorganic phosphate
PO_2	Partial pressure of oxygen
r	Pearson's correlation coefficient
r^2	Pearson's *r* squared
reps	Repetitions
RFD	Rate of force development
RMS	Root mean square of electromyography signal
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RPE	Rating of perceived exertion
RT	Resistance training without BFR in normoxia
s	Second/s
SD	Standard deviation of the mean
SENIAM	Surface EMG for non-invasive assessment of muscles
SJ	Squat jump
sO_2	Oxygen saturation
SpO_2	Arterial oxygen saturation (%)
sRPE	Session rating of perceived exertion
S6K1	Ribosomal S6 kinase 1
TSI	Tissue saturation index
TSI_{min}	Relative minimum tissue saturation index
VEGF	Vascular endothelial growth factor
VL	Vastus lateralis
VM	Vastus medialis
W	Watts
$W \cdot kg^{-1}$	Watts per kilogram
WU	Warm-up set
yr	Year/s

Abstract

Recent evidence suggests that supplemental hypoxia during resistance training can enhance muscular adaptation. However, the mechanisms underpinning augmented muscular responses to intermittent hypoxic resistance training (IHRT) and how they can be optimised remain largely unknown. Therefore, the aim of this thesis was to examine the acute physiological, physical and perceptual responses to IHRT in well-trained participants.

Study 1 quantified the inter- and intra-test reliability of electromyography (EMG) and near-infrared spectroscopy (NIRS) technologies during resistance exercise. Twelve well-trained young men (age: 24.8 ± 3.4 yr; height: 178.6 ± 6.0 cm; body mass: 84.8 ± 11.0 kg) performed high-load back squat exercise (12 sets at 70-90% of 1-repetition maximum [1RM]) on two occasions, with thigh muscle activation and oxygenation being monitored by EMG and NIRS, respectively. Intra-test reliability for EMG and NIRS variables was generally higher than inter-test reliability. NIRS-derived measures of muscle oxygenation were generally more reliable during single-repetition sets than multiple-repetition sets at the same load. Although the reliability of EMG and NIRS varied across the exercise protocol, the biological variation during multi-joint isoinertial resistance exercise may account for the fluctuations in the observed results.

Study 2 aimed to determine whether different levels of hypoxia affect physical performance during high-load resistance exercise. Using a randomised single

blind cross-over design, 12 resistance-trained males (age: 25.3 ± 4.3 yr; height: 179.0 ± 4.5 cm; body mass: 83.4 ± 9.1 kg) completed three trials of 5 x 5 repetitions of back squats and deadlifts at 80% 1RM with 180 s inter-set rest. Trials took place in normoxia (NORM; fraction of inspired oxygen [F_IO_2] = 21%), moderate-level hypoxia (MH; F_IO_2 = 16%), and high-level hypoxia (HH; F_IO_2 = 13%). Physical performance was monitored during repetitions (force and power variables), and arterial oxygen saturation (SpO_2), heart rate (HR), and a rating of perceived exertion (RPE) were obtained following each set. No differences in performance were evident between conditions. HR was higher following sets in HH than NORM (p = 0.009), while SpO_2 was lower in hypoxic conditions than in NORM (p < 0.001). There were no differences in RPE between conditions. These findings suggest that physical performance and perceived effort during high-load resistance exercise is not affected by supplemental hypoxia.

Study 3 assessed whether hypoxia during high-load resistance exercise could enhance the acute responses thought to underpin IHRT adaptation. Twelve well-trained males (age: 25.3 ± 4.3 yr; height: 179.0 ± 4.5 cm; body mass: 83.4 ± 9.1 kg) performed the same high-load resistance exercise protocol described for Study 2 in NORM, MH and HH. Muscle oxygenation and activation were monitored via NIRS and EMG, respectively. Blood lactate (BLa^-) concentration and pH levels were assessed to quantify metabolic stress. Perceived fatigue and soreness were also quantified following the exercise. HH appeared to cause the lowest levels of muscle oxygenation during exercise, though significant differences between conditions were only observed for maximal

deoxyhaemoglobin in the deadlift (p = 0.009). Metabolic stress increased from baseline following exercise ($p \leq 0.004$), however there were no consistent between-condition differences. Muscle activation, perceived fatigue and soreness also did not differ between conditions. These data suggest that high-load IHRT may not provide added benefit over the equivalent normoxic training, possibly because of its inherent design with long inter-set rest periods.

Study 4 assessed whether moderate-load IHRT with short rest periods could augment acute anabolic responses. Using a randomised single blind cross-over design, 14 well-trained male subjects (age: 24.6 ± 2.7 yr; height: 179.7 ± 5.9 cm; body mass: 84.6 ± 11.6 kg) performed resistance exercise trials in NORM and MH (3 x 10 repetitions of back squats and deadlifts at 60% 1RM with 60 s rest). SpO_2 and HR were assessed following each set, and BLa^- concentration was quantified after each exercise. Thigh circumference was measured as a marker of muscle swelling. Muscle activation and oxygenation were monitored via EMG and NIRS, respectively. Relative BLa^- concentrations were significantly higher following both squats (p = 0.041) and deadlifts (p = 0.002) in MH than NORM. SpO_2 was lower following each set in MH ($p < 0.001$), though there were no between-condition differences for HR or thigh circumference. Integrated EMG was higher in the MH trial at several time points for the back squat ($p < 0.001$), but not the deadlift. Muscle oxygenation did not differ between conditions. These data demonstrate that hypoxia during moderate-load resistance exercise with brief rest periods between sets can enhance metabolic stress in concert with increased muscle activation.

Lastly, Study 5 aimed to determine whether hypoxia can affect markers of physical performance, training stress and neuromuscular recovery during moderate-load resistance exercise. Fourteen well-trained male subjects (age: 24.6 ± 2.7 yr; height: 179.7 ± 5.9 cm; body mass: 84.6 ± 11.6 kg) performed the same moderate-load resistance exercise protocol as for Study 4 in NORM and MH. Physical performance was quantified during repetitions (velocity and power). Perceived exertion, fatigue, soreness and wellbeing were assessed during and following exercise. Neuromuscular performance was monitored using vertical jump and isometric mid-thigh pull (MTP) tasks for up to 48 h following exercise. Performance declined across sets ($p \leq 0.010$), though this was not different between conditions. Perceptual responses were also not different between conditions. Jump height and MTP peak force were decreased from pre-exercise values immediately after all trials ($p \leq 0.026$), but returned to pre-exercise values at 24 h. Despite increases in metabolic stress and muscle activation (Study 4), physical performance and markers of training stress were not impacted by hypoxia during moderate-load resistance exercise.

This collective work has highlighted the importance of structuring exercise using sufficient repetition volume and brief inter-set rest periods to elicit hypoxia-mediated benefits. Moderate-load IHRT with short rest in hypoxia was shown to enhance metabolic stress and muscle activation, which may maximise adaptation to resistance training. Importantly, supplementary hypoxia did not affect markers of training stress or recovery of neuromuscular function, making this an attractive strategy for already well-trained individuals.

Chapter 1

Introduction

Background Information

Skeletal muscle is recognised as a highly plastic tissue that can be altered in responses to a given stimulus. Most notably, resistance exercise has a potent effect on increases in the size and strength of muscle (Kraemer, Fleck, & Evans, 1996). Athletic and clinical populations alike perform resistance training to facilitate muscular development and elicit beneficial functional adaptations. Traditionally, acute resistance exercise variables have been manipulated to provide a desired training stimulus. These variables include the muscle action, loading and volume, exercise selection and performance order, inter-set rest periods, repetition velocity and training frequency (Bird, Tarpenning, & Marino, 2005). Following a bout of resistance exercise, a complex cascade of biological events occurs in response to the exercise stimulus, including metabolic and hormonal alterations, intramuscular signalling processes and subsequent protein synthesis (Spiering et al., 2008a).

It is generally accepted that moderate- or high-load resistance training should be performed using loads equivalent to at least 70% of 1-repetition maximum (1RM) to facilitate substantial hypertrophic and strength gains (ACSM, 2009). However, research has begun to investigate whether other factors, such as the intramuscular environment, can be manipulated through various practices to enhance the physiological processes associated with muscular development (Kacin & Strazar, 2011). Mounting evidence suggests that low-load resistance exercise (20-50% 1RM) can result in marked increases in muscle strength and size when combined with moderate blood flow restriction (BFR) in both clinical

(Ohta et al., 2003) and athletic (Cook, Kilduff, & Beaven, 2014; Manimmanakorn, Hamlin, Ross, Taylor, & Manimmanakorn, 2013a) cohorts. This novel strategy involves the application of an inflatable cuff (Takano et al., 2005), elastic wraps (Loenneke & Pujol, 2009) or tourniquet (Shinohara, Kouzaki, Yoshihisa, & Fukunaga, 1998) around the top of a limb, with the aim to maintain blood delivery to contracting muscles but occlude venous return during exercise. Resistance training using loads this light would not normally facilitate substantial muscular development, and as such the observed physiological responses evoked by the BFR stimulus have become of interest to researchers.

Several mechanisms are likely to underpin the augmented adaptive responses to resistance exercise combined with BFR. It is suggested that the hypoxic intramuscular environment created by the BFR stimulus is important for subsequent anabolic responses (Kacin & Strazar, 2011). Downstream of this hypoxic environment, it is likely that a greater accumulation of metabolites, resulting from both an increased production due to the hypoxia and limited removal due to the BFR itself, acts as a primary moderator of the anabolic response to this form of exercise (Schoenfeld, 2013). Importantly, this accumulation of metabolites may increase muscle cell swelling (Yasuda, Loenneke, Thiebaud, & Abe, 2012), intramuscular anabolic/anti-catabolic signalling (Fry et al., 2010; Fujita et al., 2007; Laurentino et al., 2012), and muscle fibre recruitment (Takarada et al., 2000a; Yasuda et al., 2009), which are all thought to be beneficial for muscular adaptation. Furthermore, evidence suggests that the localised hypoxic environment created during BFR may

increase the activation and proliferation of myogenic stem cells, enhancing the hypertrophic response (Nielsen et al., 2012). Significantly elevated endocrine responses have also been observed with BFR (Takarada et al., 2000a), though the role of acute exercise-induced endocrine responses in resistance training adaptation has been recently questioned (West & Phillips, 2010). However, the use of BFR limits the hypoxic stimulus to the limb muscles, and as such may not be as beneficial for those targeting adaptation in the muscles of the trunk.

In light of the augmented muscular development from BFR training, recent research has begun to examine whether a systemic hypoxic stimulus, created by breathing hypoxic air during resistance exercise, can also result in enhanced muscular adaptation. Separate to BFR training, intermittent hypoxic resistance training (IHRT) allows muscles of the trunk to be trained under the same hypoxic conditions as the limbs, and may attenuate some detrimental side-effects commonly experienced with BFR, including subcutaneous haemorrhage and limb numbness (Nakajima et al., 2006). Early research demonstrated that moderate-load IHRT (70% 1RM; fraction of inspired oxygen [F_IO_2] = 16%) resulted in greater hypertrophy and faster improvements in strength than the equivalent training in normoxia (Nishimura et al., 2010). Similarly, greater improvements in muscular strength, endurance and size have been reported following low-load IHRT (20% 1RM; F_IO_2 adjusted to maintain arterial oxygen saturation [SpO_2] at 80%) than work-matched training in normoxia (Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b). These results

indicate that supplementing resistance exercise with a hypoxic stimulus may enhance the physiological responses that underpin muscular development.

However, some research has observed limited or no additional benefit for resistance training in hypoxia, with similar hypertrophic and strength increases between IHRT and normoxic training groups (Ho, Kuo, Liu, Dong, & Tung, 2014b; Kon et al., 2014). These conflicting data are likely due to differences in the training protocols and hypoxic doses applied. Studies that have employed short inter-set rest periods and moderate-level hypoxia have shown benefits for muscular development (Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b; Nishimura et al., 2010), whereas those that have used extended inter-set rests and high-level hypoxia have reported limited or no additional benefit for muscular development (Ho et al., 2014b; Kon et al., 2014; Kurobe et al., 2015). Thus, it appears that the responses to IHRT may be highly dependent on the type of training performed and the hypoxic stimulus employed.

The potential benefits of IHRT have important applications for athletes who are required to concurrently develop several physiological qualities in conjunction with sport-specific technical skills. Training for numerous physiological adaptations can be time consuming and demanding on an athlete's body. It is possible that with appropriate implementation of IHRT, significant increases in muscle size and strength may be achieved using lower training volumes than those required in normoxia. This is important, considering that increased training loads above normal levels can lead to performance decrements, injury

and illness (Foster, 1998). However, the majority of IHRT research has used untrained participants, and it is not clear whether the results of these investigations can translate to well-trained individuals. Furthermore, it is possible that hypoxia may have negative effects on physical performance during IHRT, as well as increasing the perceived difficulty of exercise, which would limit the efficacy of this novel strategy. To understand whether IHRT strategies may be beneficial for muscular development in well-trained cohorts, research into the acute responses to various resistance exercise protocols and levels of hypoxia is therefore warranted.

Statement of the Problem

Research has demonstrated that both low-load (Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b) and moderate-load (Kurobe et al., 2015; Nishimura et al., 2010) IHRT can substantially augment strength development and/or hypertrophy. Equivocally, some authors have reported no additional benefit for body composition or strength following IHRT when compared to the equivalent normoxic training (Ho et al., 2014b; Kon et al., 2014). It is likely that the differences between studies is largely due to inconsistencies in research methodologies, particularly the inter-set recovery periods (and subsequent metabolic stress associated with exercise), and the level of hypoxia employed. Current knowledge regarding the affects of hypoxia on resistance exercise performance, and the mechanisms by which IHRT may augment muscular adaptation to training is very limited. Furthermore, while it is probable that in real-world settings only high-level athletes would have access to the hypoxic

equipment required to perform IHRT, only one investigation has used trained participants (Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b), although their resistance training history was not reported. Therefore, a comprehensive investigation of IHRT for well-trained participants is required to determine the efficacy of this novel training strategy, and to elucidate the mechanisms by which hypoxia may benefit muscular development.

Purpose of the Thesis

The primary aim of this series of research studies was to investigate the acute physiological, physical and perceptual responses to high- and moderate-load IHRT in well-trained participants. Specifically, this research thesis aimed:

1. To review existing literature detailing the adaptive responses to hypoxic resistance training strategies, and highlight the potential mechanisms by which hypoxia may augment resistance training adaptation;

2. To quantify the intra-test and inter-test reliability of telemetric methods to estimate muscle activation and oxygenation status during resistance exercise (Study 1);

3. To quantify the impact of hypoxia on physical performance during high-load resistance exercise in hypoxia (Study 2);

4. To examine the acute physiological responses to high-load resistance exercise in hypoxia (Study 3);

5. To examine the acute physiological responses to moderate-load resistance exercise in hypoxia (Study 4), and;

6. To assess the impact of hypoxia during moderate-load resistance exercise on indices of training stress (Study 5).

Significance of the Study

Athletes, coaches and sport scientists are in the continual pursuit for novel training methods that enhance the adaptive responses to an exercise stimulus. This research provides information relating to the acute physiological, physical and perceptual responses of well-trained individuals to high- and moderate-load exercise under hypoxic conditions. Such data are of great significance for athletic populations who are required to develop muscular size and strength. Indeed, if IHRT can stimulate a greater anabolic response than normoxic resistance exercise for the same mechanical load (as indicated by acute physiological responses), it is likely that this practice will gain popularity in professional sporting settings. It is anticipated that the findings of this research will elucidate whether some of the mechanisms thought to underpin adaptive responses to IHRT in untrained cohorts are enhanced in well-trained participants. This would also have strong implications for strength and conditioning practices in elite sport, as well as providing rationale for using systemic hypoxia to enhance resistance training adaptations.

Limitations and Assumptions

The following limitations and assumptions may apply to the studies presented within this thesis:

1. *Specificity of the results*

   The data collected within these studies were obtained from well-trained young men with at least two years resistance training experience. The results may lack validity for untrained or female populations, or for age groups dissimilar to those in the current studies.

2. *Technological restrictions*

   The use of surface electromyography (EMG) and near-infrared spectroscopy (NIRS) technologies are limited by their varying reliability during dynamic multi-joint resistance exercise (see Study 1).

3. *Maintenance of strength levels and exercise technique*

   At times throughout the present study, data were collected over numerous weeks for all participants. It is therefore assumed that the participants maintained their physiological capacities and technical abilities throughout the entirety of the data collection period.

4. *Inter-individual differences in responses to hypoxia and exercise*

   Although all participants were subjected to identical hypoxic conditions during experimental trials, it is possible that there were inter-individual differences in physiological responses to hypoxia. Similarly, it is possible that the homogeneous resistance exercise protocols were perceived to be of different intensity by some individuals.

5. *Capillary blood measures reflect muscular changes in metabolic stress*

   While capillary blood sampling is a common and practical technique to estimate metabolic stress following exercise, it may not completely reflect changes at the muscular level.

Delimitations

The following delimitations may apply to the present research:

1. *Limited sample size*

   Due to the time demanding nature of the studies, the number of participants was limited for each study. However, the sample size of each study is typical of previous research assessing similar performance outcomes.

2. *Gender restrictions of the participants*

   The participants recruited across the research project were restricted to males to avoid any effect of gender on endocrine responses and exercise performance.

3. *Sample representation*

   The data contained within the entire research project are based on a specific sample of participants, and therefore may not be a true representation of similar populations.

4. *Environmental conditions*

   Experimental trials were conducted in a controlled laboratory environment with consistent temperature and humidity levels. The only environmental factor that was varied was the F_IO_2 during trials, as this was manipulated depending on the trial condition.

Chapter 2

Review of the Literature

Hypoxia and Resistance Exercise: A Comparison of Localised and Systemic Methods

As per the peer-reviewed paper **Accepted and Published** in *Sports Medicine*:

Scott, B. R., Slattery, K. M., Sculley, D. V., & Dascombe, B. J. (2014). Hypoxia and resistance exercise: a comparison of localized and systemic methods. *Sports Medicine*, 44(8), 1037-1054.

This chapter has been edited from the original published manuscript to include IHRT research published since this manuscript's acceptance by Sports Medicine.

Abstract

It is generally believed that optimal hypertrophic and strength gains are induced through moderate- or high-load resistance training, equivalent at least 70% of an individual's 1RM. However, recent evidence suggests that similar adaptations are facilitated when low-load resistance exercise (~20-50% 1RM) is combined with BFR to the working muscles. Although the mechanisms underpinning these responses are not yet firmly established, it appears that localised hypoxia created by BFR may provide an anabolic stimulus by enhancing the metabolic and endocrine response, and in turn increasing cellular swelling and signalling function following resistance exercise. Moreover, BFR has also been demonstrated to increase type II muscle fibre recruitment during exercise. However, inappropriate implementation of BFR can result in detrimental effects, including petechial haemorrhage and dizziness. Further, as BFR is limited to the limbs, the muscles of the trunk are unable to be trained under localised hypoxia. More recently, the use of systemic hypoxia via hypoxic chambers and devices has been investigated as a novel way to stimulate similar physiological responses to resistance training as BFR techniques. While little evidence is available, reports indicate that beneficial adaptations, similar to those induced by BFR, are possible using these methods. The use of systemic hypoxia allows large groups to train concurrently within a hypoxic chamber using multi-joint exercises. However, further scientific research is required to fully understand the mechanisms that cause augmented muscular changes during resistance exercise with a localised or systemic hypoxic stimulus.

Introduction

Resistance exercise has a potent effect on increases in the size and strength of skeletal muscle (Kraemer et al., 1996). The efficacy of a resistance training program is determined largely by the manipulation of acute training variables, such as muscle action, loading and volume, exercise selection and order, inter-set rest periods, repetition velocity and training frequency (Bird et al., 2005). A complex cascade of biological events occurs in response to the mechanical stimulus, including metabolic and hormonal alterations, intramuscular signalling processes, and subsequent protein synthesis (Spiering et al., 2008a). For instance, local accumulation of metabolic by-products during resistance exercise, such as lactate and hydrogen ions (H^+), stimulates the release of various anabolic hormones (Gordon, Kraemer, Vos, Lynch, & Knuttgen, 1994; Goto, Ishii, Kizuka, & Takamatsu, 2005; Häkkinen, Pakarinen, Alen, Kauhanen, & Komi, 1988; Takarada et al., 2000a; Viru, Jansson, Viru, & Sundberg, 1998). These hormones are proposed to promote muscular hypertrophy by increasing protein synthesis and decreasing protein degradation (Crewther, Keogh, Cronin, & Cook, 2006; Florini, Ewton, & Coolican, 1996; McCall, Byrnes, Fleck, Dickinson, & Kraemer, 1999). Further, the accumulation of metabolites may facilitate cellular swelling and moderate subsequent signals for growth (Loenneke, Fahs, Rossow, Abe, & Bemben, 2012a). It is evident that both the mechanical stress applied and methods to up-regulate physiological responses, should be considered when designing resistance training programs. It may be possible to manipulate these physiological responses using hypoxic stimuli to accelerate strength gains from resistance exercise.

The use of a hypoxic stimulus during resistance exercise was initially studied via BFR techniques (Shinohara et al., 1998; Takarada et al., 2000a; Takarada et al., 2000b). More recently, devices that create a systemic hypoxic environment via nitrogen dilution or oxygen extraction have been used (Kon et al., 2010; Kon, Ikeda, Homma, & Suzuki, 2012; Nishimura et al., 2010). The addition of a hypoxic stimulus is suggested to increase the metabolic and hormonal responses to resistance exercise, in turn enhancing the subsequent hypertrophic and strength responses (Kon et al., 2010; Kon et al., 2012). However, both methods of hypoxic exposure (BFR and systemic) have inherent limitations. Therefore, the purpose of this review is to summarise the current body of literature that has reported on resistance exercise under hypoxic conditions. Current limitations of hypoxic techniques will also be examined. Where applicable, the underlying mechanisms that may facilitate strength and hypertrophic gains will be described.

Resistance Exercise with Blood Flow Restriction

The BFR technique involves application of a tourniquet (Shinohara et al., 1998), inflatable cuff (Takano et al., 2005) or elastic knee wraps (Loenneke, Kearney, Thrower, Collins, & Pujol, 2010) around the proximal end of a limb to occlude distal blood flow, thus inducing a localised hypoxic environment during exercise (Loenneke & Pujol, 2009). Early research utilised occlusive pressures in excess of 200 mmHg (Takarada et al., 2000a), though more recent findings have demonstrated beneficial results with pressures as low as 50 mmHg (Sumide, Sakuraba, Sawaki, Ohmura, & Tamura, 2009). Numerous factors can affect the

acute responses to BFR resistance exercise, including the occlusive pressure used, cuff location, width and type, exercise intensity, volume and inter-set rest periods, as well as the frequency and duration of training, and whether exercise is performed to volitional fatigue or not. Training with BFR (sometimes referred to as Kaatsu training) is currently promoted as a novel training method that enhances muscle hypertrophy and strength (Nakajima, Morita, & Sato, 2011). While the American College of Sports Medicine typically recommends that resistance training intensity exceed 70% 1RM to induce optimum hypertrophy (ACSM, 2009), numerous studies have demonstrated substantial increases in hypertrophy and strength following 2-16 weeks of BFR training at intensities as low as 20% 1RM (Abe et al., 2005b; Sumide et al., 2009; Takarada, Sato, & Ishii, 2002; Takarada, Tsuruta, & Ishii, 2004). Although the precise mechanisms are not yet clear, the augmented responses to resistance exercise with BFR are believed to be accounted for by a greater accumulation of metabolites and concomitant increases in anabolic hormone concentrations, intramuscular signalling, intracellular swelling and motor unit recruitment (Loenneke et al., 2012a; Takano et al., 2005; Takarada et al., 2000a). The following sections of this review will detail the adaptive and perceptual responses, potential causative mechanisms as well as the practical applications of BFR training.

Adaptive and Perceptual Responses to Blood Flow Restriction Training

Morphological Adaptations

Several researchers have observed increased muscle cross-sectional area (CSA) following BFR training (Abe et al., 2005b; Kacin & Strazar, 2011;

Madarame et al., 2008; Takarada et al., 2002; Takarada et al., 2000b; Takarada et al., 2004). Takarada et al. (2000b) reported that 16 weeks of low-load (~50-30% 1RM) elbow flexion training (twice per week) with BFR elicited greater increases in muscle CSA in older women than low-load training alone. More recently, Manimmanakorn et al. (2013a) observed that the CSA of knee extensors and flexors increased by 6.6 ± 4.5% (mean ± SD) following five weeks of low-load (20% 1RM) BFR training in netball athletes, whereas the CSA increased by 2.9 ± 2.7% in the control group. While many of these investigations recruited participants with little or unspecified resistance training experience, recent research indicates that BFR exercise may also be beneficial for resistance-trained athletic populations (Abe et al., 2005a; Cook et al., 2014; Yamanaka, Farley, & Caputo, 2012). Low-load (20% 1RM) resistance exercise combined with BFR has resulted in greater hypertrophy than in non-restricted control groups in track and field athletes (Abe et al., 2005a) and American football players (Yamanaka et al., 2012). As these participants already have achieved a high level of muscular adaptation to resistance training, low-load resistance training would not normally have facilitated hypertrophic gains. Therefore, the addition of BFR during resistance exercise appears to also benefit skeletal muscle adaptation in resistance-trained athletes.

Increases in Muscular Strength

Several authors have reported increased peak torque and maximal rate of torque development across a range of angular velocities following low-load (20-50% 1RM) BFR training, despite no significant changes in control groups

performing the equivalent training without BFR (Shinohara et al., 1998; Takarada et al., 2002; Takarada et al., 2000b; Takarada et al., 2004). These increases in strength are likely due to concomitant increases in muscle fibre CSA and neural adaptations. While general strength is largely dependent upon muscle CSA and contractile properties (Schantz, Randall-Fox, Hutchison, Tyden, & Astrand, 1983), low-load resistance exercise with BFR has also been reported to increase muscle fibre recruitment during exercise (Moore et al., 2004; Takarada et al., 2000a; Takarada et al., 2002; Yasuda et al., 2009; Yasuda et al., 2006). Numerous investigations have noted that neural drive, measured via the amplitude of muscle EMG signals, is increased following a period of traditional resistance training (Gabriel, Kamen, & Frost, 2006). As neural adaptations are predominant in strength gains during the early stages of resistance training (Behm, 1995), it is possible that strength gains in untrained subjects following BFR training are partly explained by neuromuscular adaptation, which may be augmented by consistent increases in muscle recruitment. However, it should be acknowledged that low-load BFR resistance exercise does not increase muscle activation to the same degree as higher-load resistance exercise without BFR (Cook, Murphy, & Labarbera, 2013; Manini & Clark, 2009). Therefore, it is likely that neural adaptations to BFR training are dissimilar to those experienced following traditional high-load resistance training.

Low-load BFR training has also been shown to elicit significant increases in maximal isometric strength and muscular endurance across 50 repeated

submaximal contractions compared to low-load resistance training without BFR (Takarada et al., 2002). This improvement in muscular endurance may reflect intramuscular metabolic adaptations (i.e. increases in oxidative energy metabolism and H^+ buffering) rather than enhanced neural fatigue resistance. In support of this suggestion, no changes were present in the integrated EMG pattern during the initial or the last 10 of the 50 repeated contractions (Takarada et al., 2002). Considering the available evidence, it is evident that low-load resistance exercise with BFR can enhance various indices of strength ranging from muscular endurance to maximal strength and rate of force development.

Perceptual Responses

Perceptual responses to resistance exercise are important for monitoring and regulating exercise intensity, providing general markers of the physiological demands during training. Generally, it appears that resistance exercise with BFR results in similar rating of perceived exertion (RPE) values to the equivalent exercise without BFR (2009; Wernbom, Augustsson, & Thomee, 2006; Wernbom, Jarrebring, Andreasson, & Augustsson, 2009). However, in a recent study that used knee wraps to occlude blood flow, subjects reported significantly greater RPE scores following low-load knee extension to failure with BFR than without (Loenneke, Balapur, Thrower, Barnes, & Pujol, 2011a). Further, contrasting data have been reported relating to perceived pain during BFR resistance exercise. Some authors have reported that pain was similar between BFR and control groups, following low-load resistance exercise to exhaustion (Manimmanakorn et al., 2013a; Wernbom et al., 2009), while others

observed significantly greater pain scores following low-load resistance exercise with BFR than without (Loenneke et al., 2011a; Wernbom et al., 2006). These conflicting data might be explained by differences in the method used to restrict blood flow (i.e. narrow elastic knee wraps versus inflatable cuffs), the occlusive pressure used, and the width of the occlusive cuffs (Crenshaw, Hargens, Gershuni, & Rydevik, 1988; Graham, Breault, McEwen, & McGraw, 1993; Wernbom et al., 2009).

Potential Mechanisms of Blood Flow Restriction for Hypertrophy and Strength

Concentration of Metabolites

The anabolic response to exercise-induced metabolic stress is well documented (Schoenfeld, 2013). Research suggests that when resistance exercise is performed with BFR, a significantly greater metabolic stress is observed via exaggerated phosphocreatine (PCr) depletion (Suga et al., 2009; Suga et al., 2012), increased inorganic phosphate (P_i) (Suga et al., 2009; Takada et al., 2012), pH decreases (Suga et al., 2009; Suga et al., 2012), and increased lactate production (Fujita et al., 2007; Pierce, Clark, Ploutz-Snyder, & Kanaley, 2006; Reeves et al., 2006; Takano et al., 2005; Takarada et al., 2000a). In an early investigation, Takarada et al. (2000a) examined the physiological responses to 5 sets of bilateral leg extensions (20% 1RM) to exhaustion, either with or without BFR. Immediately post exercise, plasma lactate concentration was doubled in the BFR group compared to the control (Takarada et al., 2000a). More recently, Suga et al. (2012) reported that low-load plantar flexion

exercise (3 sets of 30 repetitions at 20% 1RM) with BFR resulted in a similar metabolic stress (namely intramuscular metabolites and pH) when compared to moderate-load (65% 1RM) exercise without BFR. Furthermore, increases with muscle CSA following BFR training have been strongly correlated to metabolic stress, measured via increases in the P_i (r = 0.87) and decreases in pH (r = 0.60) (Takada et al., 2012). Taken together, these findings suggest that the decreased availability of oxygen within the working muscles during BFR increases the reliance on anaerobic metabolism (Kawada, 2005), and restricts lactate clearance. This augmented metabolic response to resistance exercise with BFR may potentially lead to greater type II muscle fibre recruitment, hormonal responses, intramuscular signalling and intracellular swelling (Schoenfeld, 2013).

Hormonal Responses

The endocrine responses to resistance training may play an important role in regulating anabolic processes (Schoenfeld, 2010). Elevated concentrations of hormones such as growth hormone (GH), insulin-like growth factor-1 (IGF-1) and testosterone increase the likelihood of hormone receptor interactions that promote anabolic processes (Crewther et al., 2006). Generally, investigations have reported an augmented hormonal response to performing resistance exercise under BFR conditions (Kon et al., 2010; Kon et al., 2012; Pierce et al., 2006; Takarada et al., 2000a). Several researchers have reported significantly greater plasma GH concentrations following resistance exercise with BFR than without (Patterson, Leggate, Nimmo, & Ferguson, 2013; Pierce et al., 2006;

Reeves et al., 2006; Takano et al., 2005; Takarada et al., 2000a; Takarada et al., 2004). In their seminal study, Takarada et al. (2000a) reported GH elevations of ~290 times greater than baseline after BFR trials, without any significant increase in the control group exercising without BFR. Furthermore, 3 sets of low-load (30% 1RM) resistance exercise with BFR facilitated a 4-fold increase in GH, despite no significant rise following moderate-load exercise (70% 1RM) without BFR (Reeves et al., 2006). The GH response to low-load resistance exercise with BFR appears to provide a significant anabolic stimulus, potentially even greater than traditional resistance exercise designed to promote hypertrophy using much higher intensities (~70% 1RM) without BFR (ACSM, 2009; Kraemer, Kilgore, Kraemer, & Castracane, 1992).

Many of the anabolic actions of GH are mediated by IGF-1 (Crewther et al., 2006; Kraemer & Ratamess, 2005), which is predominantly synthesised and released in response to circulating GH levels (Le Roith, Bondy, Yakar, Liu, & Butler, 2001). Some research has reported that IGF-1 is up-regulated following acute bouts of low-load BFR resistance exercise (Takano et al., 2005) and to high-frequency BFR training (twice daily) (Abe et al., 2005b), which are similar in magnitude to typical responses following high-load resistance training without BFR (Borst et al., 2001; Marx et al., 2001). However, despite noting enhanced GH responses following low-load (20% 1RM) resistance exercise with BFR, neither Fujita et al. (2007) or Patterson et al. (2013) observed concomitant increases in IGF-1. This discrepancy may be explained by the different time course of change in GH and IGF-1 (Kraemer & Ratamess, 2005), with peaks in

IGF-1 typically occurring at 16-28 h following GH release (Chandler, Byrne, Patterson, & Ivy, 1994; Kraemer & Ratamess, 2005). While it is likely that the high-frequency BFR training employed by Abe et al. (2005b) resulted in chronically elevated GH levels and concurrent increases in IGF-1, it is unclear at this time what mechanisms may have facilitated the higher IGF-1 levels following a single bout of resistance exercise with BFR.

Testosterone has anabolic effects on skeletal muscle directly by increasing protein synthesis and decreasing protein degradation, and indirectly by stimulating other anabolic hormones (Crewther et al., 2006). However, low-load resistance exercise with BFR appears not to augment testosterone responses (Fujita et al., 2007; Reeves et al., 2006). These results may be explained by the low volume and/or intensity of the exercise protocols used, which might have been insufficient to elicit changes in testosterone levels. Significant increases in testosterone have previously been demonstrated following non-restricted multi-joint resistance training of considerably higher volume and intensity (Kraemer et al., 1990). This suggests that the magnitude of testosterone responses might not be affected by the degree of metabolic stress during resistance exercise (Durand et al., 2003; Goto et al., 2005), but rather by factors such as the amount of muscle mass stimulated, and the intensity and volume of exercise (Pope, Willardson, & Schoenfeld, 2013).

The actions of GH and testosterone are enhanced by catecholamine release, which reflects the acute demands of exercise, as well as influencing force

production, muscle contraction rate and energy availability (Giustina & Veldhuis, 1998; Kraemer, 2000; Weltman et al., 2000). Research has reported low-load resistance exercise with BFR to increase norepinephrine secretion, in concert with GH and lactate levels, when compared to the equivalent exercise without BFR (Takano et al., 2005; Takarada et al., 2000a). However, the relationship between GH and norepinephrine levels was not significant (Takano et al., 2005). It is therefore likely that a combination of anaerobic factors such as local ischemia and accumulation of lactate and H^+ ions may stimulate peripheral afferent neural activity, resulting in an enhanced GH-releasing hormone secretion and/or inhibition of somatostatin release from the hypothalamus (Giustina & Veldhuis, 1998; Godfrey, Madgwick, & Whyte, 2003; Takano et al., 2005). As such, despite the moderating effect that catecholamines may have on other anabolic hormones, their exact role during BFR exercise is not yet fully understood.

Cortisol is released from the adrenal cortex in response to stress during exercise (Kon et al., 2010), and promotes catabolism via decreased protein synthesis and increased protein degradation (Kraemer & Ratamess, 2005). The cortisol response to resistance exercise appears to be dependent on the stress and metabolic requirements of the exercise (Goto et al., 2005; Smilios, Pilianidis, Karamouzis, & Tokmakidis, 2003). Fujita et al. (2007) reported an increase in serum cortisol after low-load resistance exercise with BFR, despite no changes in the control condition, possibly reflecting additional stress from BFR. In contrast, Reeves et al. (2006) observed no significant change in serum

cortisol in any experimental conditions. Cortisol responses to exercise primarily occur following high-load exercise, possibly due to congruous anaerobic metabolic factors (Kraemer et al., 1989). Thus, these conflicting findings may reflect differences in the exercise protocols, particularly the short 30 s inter-set rest period used by Fujita et al. (2007).

In general, low-load resistance exercise combined with BFR promotes a favourable anabolic endocrine response, similar to traditional training programs designed to promote hypertrophy (Kraemer et al., 1992). However, while GH appears to be the primary hormone affected by BFR, the direct influence of GH on strength gains remains equivocal (Godfrey et al., 2003). Indeed, the role of systemic endocrine responses in resistance training adaptation has been a point of conjecture in recent years, with some proposing that there is no evidence to suggest transient exercise-induced changes in GH have anabolic effects in healthy individuals (West & Phillips, 2010). West, Burd, Staples, and Phillips (2010) contend that exercise-mediated hypertrophy is in fact an intrinsic process dependent on intramuscular signalling, rather than systemic increases in anabolic hormones. Therefore, it must be acknowledged that systemic hormones and growth factors may not be as important for protein synthesis as once though. Beneficial adaptive responses to BFR training may therefore be moderated by other factors in addition to an increased endocrine response.

Intramuscular Signalling

Recent evidence suggests that improvements in muscular size and strength following low-load resistance training (20% 1RM) with BFR rely considerably on the proliferation and differentiation of myogenic stem cells, resulting in the addition of myonuclei to the exercised fibres (Nielsen et al., 2012). Mechanical deformation of muscle fibres during contractile processes and stretching also stimulate intramuscular signalling pathways independently of hormones and growth factors (Hornberger et al., 2004). In particular, mechanical disruptions activate the mammalian target of rapamycin (mTOR) pathway, which moderates the adaptive responses via translation initiation and muscle protein synthesis (MPS) (Wang & Proud, 2006). Activation of mTOR signalling increases MPS by enhancing translational efficiency (i.e. messenger ribonucleic acid [mRNA] translated per ribosome) (Spiering et al., 2008a), and is therefore critical for subsequent skeletal muscle hypertrophy (Bodine et al., 2001). Fujita et al. (2007) demonstrated an increased phosphorylation of ribosomal S6 kinase 1 (S6K1; a key downstream regulator of the mTOR signalling pathway), as well as significantly higher MPS at 3 h post-exercise in the BFR condition, despite no change in the control. More recently, Fry et al. (2010) reported increases in both MPS and S6K1 phosphorylation at 3 h after 4 sets of low-load (20% 1RM) bilateral knee extensions with BFR, despite no change following equivalent trials without BFR. Similarly, Wernbom et al. (2013) reported enhanced mTOR signalling at 1 h following low-load unilateral knee extensions to failure (30% 1RM) in a BFR condition and not in a non-occluded control. The authors concluded that enhanced mTOR signalling could partly explain the augmented

hypertrophic response induced by low-load resistance exercise with BFR. However, Wernbom et al. (2013) noted that while mTOR signalling was also enhanced at 24 h following exercise, there was no difference between conditions. It is therefore difficult to assess the relative contributions of mTOR signalling in concurrence with growth factors and systemic responses (Wernbom, Augustsson, & Raastad, 2008).

Production of reactive oxygen species (ROS) presents another novel mechanism that may mediate the adaptive responses to BFR training. While chronically elevated levels of ROS have been implicated in harmful biological events, acute production of ROS is important for optimum cellular function and development (Thannickal & Fanburg, 2000; Valko et al., 2007). Previously, ROS have been shown to promote growth in both smooth and cardiac muscle (Suzuki & Ford, 1999), and it is theorised that similar hypertrophic effects may occur in skeletal muscle (Takarada et al., 2000b; Tamaki, Uchiyama, Tamura, & Nakano, 1994; Wernbom et al., 2009). However, while the activity of ROS within muscle is known to increase in ischemic conditions, particularly upon reperfusion (Korthuis, Granger, Townsley, & Taylor, 1985), previous research by Takarada et al. (2000a) reported no change in lipid peroxide levels following low-load resistance exercise either with or without BFR. Similar findings were reported by Goldfarb et al. (2008), who observed no significant increase in markers of oxidative stress (glutathione status and plasma protein carbonyls) following low-load (30% 1RM) resistance exercise with BFR, despite increases following both moderate-load (70% 1RM) resistance exercise without BFR, and

BFR alone. As such, further research is required to investigate whether markers of redox signalling and exercise-induced ROS production are augmented by the addition of BFR, and if ROS play a role in subsequent cellular signalling processes for post-exercise muscle adaptations (Pope et al., 2013).

Contrary to the potential anabolic effects of augmented mTOR signalling and ROS production, myostatin acts as a negative regulator of skeletal muscle growth (Roth & Walsh, 2004). Several investigations have shown that the expression of myostatin is decreased in response to resistance exercise (Hulmi et al., 2007; Kim, Cross, & Bamman, 2005; Petrella, Kim, Cross, Kosek, & Bamman, 2006; Roth et al., 2003; Walker, Kambadur, Sharma, & Smith, 2004), and this response may be vital for optimal hypertrophic adaptation (Kim et al., 2005). Reductions in myostatin mRNA were reported by Drummond et al. (2008) following low-load resistance exercise with BFR. Similarly, Laurentino et al. (2012) demonstrated that myostatin mRNA expression was decreased following eight weeks of low-load (20% 1RM) resistance training with BFR and high-load (80% 1RM) training without BFR. However, Drummond et al. (2008) reported similar reductions in myostatin mRNA in the non-occlusive control group, indicating that the reduced myostatin was unlikely to be solely mediated by BFR. Furthermore, Manini et al. (2011) failed to detect any differences in myostatin mRNA levels following 4 sets of bilateral knee extension exercise at 20% 1RM either with or without BFR. These contrasting findings suggest significant reductions in myostatin expression may require prolonged BFR training, rather than a single exposure (Pope et al., 2013).

Intracellular Swelling

Intracellular swelling is a novel mechanism that has been proposed by numerous authors to mediate anabolic responses to resistance exercise with BFR (Abe et al., 2012; Fujita, Brechue, Kurita, Sato, & Abe, 2008; Loenneke et al., 2012a; Schoenfeld, 2013). Cell swelling is maximised during anaerobic exercise, due to the osmotic changes caused by lactate accumulation (Sjogaard, Adams, & Saltin, 1985). The localised hypoxic environment created by BFR increases the production of metabolites, while the occlusion itself limits venous outflow which promotes further metabolite accumulation (Loenneke et al., 2012a). Thus, a resultant increase in the flow of water into the cell is required to equilibrate the osmotic gradient (Loenneke et al., 2012a).

Research has demonstrated that hydration-mediated cellular swelling increases protein synthesis and decreases proteolysis in hepatocytes, osteocytes, breast cells and muscle fibres (Lang et al., 1998). In muscle, it is proposed that cell swelling may trigger the proliferation of satellite cells and facilitate their fusion to hypertrophying muscle fibres (Dangott, Schultz, & Mozdziak, 2000). Swelling-mediated increases in pressure against the cytoskeleton or cellular membrane may be perceived to threaten cellular integrity, causing the cell to initiate a signalling response to reinforce its ultrastructure (Lang, 2007; Schoenfeld, 2010, 2013). This could include anabolic signalling cascades such as mTOR and mitogen-activated protein kinase (MAPK) pathways (Loenneke et al., 2012a; Schoenfeld, 2010, 2013), which are known to be stimulated by low-load resistance exercise with BFR (Fry et al., 2010; Fujita et al., 2007).

However, it should be noted that evidence in opposition to the cell swelling theory has been recently presented. Gundermann et al. (2012) compared low-load resistance exercise (20% 1RM) with hyperemia simulated via pharmacological vasodilation against the equivalent exercise with BFR. Increases in mixed muscle fractional synthetic rate, and phosphorylation of mTOR, S6K1 and extracellular signal-regulated kinases were observed in the BFR trials, but not in the vasodilation group. However, the initial hyperemic response to pharmacological vasodilatation (first 10 minutes) did not replicate that observed following BFR exercise, and possibly did not reach a threshold required to stimulate significant anabolic signalling. Further, while decreased proteolysis may enhance net protein accretion during cellular swelling (Schoenfeld, 2013), this was not measured. While limited research that has examined precisely how cellular swelling may promote adaptation to BFR resistance exercise, this novel mechanism warrants future research attention.

Muscle Fibre Recruitment

Several investigations have reported increased levels of muscle activation during resistance exercise with BFR using surface EMG (Moore et al., 2004; Takarada et al., 2000a; Yasuda et al., 2009; Yasuda et al., 2006). Takarada et al. (2000a) noted that the BFR trial facilitated 1.8 times greater muscle stimulation than the control, despite no difference in the force generated and the mechanical work produced. This enhanced muscle activation at low levels of force may have been a result of the hypoxic intramuscular environment, where low-threshold type I motor units will readily fatigue, and an increased activation

of more glycolytic (i.e. type II) motor units is required to maintain the same level of force generation (Moritani, Sherman, Shibata, Matsumoto, & Shinohara, 1992; Sundberg, 1994). The size principle suggests that type I fibres are recruited first, with type II fibres being recruited with increasing exercise loads (Henneman, Somjen, & Carpenter, 1965). Given that the hypoxic condition and metabolite accumulation which occur during BFR exercise can stimulate group III and IV afferents (Loenneke, Fahs, Wilson, & Bemben, 2011b; Rotto & Kaufman, 1988; Yasuda et al., 2010a), mechanistically speaking, a reflexive net inhibitory effect on the α-motor neuron may result (Leonard et al., 1994), increasing fibre recruitment to maintain force and protect against conduction failure (Loenneke et al., 2011b; Yasuda et al., 2010a). Therefore, the potential for hypertrophic and strength gains may be augmented by BFR, even at very low training intensities, due to the increased recruitment of type II motor units.

However, it must be acknowledged that some investigations have failed to demonstrate increased muscle activation during resistance exercise with BFR. Both Wernbom et al. (2009) and Kacin and Strazar (2011) have reported similar EMG patterns between BFR and control conditions during low-load (30% 1RM and 15% maximum voluntary contraction [MVC], respectively) unilateral knee extension. Importantly, participants in these investigations exercised to volitional fatigue in both BFR and non-restricted conditions, suggesting that BFR does not increase muscle activation above non-restricted levels when exercise is performed to failure. While increased motor unit activation is likely to contribute strongly to enhanced morphological adaptations following BFR training

(Loenneke et al., 2011b), other factors related to the occlusive stimulus are also likely to play a role. Indeed, research suggests that low-load BFR resistance exercise does not facilitate muscle activation of the same magnitude as higher-load resistance exercise without BFR, when performed to volitional fatigue (Cook et al., 2013; Manini & Clark, 2009). As such, it is unlikely that low-load BFR resistance exercise will stimulate the complete pool of high-threshold motor units, resulting in dissimilar neuromuscular responses to that experienced following high-load training without BFR. Table 2.1 summarises the potential mechanisms underpinning BFR resistance exercise discussed in this section.

Practical Applications and Limitations of Blood Flow Restriction

The muscular adaptations to BFR training may benefit populations such as the elderly or post-surgery rehabilitation patients that exhibit compromised strength and/or joint stability (Wernbom et al., 2008). Low-load training combined with BFR could reduce joint articular and ligament stress forces when compared with higher-load resistance training (> 70% 1RM), decreasing the incidence of injury whilst still promoting strength and hypertrophic increases (Shinohara et al., 1998; Takarada et al., 2002; Takarada et al., 2000b). Furthermore, low-load BFR training does not require extensive recovery time between training sessions (Abe, 2004), due to the low mechanical stress and reduced muscle damage and inflammation (Takarada et al., 2000a). It may therefore be possible to employ higher training frequencies than traditional resistance training programs (Abe et al., 2005b).

Table 2.1. Summary of the current understanding of physiological responses to resistance exercise with BFR, and factors influencing the magnitude of these responses.

Mechanism	Responses (when compared to the equivalent training without BFR)	Potential factors influencing the magnitude of responses
Metabolic stress	↑ [BLa^-] ↑ PCr depletion ↑ P_i ↓ pH	Inter-set recovery period Occlusion maintained during inter-set recovery Occlusive pressure used (i.e. degree of vascular occlusion)
Hormonal responses	↑ GH ↔ testosterone ? IGF-1 ? catecholamine ? cortisol	The degree of metabolic stress associated with exercise Amount of muscle mass recruited during exercise
Intramuscular signalling	↑ phosphorylation of S6K1 (mTOR signalling) ↑ proliferation and differentiation of satellite cells ↔ ROS ↔ myostatin	Mechanical stress applied (volume and intensity) Level of muscular ischemia
Intracellular swelling	? cell swelling stimulates intrinsic volume sensors ? cell swelling triggers anabolic signalling and MPS	Degree of occlusion and subsequent venous pooling Metabolic stress associated with exercise
Muscle fibre recruitment	↑ motor unit activation ↓ motor unit activation when compared to high-load exercise without BFR	The degree of metabolic stress associated with exercise Possibly no effect when exercise is performed to failure
Reactive hyperaemia	↑ blood flow to muscles following cuff release ? MPS	Level of muscular ischemia Magnitude of hyperaemia likely affects signalling and MPS

BFR blood flow restriction, *[BLa^-]* blood lactate concentration, *PCr* phosphocreatine, *P_i* inorganic phosphate, *GH* growth hormone, *IGF-1* insulin like growth factor-1, *S6K1* ribosomal S6 kinase 1, *mTOR* mammalian target of rapamycin, *ROS* reactive oxygen species, *MPS* muscle protein synthesis, ↑ increase, ↓ decrease, ↔ no significant change, *?* equivocal or currently unknown response

While BFR training appears beneficial for facilitating hypertrophic and strength gains, it is important to recognise its limitations. Evidence suggests that low-load resistance exercise with BFR results in lower motor unit recruitment than higher-load exercise without BFR, therefore providing a lesser neurological stimulus (Cook et al., 2013; Manini & Clark, 2009). It is also possible that while low-load resistance exercise with BFR can result in increased strength and CSA of skeletal muscle, a concomitant increase in the strength of connective tissues may not occur due to the decreased mechanical loading. The possibility exists that the strength of muscle and connective tissues will adapt disproportionately to BFR resistance training, increasing the likelihood of musculotendinous injury if heavy exercise loads are subsequently used.

Furthermore, the logistics of using BFR cuffs with large groups of individuals across a range of exercises may prove impractical, due to equipment and expertise requirements. While the use of elastic wraps to occlude blood flow may simplify BFR application (Loenneke & Pujol, 2009), the occlusive pressure is difficult to monitor with this technique. Importantly, without abundant experience, petechial haemorrhage beneath the skin, chills, numbness and dizziness can result from inappropriately applying cuffs or elastic wraps (Nakajima et al., 2011). Additionally, as BFR is limited to the limbs, muscles of the trunk are unable to be trained under localised hypoxic conditions (Nishimura et al., 2010). Although some evidence suggests that hypertrophic responses are possible in non-occluded muscles following BFR training (Abe et al., 2005b; Yasuda, Fujita, Ogasawara, Sato, & Abe, 2010b; Yasuda, Ogasawara,

Sakamaki, Bemben, & Abe, 2011), the relationship between limb and trunk hypertrophy was not significant (r = 0.54; p = 0.13) (Yasuda et al., 2011). Further research is required before the efficacy of BFR training for increasing the size and strength of the trunk muscles can be established. To overcome these limitations, the use of systemic hypoxia via normobaric hypoxia instead of BFR may provide an attractive alternative for many individuals. Figure 2.1 presents a simplified flowchart of the interplay between well-understood and proposed mechanisms that may affect adaptations to BFR resistance exercise and IHRT.

Resistance Exercise with Systemic Hypoxia

Intermittent hypoxic training, whereby the amount of oxygen available in inspired air is reduced during training, has been shown to improve both aerobic (Meeuwsen, Hendriksen, & Holewijn, 2001) and anaerobic (Bonetti, Hopkins, & Kilding, 2006) performance in athletes. Enhanced metabolic function (i.e. molecular and structural adaptations favouring oxygen transport and utilisation) has also been demonstrated following high-load cycling training in hypoxia, compared to training in normoxia (Roels et al., 2007; Vogt et al., 2001), with muscle adaptations being dependent on the degree of hypoxia and the duration of exposure (Lundby, Calbet, & Robach, 2009). Given that the localised hypoxic environment created by BFR enhances both acute (Fujita et al., 2007; Reeves et al., 2006; Takano et al., 2005; Takarada et al., 2000a) and adaptive (Abe et al., 2005b; Moore et al., 2004; Sumide et al., 2009; Takarada et al., 2002) responses to resistance exercise, it is plausible that similar benefits may result

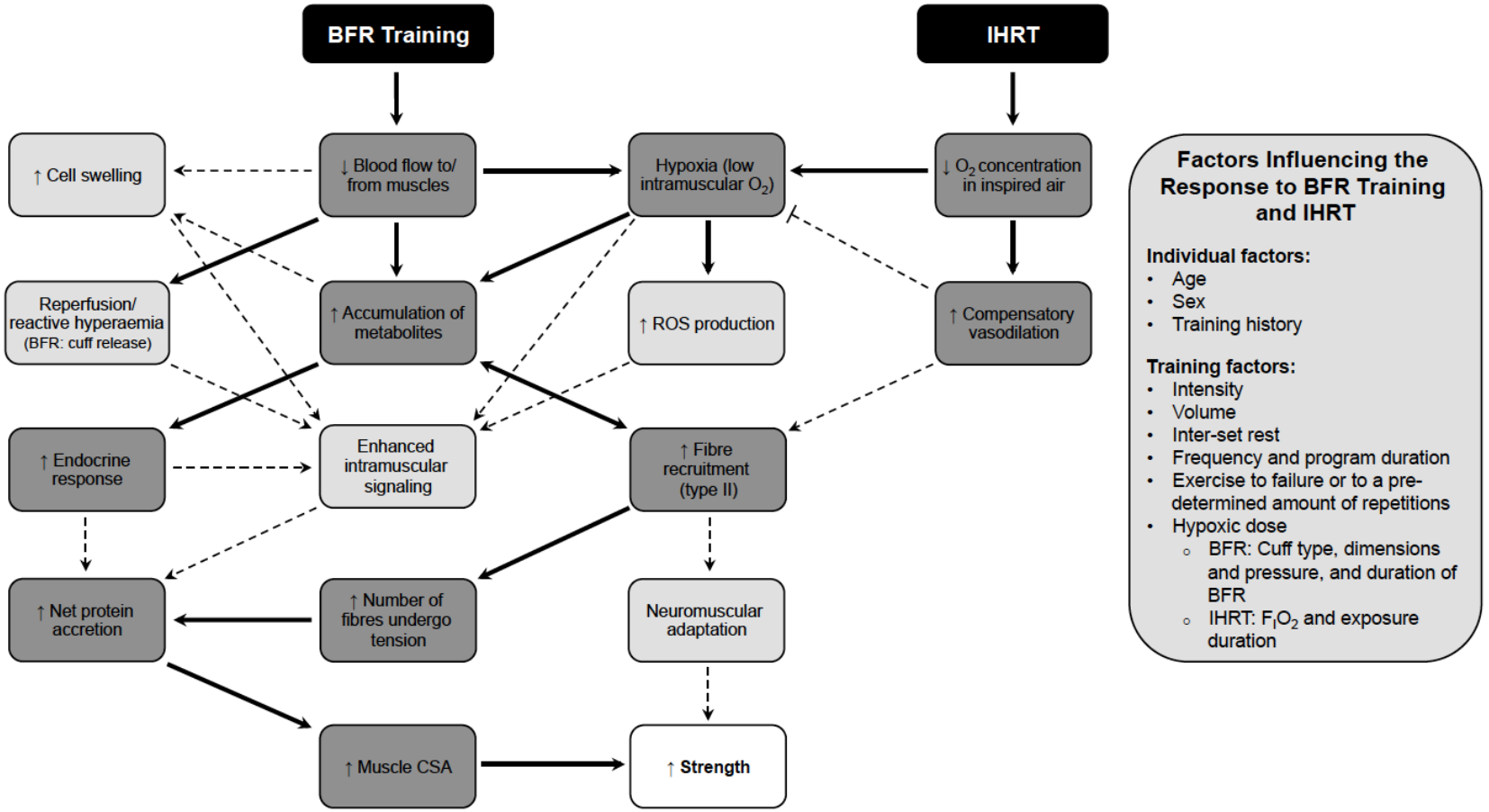

Figure 2.1. Simplified schematic of the proposed interplay between potential mechanisms that may mediate the adaptive responses to BFR training and IHRT.

Note: Likely mechanisms are represented by dark shaded boxes, whereas possible mechanisms that require further research are represented by light shaded boxes. Outcomes of training are represented by white boxes with bold text. Bold arrows indicate a likely link between proposed mechanisms, and dotted arrows indicate a possible link requiring further investigation. Blunted arrow heads indicate an inhibitory effect. While increased muscle CSA is represented here as a mechanism underpinning increases in strength, it may also be considered as a training outcome if hypertrophy is the desired goal. *BFR* blood flow restriction, *IHRT* intermittent hypoxic resistance training, O_2 oxygen, *ROS* reactive oxygen species, *CSA* cross-sectional area, F_IO_2 fraction of inspired oxygen, ↓ decrease, ↑ increase

from performing resistance exercise in systemic hypoxia (Manimmanakorn et al., 2013a). Researchers have recently begun to investigate the use of hypoxic devices, which typically provide a systemic normobaric hypoxic environment via nitrogen dilution or oxygen extraction (Millet, Roels, Schmitt, Woorons, & Richalet, 2010). This allows trunk musculature to be trained under hypoxic conditions with the limbs, providing a novel training strategy for individuals requiring development of these muscles (Nishimura et al., 2010). While the combination of localised hypoxia (via BFR) and resistance exercise has been found to induce beneficial muscular responses (Abe et al., 2005b; Kacin & Strazar, 2011; Madarame et al., 2008; Pierce et al., 2006; Sumide et al., 2009; Takano et al., 2005; 2000a; Takarada et al., 2002; Takarada et al., 2000b; Takarada et al., 2004), few studies to date have examined the physiological responses to IHRT (Table 2.2 and Table 2.3). The following sections of this review will report on the adaptive and perceptual responses following IHRT, before discussing the suggested mechanisms that might underpin these responses.

Table 2.2. Summary of research examining the acute responses to resistance exercise with systemic hypoxia.

Study	Subjects	Testing protocol				Physiological/physical response	Main findings
		Hypoxic dose	Exercise (load)	Sets x reps (inter-set rest; s)	Control group		
Kon et al. (2010)	Healthy males (n=14)	Acute hypoxia (F_IO_2 = 13%); including 15 minutes pre- and 60 minutes post-exercise	Bench press and leg press (70% 1RM)	5 x 10 (60)	Normoxia	↓ SpO_2 in hypoxia Greater ↑ [BLa^-] after hypoxic condition ↑ GH release at 15 and 30 minutes post-exercise after hypoxic condition only ↔ Epinephrine and norepinephrine (though a trend for higher values after hypoxic condition)	Moderate-load resistance exercise in hypoxic conditions can induce greater accumulation of metabolites and a stronger anabolic hormone response than equivalent training in normoxia.
Etheridge et al. (2011)	Healthy males (n=7)	Extended acute hypoxia (F_IO_2 = 12%); 1 h pre-exercise and until 3.5 h exposure	Isometric knee extension (70% MVC)	8 x 6 (120)	Normoxia	↔ peak MVC in hypoxia and normoxia ↑ MPS 2.5 h following resistance exercise in normoxia, but not hypoxia Linear relationship between MPS 2.5 h after resistance exercise in hypoxia and mean arterial blood O_2 saturation during hypoxia (r^2 = 0.49)	MVC is unchanged in hypoxia. 3.5 h hypoxic exposure blunts MPS following resistance exercise. Suppression of MPS correlates with blood O_2 saturation levels.
Kon et al. (2012)	Healthy males (n=8)	Acute hypoxia (F_IO_2 = 13%); including 15 minutes pre- and 30 minutes post-exercise	Bench press and leg press (50% 1RM)	5 x 14 (60)	Normoxia	↓ SpO_2 and muscle O_2 saturation in hypoxia ↑ area under the curve for [BLa^-] after hypoxic condition ↑ GH release at 0 and 15 minutes post-exercise after hypoxic condition only No difference in subjective fatigue between conditions ↔ Epinephrine, norepinephrine and cortisol in either condition	Low-load resistance exercise in hypoxic conditions induced a greater GH response and a trend for increased accumulation of metabolites than equivalent training in normoxia, despite no change in subjective fatigue between conditions.
Ho et al. (2014a)	Healthy males (n=10)	Acute hypoxia (F_IO_2 = 13%); including 10 minutes pre-exercise	Back squat (30% 1RM)	5 x 15 (90)	Normoxia	↓ SpO_2 in hypoxia ↑ [BLa^-] in both trials (no difference between conditions) ↑ GH and testosterone in both trials (no difference between conditions)	Low-load resistance exercise in hypoxia with relatively long rest between sets does not increase accumulation of metabolites or hormonal responses above the equivalent normoxic training.

Table 2.2 (Cont'd). Summary of research examining the acute responses to resistance exercise with systemic hypoxia.

Study	Subjects	Testing protocol				Physiological/physical response	Main findings
		Hypoxic dose	Exercise (load)	Sets x reps (inter-set rest; s)	Control group		
Kon et al. (2014)	Healthy males (n=16)	Acute hypoxia (F_IO_2 = 14.4%); including 10 minutes pre- and 30 minutes post-exercise	Bench Press and leg press (70% 1RM)	5 x 10 (90)	Normoxia	↓ SpO_2 in hypoxia GH concentration appears higher after hypoxic trials (not significant)	Moderate-load resistance exercise in hypoxia causes decreased SpO_2, though GH results were equivocal.
Kurobe et al. (2015)	Healthy males (n=13)	Acute hypoxia (F_IO_2 = 12.7%); for 95 minutes	Unilateral elbow extension (10RM)	3 x to failure (60)	Normoxia	↑ [BLa^-] in both trials (no difference between conditions) GH concentration significantly higher after hypoxic trials	Hypoxia caused increases in GH concentration, though this was not related to [BLa^-].
Yan et al. (In Press)	Healthy males (n=25)	Acute hypoxia (F_IO_2 = 12.6% and 16%); including 30 minutes post-exercise	Back squat (70% 1RM)	5 x 10 (60)	Normoxia	↓ SpO_2 in hypoxia ↑ [BLa^-] following exercise in all conditions, but no large differences between conditions ↑ GH and in both hypoxic conditions compared to normoxia (attenuated across a 5-week training program)	Moderate-load resistance exercise in hypoxia increases GH concentrations, though this was not related to [BLa^-].

reps repetitions, F_IO_2 fraction of inspired oxygen, *1RM* 1 repetition maximum, SpO_2 arterial oxygen saturation, *[BLa^-]* blood lactate concentration, *GH* growth hormone, *MVC* maximal voluntary contraction, *MPS* muscle protein synthesis, O_2 oxygen, ↑ increase, ↓ decrease, ↔ no significant change

Table 2.3. Summary of research examining the morphological and strength responses to resistance training programs with systemic hypoxia.

Study	Subjects	Training protocol						Physiological/physical response	Main findings
		Training conditions	Hypoxic dose	Exercise (load)	Sets x reps (inter-set rest; s)	Frequency (sessions/ week)	Protocol duration (days)		
Friedmann et al. (2003)	Untrained males (n=19)	IHRT	F_IO_2 = 12%/ ~4500 m (only during training)	Unilateral knee extension (30% 1RM)	6 x 25 (60)	3	28	↔ Maximal isokinetic strength in both groups ↔ Muscle CSA in both groups ↑ Strength endurance capacity in both groups (not different between groups) ↔ Muscle fibre type parameters	Low-load resistance exercise in hypoxia did not induce greater increases in strength or hypertrophy.
		RT	~120 m	Unilateral knee extension (30% 1RM)	6 x 25 (60)	3	28		
Nishimura et al. (2010)	Untrained males (n=14)	IHRT	F_IO_2 = 16% (30 min exposure pre- and post-training)	Bilateral elbow extension and flexion (70% 1RM)	4 x 10 (60)	2	42	↑ in Muscle CSA only after IHRT ↑ 1RM after 3 weeks only following IHRT ↑ 1RM after 6 weeks in both groups No between-group differences in RPE at any stage	Moderate-load IHRT induced greater hypertrophic gains, and faster strength increases than the training in normoxia, despite no differences in RPE.
		RT	F_IO_2 = 21%	Bilateral elbow extension and flexion (70% 1RM)	4 x 10 (60)	2	42		
Manimmanakorn et al. (2013a)	Female netball athletes (n=30)	BFR	BFR (160-230 mmHg)	Bilateral knee extension and flexion (20% 1RM)	6 x to failure (30)	3	35	Greater ↑ MVC_3 after IHRT than RT Greater ↑ MVC_{30} after IHRT and BFR than RT Greater ↑ maximum Rep_{20} after IHRT and BFR than RT Substantially greater ↑ in CSA following IHRT and BFR than RT ↑ Perceived muscle pain during days 4-8 in IHRT than BFR and RT Greater ↑ netball specific performance following IHRT and BFR than RT (possibly greater ↑ after BFR than IHRT)	Low-load IHRT facilitated greater improvements in muscle strength and size than training in normoxia, which translated into improved sport-specific performance. However, differences in sport-specific performance between IHRT and BFR groups are not understood.
		IHRT	Not stated (SpO_2 maintained at ~80%)	Bilateral knee extension and flexion (20% 1RM)	6 x matched with BFR (30)	3	35		
		RT	Ambient air	Bilateral knee extension and flexion (20% 1RM)	6 x matched with BFR (30)	3	35		

Table 2.3 (Cont'd). Summary of research examining the morphological and strength responses to resistance training programs with systemic hypoxia.

Study	Subjects	Training protocol						Physiological/physical response	Main findings
		Training conditions	Hypoxic dose	Exercise (load)	Sets x reps (inter-set rest; s)	Frequency (sessions/ week)	Protocol duration (days)		
Manimmanakorn et al. (2013b)	Female netball athletes (n=30)	BFR	BFR (160-230 mmHg)	Bilateral knee extension and flexion (20% 1RM)	6 x to failure (30)	3	35	Moderate effect for ↑ RMS during MVC_3 for BFR versus IHRT and RT (0.80) Moderate effect for ↑ RMS during MVC_{30} for BFR versus IHRT (0.50), and small effect for BFR versus RT (0.30) Moderate effect for ↓ RMS during Rep_{20} for RT versus BFR and IHRT (0.50 and 0.60, respectively)	Low-load IHRT resulted in neuromuscular adaptation, evidenced via increased RMS during MVC_3 and MVC_{30}. Improved performance during Rep_{20} following BFR and IHRT was likely due to improved contractile efficiency.
		IHRT	Not stated (SpO_2 maintained at ~80%)	Bilateral knee extension and flexion (20% 1RM)	6 x matched with BFR (30)	3	35		
		RT	Ambient air	Bilateral knee extension and flexion (20% 1RM)	6 x matched with BFR (30)	3	35		
Ho et al. (2014b)	Healthy males (n=18)	IHRT	F_IO_2 = 15%	Bilateral back squats (10RM)	3 x to failure (120)	3	42	Squat 1RM ↑ significantly in both groups, but changes were not different between conditions ↔ isometric and isokinetic leg strength in either group ↔ body composition in either group	Moderate-load IHRT did not provide additive effects on tests of muscle strength or body composition. It should be noted that the exercise stimulus was not work matched between conditions.
		RT	F_IO_2 = 21%		3 x to failure (120); not work-matched with IHRT	3	42		
Kon et al. (2014)	Healthy males (n=16)	IHRT	F_IO_2 = 14.4%	Bench Press and leg press (70% 1RM)	5 x 10 (90)	2	56	↑ lean body mass and thigh CSA in both groups, but changes were not different between conditions ↑ 1RM for both groups (not different between conditions) ↑ muscle endurance for both groups but significantly higher in IHRT ↑ plasma VEGF and capillary-to-fibre ratio only in IHRT	IHRT did not augment muscle hypertrophy or strength to a greater extent than the equivalent training in normoxia. However, IHRT appeared to facilitate greater muscular endurance in conjunction with increased angiogenesis compared to normoxic training.
		RT	F_IO_2 = 21%	Bench Press and leg press (70% 1RM)	5 x 10 (90)	2	56		

Table 2.3 (Cont'd). Summary of research examining the morphological and strength responses to resistance training programs with systemic hypoxia.

Study	Subjects	Training protocol						Physiological/physical response	Main findings
		Training conditions	Hypoxic dose	Exercise (load)	Sets x reps (inter-set rest; s)	Frequency (sessions/ week)	Protocol duration (days)		
Kurobe et al. (2015)	Healthy males (n=13)	IHRT	F_IO_2 = 12.7%/ ~4000 m for 95 minutes	Unilateral elbow extension (10RM)	3 x to failure (60)	3	56	Triceps brachii thickness ↑ for both groups, with significantly larger ↑ for IHRT 10RM ↑ for both groups, though not different between groups	Moderate-load IHRT resulted in greater hypertrophy than training in normoxia, though changes in strength were not affected by hypoxia. While pre-training 10RM loads were not different between conditions, training was not work-matched between groups.
		RT	F_IO_2 = 21%	Unilateral elbow extension (10RM)	3 x to failure (60); not work-matched with IHRT	3	56		
Yan et al. (In Press)	Healthy males (n=25)	IHRT	F_IO_2 = 12.6%	Back squats (70% 1RM)	5 x 10 (60)	2	35	↑ 1RM back squat in all groups (not different between conditions) ↑ isometric leg press in both IHRT groups, but not in RT (relative change higher in 12.6% IHRT compared to RT) ↑ lean body mass in IHRT groups, but not in RT	IHRT appeared to increase isometric strength and lean body mass to a greater extent than training in normoxia. However, exercise loads were not increased uniformly across groups, and differences may be affected by slightly different training doses.
		IHRT	F_IO_2 = 16%	Back squats (70% 1RM)	5 x 10 (60)	2	35		
		RT	F_IO_2 = 21%	Back squats (70% 1RM)	5 x 10 (60)	2	35		

The study of Pesta et al. (2011) was not included in this table, as morphological and strength response data were not presented.
reps repetitions, *IHRT* intermittent hypoxic resistance training, *RT* resistance training without blood flow restriction in normoxia, *BFR* blood flow restriction, F_IO_2 fraction of inspired oxygen, *1RM* 1-repetition maximum, *CSA* cross sectional area, *RPE* rating of perceived exertion, MVC_3 3 second maximum voluntary contraction, MVC_{30} 30 second maximum voluntary contraction, Rep_{20} maximum number of repetitions at 20% 1RM, *RMS* root mean square of electromyography signal, SpO_2 arterial oxygen saturation, *10RM* 10-repetition maximum, *VEGF* vascular endothelial growth factor, ↑ increase, ↓ decrease, ↔ no significant change

Adaptive and Perceptual Responses to Intermittent Hypoxic Resistance Training

Morphological Responses

Few researchers have detailed the morphological responses to IHRT using systemic hypoxia. In an early study, Friedmann et al. (2003) reported that four weeks of low-load (30% 1RM) knee extension exercise in a normobaric hypoxic environment (F_IO_2 = 12%) did not induce significant gains in muscle or fibre CSA. However, no significant gains were made in the control group either, suggesting that the low-load resistance training in systemic hypoxia was not superior or inferior to equivalent normoxic training. In contrast, Manimmanakorn et al. (2013a; 2013b) reported that five weeks of low-load (20% 1RM) IHRT elicited greater increases in the combined CSA of the knee extensor and flexor muscles than the equivalent training in normoxia (6.1 ± 5.1% versus 2.9 ± 2.7%, respectively) in female netball athletes. Interestingly, similar gains were observed in the CSA of subjects who performed training with BFR (6.6 ± 4.5%) (Manimmanakorn et al., 2013a).

Nishimura et al. (2010) also reported significant increases in the CSA of the elbow flexors and extensors of untrained males after six weeks of moderate-load resistance training (70% 1RM) in hypoxia (F_IO_2 = 16%), despite no significant morphological changes after equivalent training in normoxia. Similar findings have been recently presented by Kurobe et al. (2015) and Yan, Lai, Yi, Wang, and Hu (In Press), with significantly greater muscle thickness in trained muscles and lean body mass, respectively, following IHRT compared to the

equivalent training in normoxia. Conversely, Ho et al. (2014b) observed no significant changes in body composition (estimated via bioelectrical impedance analysis) following six weeks of moderate-load back squat exercise (10RM) in hypoxia (F_IO_2 = 15%), though significant changes were also not observed following the equivalent training in normoxia.

The discrepancy between some of these investigations may be accounted for by methodological differences, particularly in the prescription of exercise. While Friedmann et al. (2003) and Manimmanakorn et al. (2013a; 2013b) employed similar low-load loads and repetition schemes, the inter-set recovery period varied (60 s and 30 s, respectively). Furthermore, the studies using moderate loads (70% 1RM and 10RM) combined with 60 s rest periods reported enhanced morphological responses following resistance training in hypoxia (Kurobe et al., 2015; Nishimura et al., 2010; Yan et al., In Press), whereas research using similar loads but longer inter-set rest periods (Ho et al., 2014b; Kon et al., 2014) observed no significant changes in body composition. It is likely that if recovery periods between sets are too long, there may be sufficient time for the removal of metabolites, thus limiting the metabolic and hormonal responses, and subsequent morphological changes (see Appendix G and Appendix H for additional information). Taken together, these data suggest that IHRT may provide benefits for skeletal muscle hypertrophy beyond those achieved by training in normoxia, and similar to previously described BFR techniques. Future research is required to examine the effect of varying

exercise loads, inter-set recovery periods, as well as the level of hypoxia and duration of exposure, on the morphological adaptations to resistance training.

Increases in Muscular Strength

Increases in muscular strength following IHRT have recently been reported using both isoinertial (Nishimura et al., 2010; Yan et al., In Press) and isokinetic (Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b) training models. For example, Nishimura et al. (2010) reported that muscle strength increased significantly after only three weeks of IHRT, whereas a significant increase in strength in the control (normoxia) group took six weeks. Likewise, Manimmanakorn et al. (2013a) observed substantially greater increases in strength (3 s MVC) and muscular endurance (30 s MVC and number of repetitions to failure at 20% 1RM), following five weeks of training in the IHRT group than a normoxic control group. Similar results were presented in a subsequent study (Manimmanakorn et al., 2013b), with a small effect (effect size [ES] = 0.44) for IHRT versus the control condition for 3 s MVC, and moderate effects (0.64 and 0.70) for 30 s MVC and number of repetitions to failure at 20% 1RM between these conditions, respectively. While these data suggest that IHRT can accelerate increases in muscle strength, Friedmann et al. (2003) and Ho et al. (2014b) observed no significant changes in maximum strength or muscular endurance following such training. However, this is likely explained by variations in the training dose discussed previously (see pg. 44).

An interesting aspect of the study by Manimmanakorn et al. (2013a) was the inclusion of sport-specific tests to determine whether adaptive responses to IHRT translated into improved physical performance. IHRT was shown to enhance change of direction ability (as assessed by the 505 test), and possibly improve predicted peak speed when compared to training in normoxia. However, improvements in the sport-specific tests were greater following BFR training than IHRT. It is possible that due to the relatively high occlusive pressures used (up to 230 mmHg), the BFR condition caused lower levels of muscle oxygenation than the IHRT group, enhancing training adaptations. However, as muscle oxygenation status was not monitored by Manimmanakorn et al. (2013a), it is difficult to explain these results based on muscle hypoxia alone. It is also possible that cellular swelling was increased via occluded venous outflow in the BFR group, triggering an anabolic signalling cascade not replicated in the IHRT group (Loenneke et al., 2012a). Further research examining the mechanisms underlying hypertrophic responses to IHRT is warranted before these changes can be comprehensively described.

Perceptual Responses

Currently, little data have described the perceptual responses to IHRT. Nishimura et al. (2010) and Kon et al. (2012) reported no significant differences in RPE and subjective fatigue, respectively, between groups completing resistance exercise in hypoxia and normoxia. In contrast, Manimmanakorn et al. (2013a) reported that subjective pain was significantly higher in subjects performing IHRT than those in the control, or BFR groups. Previous research

has noted pain increases in direct correlation with H^+ concentration during tourniquet ischemia (Issberner, Reeh, & Steen, 1996). However, if the increased pain reported by Manimmanakorn et al. (2013a) was associated with additional metabolic stress, greater hypertrophic and strength improvements would also be expected from IHRT compared to the BFR group. It is therefore difficult to explain the mechanisms causing the differences in pain between the training conditions based on current understanding, and further research is required before the perceptual responses to IHRT can be fully described.

Potential Mechanisms of Intermittent Hypoxic Resistance Training for Hypertrophy and Strength

Concentration of Metabolites

The accumulation of metabolites is suggested to mediate, at least in part, many of the mechanisms that effect muscle hypertrophy (Schoenfeld, 2013). Kon et al. (2010) examined the metabolic responses to 5 sets of 10 repetitions of bench press and leg press exercises (70% 1RM) in systemic hypoxia (F_IO_2 = 13%) and normoxia (F_IO_2 = 21%), in active young men. Greater blood lactate (BLa^-) responses were reported for the hypoxia group than the normoxia group (1.2-fold higher). Similar findings were reported by the same researchers using lower load exercise (5 sets of 14 repetitions at 50% 1RM; F_IO_2 = 13%) (Kon et al., 2012). However, some more recent investigations have not observed consistent increases in BLa^- concentration following resistance exercise in hypoxia (Ho, Huang, Chien, Chen, & Liu, 2014a; Kurobe et al., 2015; Yan et al., In Press). At this point in time the reasons underpinning these

divergent responses are not clear, though it is likely that methodological differences including the duration of inter-set rest periods and the level of hypoxia employed may have contributed (see pg. 44). Considering that an increased accumulation of metabolites is proposed as a primary moderator for downstream hypertrophic responses (Schoenfeld, 2013), further research is required to quantify the metabolic responses during IHRT.

Hormonal Responses

Several investigations have now assessed the hormonal responses to resistance exercise in systemic hypoxia (Ho et al., 2014a; Kon et al., 2010; Kon et al., 2012; Kon et al., 2014; Kurobe et al., 2015; Yan et al., In Press). Similar to BFR research, augmented GH responses have been reported following low- (Kon et al., 2012) and moderate-load (Kon et al., 2010; Kurobe et al., 2015; Yan et al., In Press) resistance exercise in systemic hypoxia. However, some recent investigations have not observed significant differences between hypoxic and normoxic conditions (Ho et al., 2014a; Kon et al., 2014). These findings are likely related to methodological differences described previously (duration of inter-set rest periods and the associated effects on metabolic stress), given that metabolic acidosis is thought to stimulate GH release (Schoenfeld, 2013). Indeed, studies which have observed hypoxia to enhance GH concentration have used 60 s rest periods (Kon et al., 2010; Kon et al., 2012; Kurobe et al., 2015; Yan et al., In Press), whereas those which have not employed longer recovery between sets (90 s) (Ho et al., 2014a; Kon et al., 2014). In addition, while serum IGF-1 increased immediately following resistance exercise in both

hypoxia and normoxia, there were no significant differences between conditions (Kon et al., 2010). This is in agreement with previous BFR research, where GH response is typically augmented following resistance exercise with BFR, while the IGF-1 response appears equivocal (Fujita et al., 2007; Patterson et al., 2013). Similarly, whilst serum testosterone levels were significantly increased following resistance exercise in both hypoxia and normoxia, no significant differences were found between conditions (Ho et al., 2014a; Kon et al., 2010; Kon et al., 2012). These findings are also in agreement with BFR research, and might reflect dissociation between the magnitude of metabolic stress and testosterone responses (Durand et al., 2003; Goto et al., 2005).

Kon et al. (2010) reported significantly larger increases in epinephrine (1.5-fold) and norepinephrine (1.2-fold) following moderate-load resistance exercise in hypoxia than in normoxia, as well as significant increases in cortisol (1.5-fold) only in the hypoxia group. These factors suggest that a hypoxic stimulus may increase the physiological and/or psychological stress, which in turn could potentially facilitate a catabolic effect (Kraemer & Ratamess, 2005). However, contrasting results have also been reported with Kon et al. (2012) observing no differences in plasma norepinephrine or cortisol levels after low-load resistance exercise in either hypoxia or normoxia. This disagreement may be explained by differences in the loads used during exercise, as catecholamine and cortisol responses typically reflect the acute demands of exercise, and may be dependent upon the force of muscle contractions (Goto et al., 2005; Kraemer & Ratamess, 2005; Smilios et al., 2003). Nonetheless, as described previously, it

is important to note that the role of systemic endocrine responses in muscular hypertrophy is currently a point of debate among scientists. Further research regarding the mechanisms by which hormones can augment hypertrophic responses is necessary before this potential mechanism can be elucidated.

Intramuscular Signalling Pathways

Previous research has demonstrated that chronic exposure to hypoxia adversely affects protein kinase B/mTOR signalling (Favier et al., 2010), up-regulates myostatin expression (Hayot et al., 2011), and subsequently leads to atrophy in skeletal muscle (MacDougall et al., 1991). However, the response of intramuscular signalling pathways to acute hypoxic exposure during resistance exercise remains unclear. Etheridge et al. (2011) reported that following moderate-load IHRT (6 sets of 8 repetitions at 70% 1RM; 3.5 h exposure to F_IO_2 = 12%), MPS was blunted, despite an increase in S6K1 phosphorylation. These results are somewhat disparate with BFR research, where increases in S6K1 phosphorylation have been reported in congruence with increases in MPS (Fry et al., 2010; Fujita et al., 2007). While speculative, Etheridge et al. (2011) proposed that other currently unknown signalling processes might override mTOR signalling in hypoxia, affecting the physiological responses to resistance exercise in hypoxia (Etheridge et al., 2011). Additionally, the suppression of MPS following resistance exercise in hypoxia was correlated with the magnitude of decreases in SpO_2 (r^2 = 0.49; $p < 0.05$). This indicates that subjects who exhibited a lesser degree of hypoxemia had a greater capacity to maintain post-exercise MPS. However, while the results of Etheridge et al. (2011) indicate that

resistance exercise in acute systemic hypoxia may elicit a diminished MPS response, it is important to recognise that the duration of hypoxic exposure (3.5 h) in this study was longer than typical IHRT research (Kon et al., 2010; Kon et al., 2012; Nishimura et al., 2010). When considering the atrophying effects of chronic hypoxia on skeletal muscle (MacDougall et al., 1991), it may be that exposure for as little as 3.5 h can mitigate the potentially anabolic effects of IHRT.

Hypoxic exposure is known to activate hypoxia inducible factor-1α (HIF-1α), which acts as the primary transcriptional response factor for hypoxic adaptation (Mason & Johnson, 2007). In turn, HIF-1α stimulates expression of vascular endothelial growth factor (VEGF) (Semenza, 2009), which promotes angiogenesis (Takano et al., 2005). While expression of HIF-1α and VEGF might have benefits in bone remodelling and repair (Arsic et al., 2004; Loenneke et al., 2012c), the role of this pathway in hypertrophy is not yet understood (Kawada, 2005). Arsic et al. (2004) reported that VEGF could stimulate skeletal muscle fibre regeneration and growth *in vivo*. However, this research utilised an animal model, and the effects of VEGF on hypertrophy in human subjects is less clear. Interestingly, Kon et al. (2014) recently reported that moderate-load IHRT results in significantly higher plasma VEGF after eight weeks of training, whereas no changes were observed in a normoxic training group. Significantly increased capillary-to-fibre ratios were also observed only in the IHRT group, in concert with enhanced performance in muscular endurance

tasks. However, muscle hypertrophy was not enhanced by hypoxia, and the role of VEGF in IHRT requires further research attention.

A novel factor which might contribute to intracellular signalling is whether hypobaric or normobaric hypoxia is employed during exercise. Research indicates that hypobaric hypoxia (e.g. terrestrial altitude) causes a decrease in plasma nitric oxide concentration, whereas normobaric hypoxia (e.g. via nitrogen dilution hypoxic generators) does not (Faiss et al., 2013b). Nitric oxide is a potent reactive species and has been proposed to mediate the activation of skeletal muscle satellite cells and subsequent hypertrophy (Anderson, 2000). It is therefore possible that the type of hypoxia employed during IHRT may influence the subsequent physiological responses. However, no research has yet compared the responses to resistance exercise under hypobaric and normobaric hypoxia.

Skeletal muscle responses to hypoxic resistance exercise might also be affected by an up-regulation of autophagy-lysosomal pathways during periods of metabolic or hypoxic stress (Masiero et al., 2009). The autophagy-lysosomal pathway contributes largely to catabolic processes in atrophying muscle (Sandri, 2010). While the signalling pathways by which autophagic processes occur in skeletal muscle are complex (Schiaffino, Dyar, Ciciliot, Blaauw, & Sandri, 2013), it appears that mTOR complex 1 can promote both protein synthesis and autophagy. Indeed, despite the proteolytic effects of autophagic processes, it appears that they are crucial to the maintenance of muscle mass

(Masiero et al., 2009). Collectively, the effects of hypoxia on both anabolic and catabolic signalling pathways remain largely unknown (Bigard, 2013). Further research is therefore required before a comprehensive understanding of how hypoxic exposure can affect intramuscular signalling pathways can be reached.

Skeletal Muscle Function

While skeletal muscle function and activation has been extensively researched using BFR techniques, only Manimmanakorn et al. (2013b) have reported on the responses of skeletal muscle to resistance exercise in systemic hypoxia. Electrical activity of the knee extensors was assessed during tests of muscular strength and endurance in netball athletes prior to, and following, five weeks of low-load resistance training with either BFR, systemic hypoxia, or no additional stimulus (i.e. control). While all groups demonstrated significantly greater muscle activation during strength and endurance tasks following training, the largest increases were reported in the BFR group (Manimmanakorn et al., 2013b). This may suggest that the neuromuscular changes were most influenced by the restriction of blood flow, rather than hypoxia *per se*. Nonetheless, it is also possible that oxygen delivery to the muscles in the IHRT group could have been maintained by increased peripheral blood flow, despite a reduced SpO_2, thus limiting the hypoxic stimulus experienced by the muscles (Manimmanakorn et al., 2013b). Previous research has also demonstrated that breathing hypoxic air during cycle ergometer exercise increases recruitment of type II muscle fibres compared to normoxia (Melissa, MacDougall, Tarnopolsky, Cipriano, & Green, 1997). It is therefore plausible to hypothesise that systemic

hypoxia could stimulate an increased type II motor unit recruitment, as has been reported in BFR exercise. Nevertheless, the level of intramuscular hypoxia created and the resultant muscle activation may be of a lesser magnitude during exercise with systemic hypoxia than with BFR. Further research is required to fully elucidate how breathing hypoxic air during resistance exercise can alter the oxygenation status of skeletal muscle, and influence subsequent motor unit recruitment.

Differences Between Blood Flow Restriction and Intermittent Hypoxic Resistance Training Methods

While the adaptation to resistance training with BFR or IHRT may be largely mediated by the hypoxic stimulus, it is important to note how these specific methods may differ. Perhaps the most pertinent of these differences is the hemodynamic response, which is facilitated during BFR via the application of external pressure to limb vasculature, and via systemic hypoxia during IHRT. Indeed, ischemia/reperfusion and hypoxia/reoxygenation have resulted in different genetic responses in an animal model (Aravindan, Williams, Riedel, & Shaw, 2005). Reductions in blood flow via BFR result in lower muscle oxygenation levels during resistance exercise, and greater reperfusion following exercise, than observed during high-load exercise without BFR (Takano et al., 2005). As such, it would be expected that a greater reactive hyperemic response would follow BFR than IHRT, which may potentially mediate greater cellular swelling and anabolic signalling (Loenneke et al., 2012a). However, it should be recognised that some evidence does not support an anabolic role of

reactive hyperemia in MPS following BFR resistance exercise (Gundermann et al., 2012). These findings might reflect a decreased delivery of nutrients, growth factors and hormones to the limb during BFR (which is not experienced during IHRT), though the role of systemic responses in hypertrophy has also been questioned (West et al., 2010; West & Phillips, 2010).

Exercising in hypoxia is known to trigger a compensatory vasodilation to match an increased oxygen demand at the muscular level (Casey & Joyner, 2012). As mechanical pressure is not applied to the vasculature during IHRT, this compensatory vasodilation might attenuate the decrease in tissue oxygenation, thus reducing the localised hypoxic stimulus in working muscle. Furthermore, it is likely that type II muscle fibres may be more sensitive to increased perfusion (i.e. via hypoxia-induced vasodilation) than type I fibres (Faiss, Girard, & Millet, 2013a), owing to their greater fractional oxygen extraction if highly perfused (McDonough, Behnke, Padilla, Musch, & Poole, 2005). This increased microvascular oxygen delivery may cause type II fibres to behave more like their oxidatively efficient type I counterparts (Cleland, Murias, Kowalchuk, & Paterson, 2012), potentially attenuating contractile fatigue. Also, as severe hypoxia has been demonstrated to alter function of the central nervous system (Downing, Mitchell, & Wallace, 1963), the possibility exists that systemic hypoxia may affect motor unit recruitment, though this is yet to be clarified in IHRT research. Taken together, these data suggest that IHRT may be fibre type selective, having the greatest effect on type II fibres. While this hypothesis remains speculative, it is possible that IHRT may be optimised by higher-

intensities than typically used during BFR training, due to its potential impact on type II fibres.

The practical implications of the two hypoxic resistance training strategies discussed may differ greatly. It is proposed that BFR resistance training is most useful for individuals who are unable to train at moderate- or high-load (e.g. the elderly, rehabilitation patients or athletes seeking to manage total training load), yet may still benefit from increases in muscular strength and size. Separately, IHRT may be of more benefit for athletic populations, as multi-joint exercises can be performed under hypoxic conditions, and there is the potential to train at a much higher intensity under increased neurological demand.

Conclusions

In the past decade, research has established that low-load resistance training with BFR can facilitate greater muscular gains than equivalent training without BFR. It appears that this is influenced by the anabolic environment that results from localised hypoxia during BFR, though the exact mechanisms at play remain unclear. It is likely that these adaptations are dependent on a multitude of factors, including mechanical stress, neuromotor control, metabolic demands, endocrine activities, cellular swelling and intramuscular signalling (Kon et al., 2010; Loenneke et al., 2012a; Takarada et al., 2000a). Recently, research has suggested that IHRT elicits similar responses, while offering several practical benefits over BFR methods, particularly for athletic populations. However, few investigations have examined this technique, and as such it is difficult to make

recommendations for implementing IHRT based on current understanding. It should also be acknowledged that a number of mechanisms proposed to facilitate the augmented responses to both BFR training and IHRT remain poorly understood, particularly the systemic role of hormonal responses and cellular swelling. Future research should examine the physiological, performance and perceptual responses to resistance exercise and training in systemic hypoxia, as well as the mechanisms underpinning adaptation to both BFR resistance exercise and IHRT.

Chapter 3

Study 1

Reliability of Telemetric Electromyography and Near-Infrared Spectroscopy during High-Load Resistance Exercise

As per the peer-reviewed paper **Accepted and Published** in the *Journal of Electromyography and Kinesiology:*

Scott, B. R., Slattery, K. M., Sculley, D. V., Lockie, R. G., & Dascombe, B. J. (2014). Reliability of telemetric electromyography and near-infrared spectroscopy during high-intensity resistance exercise. *Journal of Electromyography and Kinesiology,* 24(5), 722-730.

Abstract

This study quantified the inter- and intra-test reliability of telemetric surface EMG and NIRS during resistance exercise. Twelve well-trained young men (age: 24.8 ± 3.4 yr; height: 178.6 ± 6.0 cm; body mass: 84.8 ± 11.0 kg) performed high-load back squat exercise (12 sets at 70-90% 1RM) on two occasions, during which surface EMG and NIRS continuously monitored muscle activation and oxygenation of the thigh muscles. Intra-test reliability for EMG and NIRS variables was generally higher than inter-test reliability. EMG median frequency (MDF) variables were generally more reliable than amplitude-based variables. The reliability of EMG measures was not related to the intensity or number of repetitions performed during the set. No notable differences were evident in the reliability of EMG between different agonist muscles. NIRS-derived measures of oxyhaemoglobin (HbO_2), deoxyhaemoglobin (HHb) and tissue saturation index (TSI) were generally more reliable during single-repetition sets than multiple-repetition sets at the same intensity. Although the reliability of the EMG and NIRS measures fluctuated across the exercise protocol, the precise causes of this variability are not yet understood. However, it is likely that biological variation during multi-joint isoinertial resistance exercise may account for some of the variation in the observed results. Together, these findings highlight the need to emphasise consistent device placement and exercise technique to optimise the reliability of test-retest EMG and NIRS assessments.

Introduction

Surface EMG provides a non-invasive, objective method to measure physiological processes occurring during muscular contraction. The EMG signal is influenced by a number of factors, such as motor unit discharge rates, muscle fibre membrane characteristics, and non-physiological properties including electrode size, shape and placement (Farina, Merletti, & Enoka, 2004). Daily variations in EMG recording may be associated with differences in electrode re-application, including alterations in electrode position and differences in skin preparation. Although EMG has demonstrated moderate to high levels of reliability during quadriceps contractions (Larsson, Karlsson, Eriksson, & Gerdle, 2003; Mathur, Eng, & MacIntyre, 2005), the systems typically used have required wired attachment between electrodes and an EMG amplifier. Such systems are cumbersome when performing complex movements, limiting their application during field-based testing. Furthermore, the risk of leads becoming dislodged from their electrode increases the chance for data loss.

Recent technological advances have resulted in the development of telemetric EMG systems to mitigate these limitations. These systems require only one electrode for targeted muscles, compared to wired systems requiring up to two electrodes per muscle and an additional reference electrode. However, whether this telemetric configuration results in reliable data is not well understood. While one investigation has reported the reliability for telemetric EMG during uphill running activity as 0.98 (presumably correlation coefficient) (Lowery et al.,

2014), the methods and analyses employed to assess reliability were not stated.

It must also be acknowledged that research examining the reliability of EMG technologies has not typically used dynamic multi-joint exercises, or assessed whether the loads lifted can affect the reproducibility of EMG variables. As such, the current body of reliability research in this field may be limited in its application to common resistance training methods. For increases in muscular size and strength, resistance training is typically performed using moderate-high loads, and with a set volume (i.e. number of repetitions in a set) that corresponds to the exercise intensity (Bird et al., 2005). However, it is not currently clear whether manipulating these training variables affects the reliability of EMG measurements.

Muscle oxygenation status has also been investigated during resistance exercise models to describe the intramuscular metabolic environment (Azuma, Homma, & Kagaya, 2000; Hoffman et al., 2003). During resistance exercise, increases in intramuscular mechanical pressure lead to reduced blood flow (MacDougall, Tuxen, Sale, Moroz, & Sutton, 1985), resulting in transient muscle hypoxia (Spiering et al., 2008b), which may facilitate increased hypertrophy (Hoffman et al., 2003). Indeed, localised muscular hypoxia induced via BFR has resulted in enhanced anabolic responses to resistance exercise (Abe et al., 2005b; Takarada et al., 2000a). While the effects of muscle oxygenation status on resistance training adaptations are not fully understood, the use of NIRS to

monitor muscle oxygenation has become popular among exercise scientists (Quaresima, Lepanto, & Ferrari, 2003). NIRS is non-invasive, and reflects the balance of oxygen delivery to working muscles and oxygen consumption in capillary beds. This may be especially important for research investigating resistance training with BFR or additional systemic hypoxia, which is currently promoted as a beneficial new training stimulus (see Review of the Literature).

NIRS values during isometric *erector spinae* activity have demonstrated a moderate to strong intraclass correlation coefficient (ICC; 0.69-0.84) (Kell, Farag, & Bhambhani, 2004). NIRS data during knee extension exercise has also yielded moderate to strong reliability during both isokinetic (ICC = 0.73-0.97) (Pereira, Gomes, & Bhambhani, 2005) and isoinertial (ICC = 0.85 and coefficient of variation [CV] = 0.07%) (Tanimoto & Ishii, 2006) exercise protocols. More recently, significant ICC values (0.39-0.87) have been shown for NIRS measures in the forearm muscles during handgrip exercise at various intensities (Celie, Boone, Van Coster, & Bourgois, 2012). While these studies have reported the reliability of NIRS during single-joint resistance exercise, no data have reported on the reliability of NIRS measures during multi-joint isoinertial resistance exercise. Furthermore, these studies reporting on the reliability of NIRS have utilised static or seated exercises, and it remains unknown whether the greater postural control required during dynamic exercises might affect its reliability. It is possible that different contributions from synergistic muscles during dynamic multi-joint exercise may alter the reliability of muscle oxygenation within a single muscle.

It is evident that the reliability of telemetric EMG and NIRS devices during dynamic multi-joint resistance exercise across various intensities is not yet known. Understanding the reliability of these technologies during resistance exercise is important to ensure that any measured differences in muscle activation or oxygenation status are indicative of actual change, rather than error in the measurement itself. In addition, many studies investigating the reliability of EMG and NIRS variables have focused largely on ICC values to test reliability. The ICC is a relative measure of reliability, and is affected by the range of the values being assessed (Atkinson & Nevill, 1998). For a more detailed reliability analysis, further statistics such as typical error (often expressed as a percentage CV) should be included (Atkinson & Nevill, 1998). Therefore, the purpose of this study was to comprehensively investigate the intra- and inter-test reliability of a telemetric EMG system and NIRS device during high-load dynamic multi-joint resistance exercise. The research also aimed to determine if the load or number of repetitions performed within a set affected reliability.

Methods

Experimental Design

Subjects visited the laboratory on four occasions, each separated by one week. Sessions one and two involved familiarisation and 1RM testing using a modified harness back squat (HBS) exercise (Figure 3.1). Sessions three and four were identical, and involved experimental trials of a high-load resistance exercise protocol using the HBS. During experimental trials, muscle activation and

oxygenation status were continuously monitored to determine the reliability of telemetric EMG and NIRS technologies.

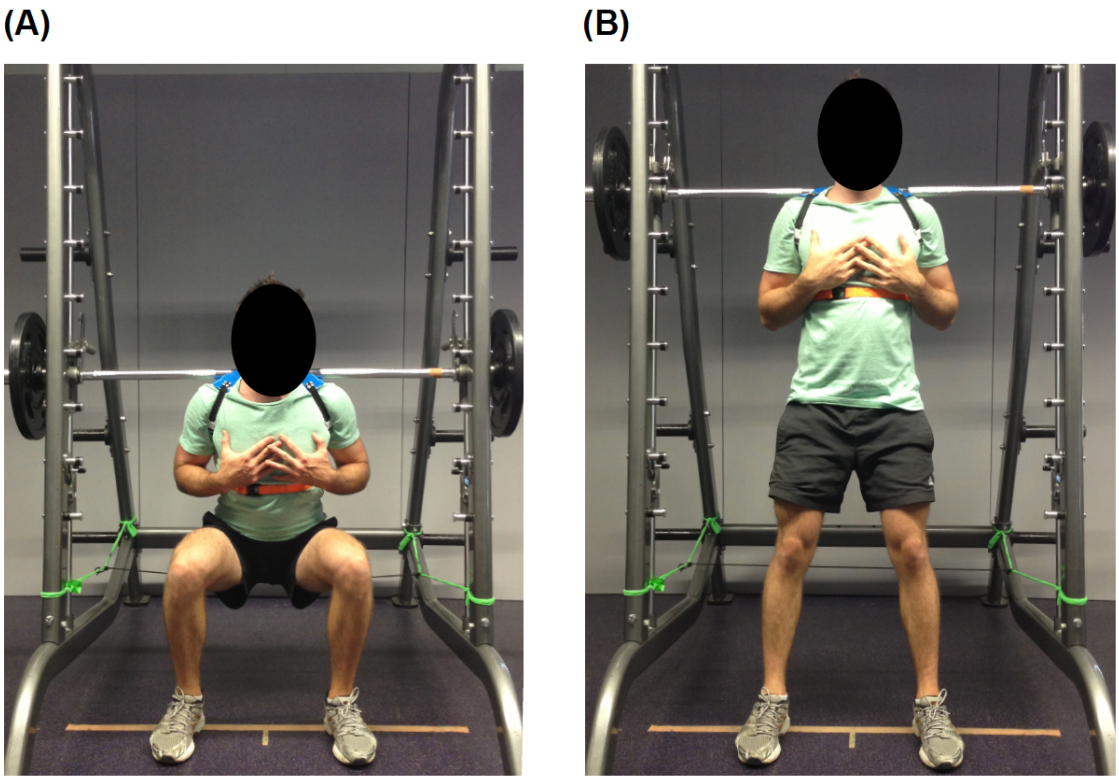

Figure 3.1. Example of a subject performing the harness back squat exercise, highlighting the squat position at the end of the eccentric/beginning of the concentric phase (A), and at the end of the concentric phase (B).

Subjects

Twelve healthy males (age: 24.8 ± 3.4 yr; height: 178.6 ± 6.0 cm; body mass: 84.8 ± 11.0 kg; HBS 1RM: 148.0 ± 21.4 kg) participated in this study. All subjects had at least two years resistance training experience and were free of musculoskeletal disorders. Prior to commencement, subjects were informed of the nature of the research, provided informed consent, and were screened for medical contraindications. They were instructed to abstain from alcohol and

caffeine for 24 h before each testing session, and avoid strenuous activity for the duration of the research. Subjects were also instructed to replicate their food and liquid intake for the 24 h period prior to each test. The University of Newcastle Human Ethics Committee approved the study and its methods.

Familiarisation and Maximal Strength Testing

Prior to experimental trials, subjects familiarised with the HBS exercise, which has been described previously (Scott et al., 2014). This exercise was chosen as it is a dynamic multi-joint exercise, and has both clinical and athletic applications. Within one week of familiarisation, subjects were tested for 1RM of the HBS exercise following procedures that have demonstrated high reliability in our laboratory (CV = 2.6%, ICC = 0.98) (Scott et al., 2014). Subjects completed a general warm-up (five minutes on a cycle ergometer at a moderate intensity), before performing three specific warm-up sets of the HBS, comprised of 10 repetitions at 50% of predicted 1RM weight (as estimated by the subject), 5 repetitions at 70%, and 1 repetition at 90%. Following the warm-up sets, weight was increased by ~5% and subjects performed a single repetition. This process continued until subjects were unable to successfully perform a lift, with 180 s rest between attempts. Subjects' 1RM was defined as their heaviest completed repetition, and was determined within 3-6 sets.

Experimental Trials

Subjects reported to the laboratory on two further occasions to perform identical resistance exercise trials. Upon arrival, EMG and NIRS devices were affixed

before subjects completed a general warm-up, followed by 3 specific warm-up sets (10 repetitions at 50% of measured 1RM, 5 repetitions at 65%, and 1 repetition at 80%). Following the warm-up, subjects rested for 180 s, before beginning 12 sets of a high-load resistance exercise protocol (Table 3.1) during which muscle activation and oxygenation status were monitored via EMG and NIRS, respectively. Subjects wore the same footwear for each trial, which was conducted under the same temperature conditions (~22 °C).

Table 3.1. Summary of the resistance exercise protocol used during experimental trials.

	% 1RM	Repetitions performed
Set 1-2	90	1
Set 3-4	90	3
Set 5-6	80	1
Set 7-8	80	6
Set 9-10	70	1
Set 11-12	70	10

The exercise protocol was designed to assess muscle function within both single and multiple repetition sets at various heavy loads, in order to provide a comprehensive examination of device reliability. Single-repetition sets were included to examine EMG and NIRS reliability during a maximal effort at a given load, as subjects were instructed to perform the concentric phase of the lift as quickly as possible. Multiple-repetition sets were included to quantify reliability during sets at various high-loads when a typical set volume was employed. That is, for individuals completing strength training it is common to perform 3, 6, and

10 repetitions at intensities of 90, 80 and 70% 1RM, respectively. Subjects rested passively for 120 s between sets.

Subjects squatted down to an elastic stringline each repetition, which was set so that each individual's superior hamstrings came in contact with it when the top of the thighs were parallel with the ground (Cotterman, Darby, & Skelly, 2005). This was visually assessed by a researcher positioned adjacent to the participant. Subjects were also given verbal cues on when they were to halt the down phase, and begin the up phase, of the squat (Cronin & Hansen, 2005). To ensure consistent feet placement, subjects positioned the posterior edge of their shoes on a horizontal line marked on the floor beneath the Smith machine, with their feet equidistant from a centre marking on this line (Hori & Andrews, 2009). Throughout the movement, heels of the feet remained flat on the ground, the spine was maintained in a neutral position and the head was kept level.

Electromyography Monitoring

Muscle activation during each set of the HBS exercise was monitored via EMG recordings from the left *gluteus maximus* (GM), *vastus lateralis* (VL), *vastus medialis* (VM) and *biceps femoris* (BF) muscles. These muscles have previously been used to examine the myoelectric activity of hip and thigh muscles during the back squat in well-trained weightlifters (Caterisano et al., 2002). Prior to electrode placement, the skin was shaved, lightly abraded and cleaned with alcohol to ensure optimal electrical conductance. Telemetric surface electrodes (Trigno™ Wireless, Delsys Inc., Boston, USA) were

positioned on the belly of the muscle, and according to the recommendations of surface EMG for non-invasive assessment of muscles (SENIAM) (Hermens & Freriks, 1997). The position of electrodes is shown in Figure 3.2. Electrodes were affixed with double-sided adhesive, running parallel with the muscle fibres. To ensure consistent electrode placement between trials, the position of each electrode was outlined using a permanent marking pen during the first experimental trial. Subjects were provided with pens and instructed to re-outline the marker position if it faded prior to the second experimental trial (Kacin & Strazar, 2011).

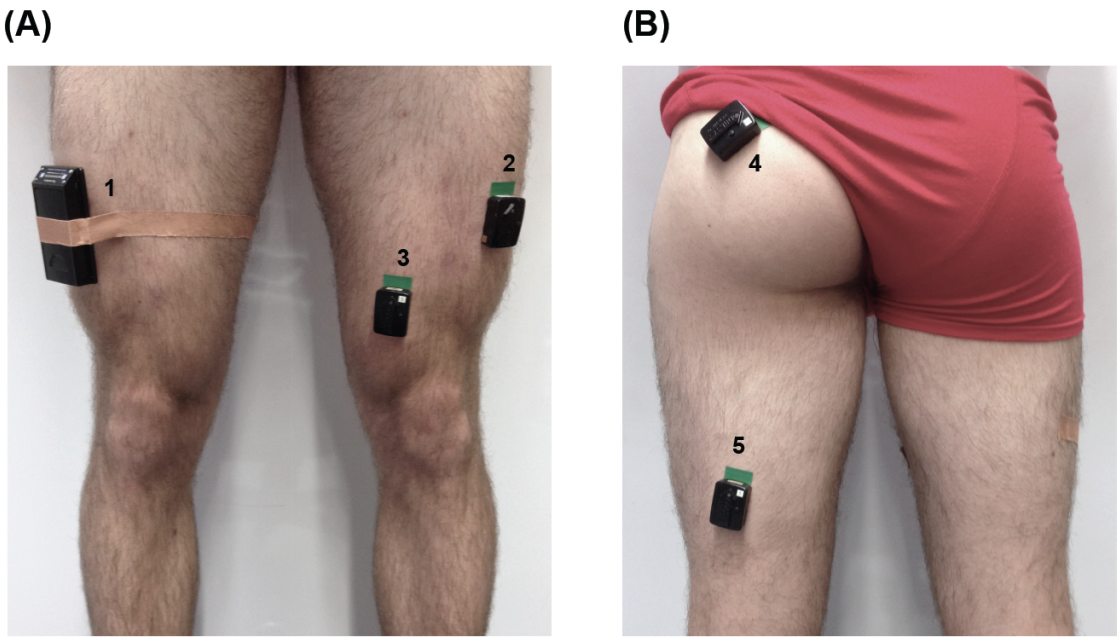

Figure 3.2. Placement positions the NIRS device on the right *vastus lateralis* (1), and EMG electrodes on the left *vastus lateralis* (2), *vastus medialis* (3), *gluteus maximus* (4) and *biceps femoris* (5) from an anterior (A) and posterior (B) view.

Data were sampled at 4000 Hz, passed through a differential amplifier at a gain of 300, and band-pass filtered (fourth order Butterworth filter) at 16-500 Hz. The

concentric phase was selected for EMG analysis, and was identified using data derived from a triaxial accelerometer housed within the EMG devices. The root mean square (RMS) of vertical acceleration (with reference to the orientation of the electrode when standing) at the VL electrode was used to identify the change in acceleration direction at the bottom (beginning of concentric phase) and top (end of concentric phase) of each repetition. The identification of lifting cycles from RMS acceleration data was performed offline by the same investigator and was highly reliable (CV = 2.2%, ICC = 1.00). An example trace of the accelerometer data, and corresponding EMG signal from the muscles assessed is illustrated in Figure 3.3.

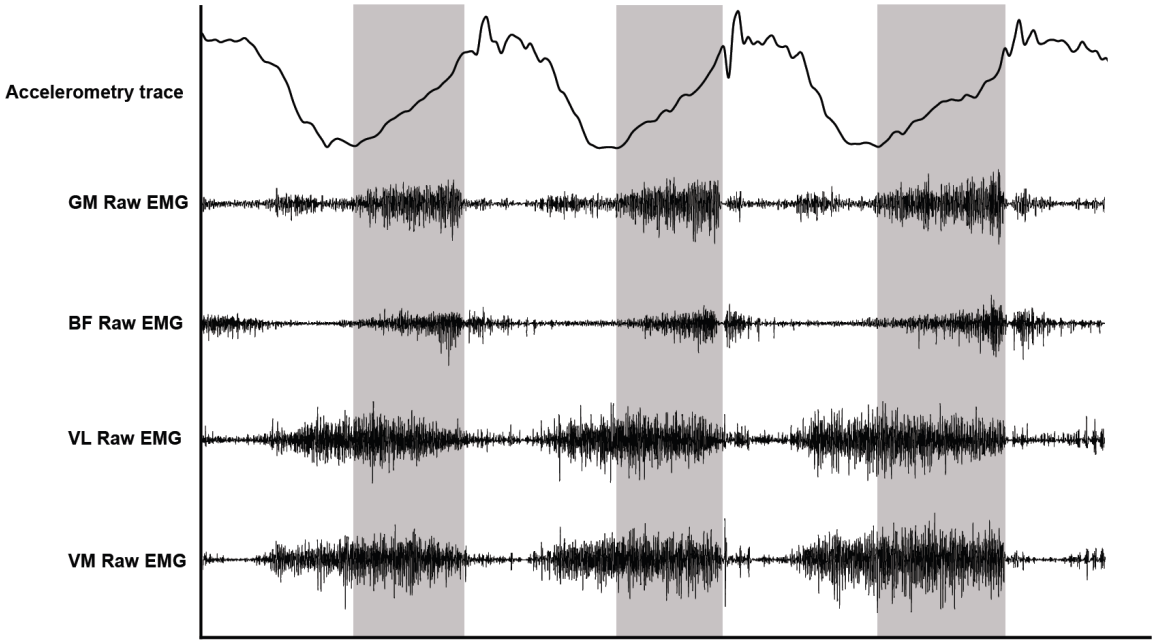

Figure 3.3. Example of the accelerometry trace from the VL electrode and typical raw EMG traces from the *gluteus maximus* (GM), *biceps femoris* (BF), *vastus lateralis* (VL) and *vastus medialis* (VM) muscles during three repetitions of the harness back squat exercise. Shaded sections represent the concentric phase of the lift.

The mean signal amplitude during the concentric phase of each repetition was calculated using a sliding RMS filter, with a window of 0.125 s and overlap window of 0.065 s. The integrated EMG (iEMG) signal across the duration of each set (from the beginning of the first repetition to the end of the last) was also calculated using the same windows. Concentric EMG MDF was obtained by Fast Fourier Transformation, using a window of 512 points with a 256-point overlap. EMG data were analysed using EMGworks software (v4.01, Boston, Delsys Inc, USA).

Near-infrared Spectroscopy Monitoring

Muscle oxygenation of the right VL was monitored continuously during experimental trials using a portable NIRS device (Portamon, Artinis Medical System, BV, The Netherlands). The device was positioned on the belly of the VL, following the same placement guidelines as previously described for VL EMG assessment, though on the opposite leg (Figure 3.2). As described for EMG electrodes, the NIRS apparatus was outlined on subjects' skin to ensure consistent placement. The device was wrapped in transparent plastic to eliminate direct contact with the skin and sweat and was affixed using dark tape to prevent contamination from ambient light.

During experimental trials, data were sampled at 20 Hz and transferred live via Bluetooth connection to a personal computer for analogue-to-digital conversion, storage, and analysis using Oxysoft software (Oxysoft, Artinis Medical Systems, BV, The Netherlands). Changes in tissue concentrations of HbO_2 ([HbO_2]) and

HHb ([HHb]) were measured using their chromophoric properties at 750 and 860 nm. The TSI was also calculated automatically by Oxysoft software, to represent the relative concentration of HbO_2 in relation to the total amount of haemoglobin as an absolute parameter.

To obtain maximal HHb and minimum HbO_2 values, cuff ischemia was performed after five minutes of passive recovery following each experimental trial. This process involved placing a thigh cuff superior to the NIRS apparatus, which was rapidly inflated to 250 mmHg for eight minutes, or until a nadir in [HHb] was reached. The cuff was administered with subjects lying supine and the instrumented leg extended horizontally. Following cuff ischemia, the cuff was deflated and subjects rested passively for five minutes. NIRS data were smoothed using a 0.5 s moving average, before relative HHb and HbO_2 for each resistance exercise set were calculated (Hoffman et al., 2003). To report on changes in peripheral oxygenation state during resistance exercise, data are reported as relative minimum [HbO_2] (HbO_{2min}) and maximum [HHb] (HHb_{max}) values, as well as the absolute minimum TSI (TSI_{min}) during each set.

Statistical Analyses

Data distribution was tested for normality using the Shapiro-Wilk test. As not all data were normally distributed, data were transformed by taking the natural logarithm to allow parametric statistical comparisons that assume a normal distribution. This treatment of data and reliability analyses were performed using a custom made spreadsheet designed for this purpose (Hopkins, 2002). Intra-

test reliability analyses were performed on selected EMG and NIRS variables between paired identical sets at each load and repetition range within the first experimental trial. Inter-test reliability analyses were performed on these same variables, between the first matching set at each intensity and repetition range, between the two experimental trials. Reliability was calculated as the typical error (expressed as a percentage CV), ICC and 95% confidence intervals (CI). The ICC was interpreted as per previous research (Dascombe, Reaburn, Sirotic, & Coutts, 2007): 0.0-0.2: very week; 0.2-0.4: weak to low; 0.4-0.7: moderate; 0.7-0.9: strong; 0.9-1.0: very strong. Paired sample *t*-tests were used to test if differences existed between the repeated tests. Significance was set at $p \leq 0.05$. Paired sample *t*-tests analyses were performed using Statistical Package for the Social Sciences v20.0 (IBM Corporation, Somers, USA).

Results

Reliability of Electromyography Measures

Significant intra-test differences were only observed between MDF values for sets 1 and 2 of single-repetition sets at 80% 1RM for GM ($p = 0.035$). The intra-test and inter-test reliability of EMG data during resistance exercise is shown in Figure 3.4 and Figure 3.5, respectively. The intra-test CV data demonstrated varied reliability for the GM (1.6-18.3%), BF (5.6-22.0%), VL (2.8-31.2%) and VM (4.2-21.2%). Further, the ICC ranged from moderate to very strong for the GM (0.77-0.99), BF (0.43-0.94), VL (0.66-1.00) and VM (0.61-0.98).

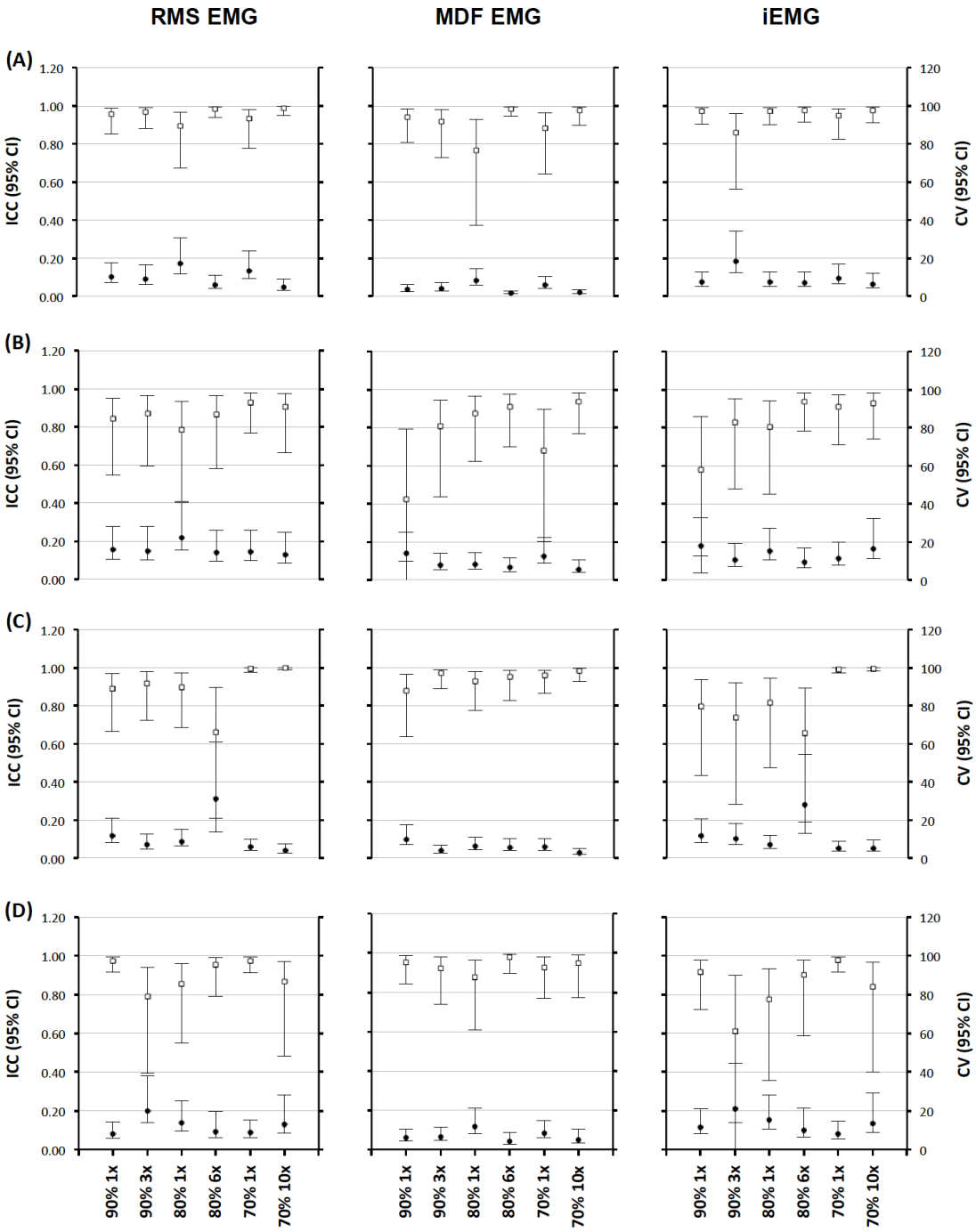

Figure 3.4. The ICC (± 95% CI; unfilled boxes) and CV (± 95% CI; filled circles) of RMS, MDF and iEMG data between identical repeated sets of harness back squat exercise within a single testing session from the (A) *gluteus maximus*, (B) *biceps femoris*, (C) *vastus lateralis* and (D) *vastus medialis* muscles.
ICC intraclass correlation coefficient, *CV* coefficient of variation, *CI* confidence interval.

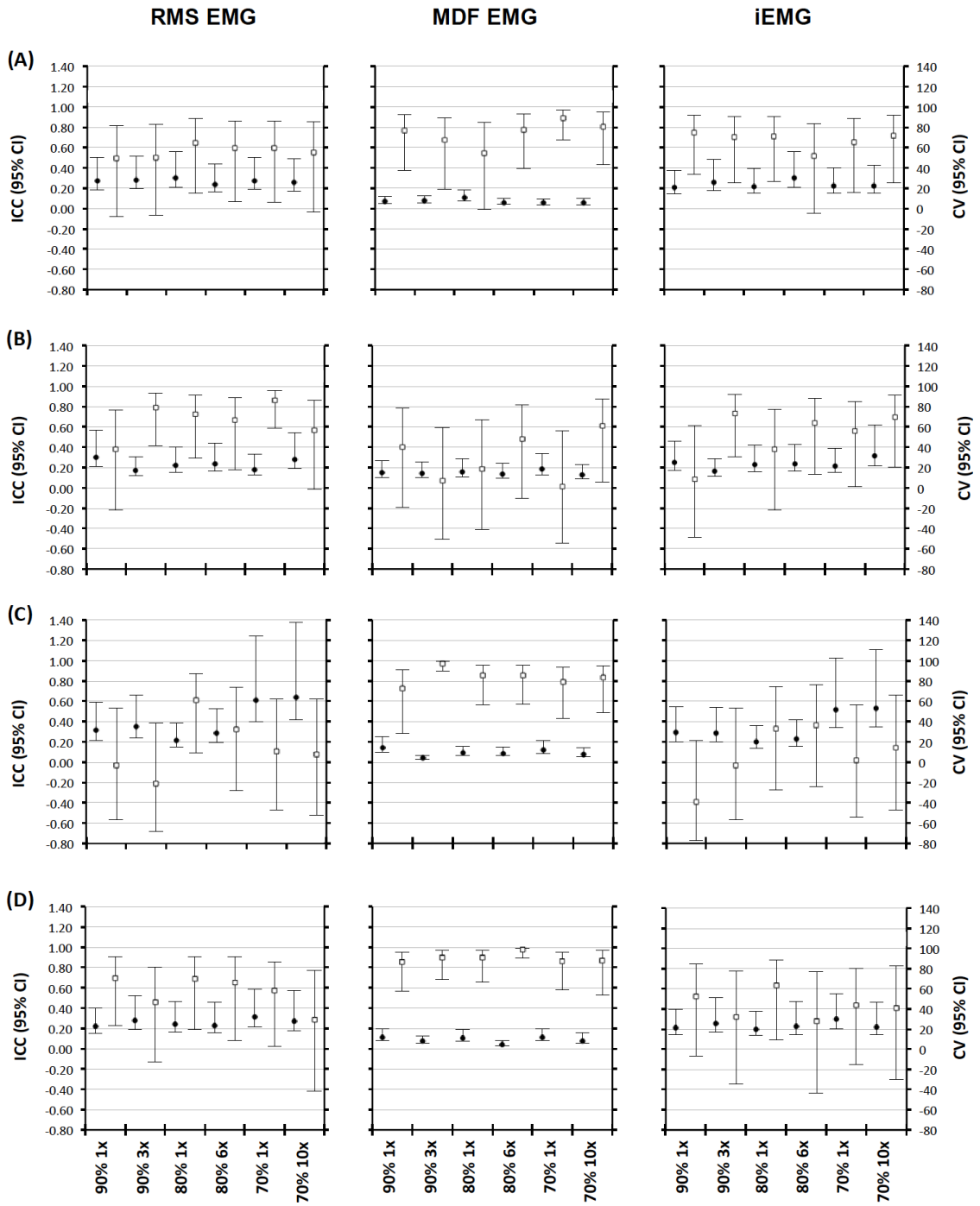

Figure 3.5. The ICC (± 95% CI; unfilled boxes) and CV (± 95% CI; filled circles) of RMS, MDF and iEMG data between identical matched sets of harness back squat exercise in separate testing sessions from the (A) *gluteus maximus*, (B) *biceps femoris*, (C) *vastus lateralis* and (D) *vastus medialis* muscles.
ICC intraclass correlation coefficient, *CV* coefficient of variation, *CI* confidence interval.

Significant inter-test differences were present between iEMG values from VL in the single-repetition sets at 90% and 70% 1RM (p = 0.049 and 0.041, respectively), and in multiple-repetition sets at 80% and 70% 1RM (p = 0.044 and 0.009, respectively). The inter-test CV data demonstrated some variability for the GM (5.2-30.0%), BF (12.4-31.6%), VL (3.8-64%) and VM (4.3-31.4%). Further, the ICC ranged from weak to very strong for the GM (0.49-0.89), BF (0.01-0.86), VL (-0.39-0.97) and VM (0.27-0.97). The frequency component of EMG signals generally displayed higher levels of reliability than amplitude components.

Reliability of Near-Infrared Spectroscopy Measures

Significant intra-test differences were observed between HHb_{max} values for multiple-repetition sets 90%, 80% and 70% 1RM (p = 0.001, 0.015 and 0.009, respectively). The intra-test and inter-test reliability of the NIRS device to monitor HbO_{2min}, HHb_{max} and TSI_{min} during the resistance exercise protocol are shown in Figure 3.6 and Figure 3.7, respectively. The intra-test CV and ICC values for NIRS variables between the paired sets within the same testing session ranged from 1.8-25.3% and 0.75-0.98, respectively. Generally, HbO_{2min}, HHb_{max} and TSI_{min} were more reliable during single-repetition sets, than the multiple-repetition sets at each intensity. However, this trend was reversed for CV values from HHb_{max} data.

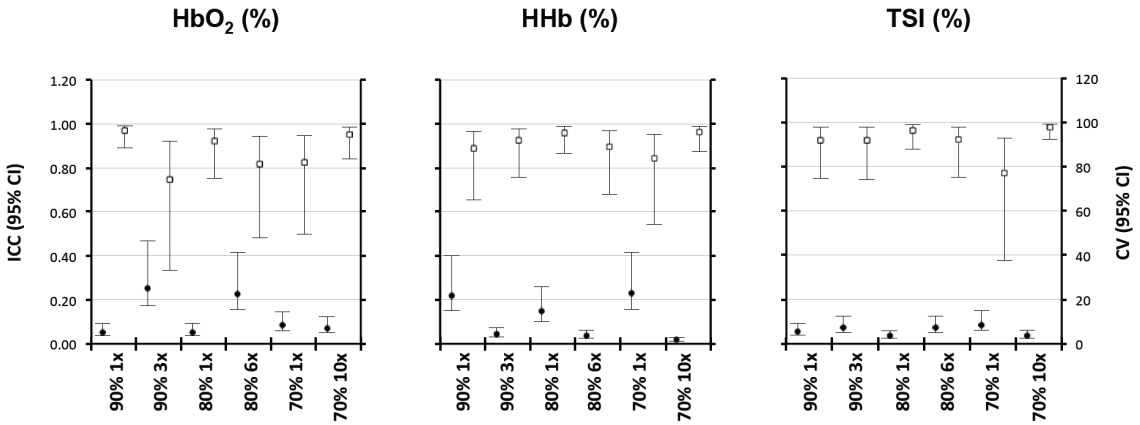

Figure 3.6. The ICC (± 95% CI; unfilled boxes) and CV (± 95% CI; filled circles) of HbO_2 (%), HHb (%) and TSI (%) data between identical repeated sets of harness back squat within a single testing session from the *vastus lateralis*.
ICC intraclass correlation coefficient, *CV* coefficient of variation, *CI* confidence interval.

There were no significant inter-test differences for NIRS variables. The CV and ICC values for NIRS variables between corresponding sets within the two testing sessions ranged from 6.1-43.5% and 0.44-0.87, respectively. As with intra-test reliability of NIRS variables, HbO_{2min}, HHb_{max} and TSI_{min} were more reliable during single-repetition sets, than multiple-repetition sets, with the exception of CV values from maximum HHb_{max} data.

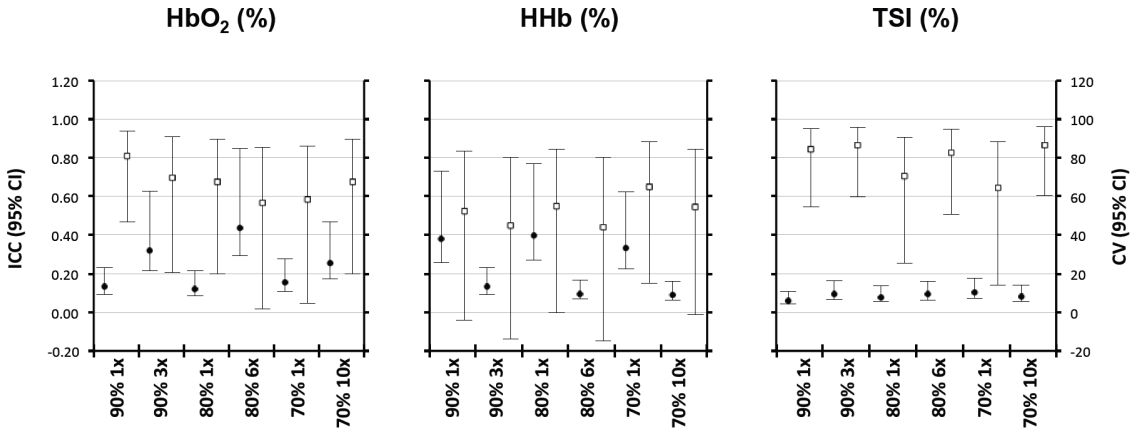

Figure 3.7. The ICC (± 95% CI; unfilled boxes) and CV (± 95% CI; filled circles) of HbO_2 (%), HHb (%) and TSI (%) data between identical matched sets of harness back squat in separate testing sessions from the *vastus lateralis*.
ICC intraclass correlation coefficient, *CV* coefficient of variation, *CI* confidence interval.

The magnitude of CV and ICC measures for both EMG and NIRS data demonstrated greater levels of reliability for paired sets within the same session, than for matched sets between experimental trials. There was also a trend for smaller ranges of 95% CI for intra-test reliability values than for inter-test values.

Discussion

The major findings of this research indicate that intra-test reliability was higher than inter-test reliability for EMG and NIRS variables. Generally, MDF was more reliable than the RMS and iEMG variables. Furthermore, the reliability of NIRS variables during resistance exercise appears to be affected by the volume of each set. These findings have important implications for both practitioners and researchers, as this is the first study to comprehensively assess the reliability of these telemetric EMG and NIRS technologies during dynamic multi-joint resistance exercise at various loads.

The current data demonstrate that each EMG variable differed in reliability across the various loads of resistance exercise, with RMS, MDF and iEMG values generally displaying moderate to strong levels of intra-test reliability. While these findings are similar to previous research reporting intra-test ICC values for mean RMS and frequency during single-joint isokinetic tests (Larsson, Karlberg, Elert, & Gerdle, 1999a; Larsson et al., 1999b), these previous investigations have demonstrated a smaller range of ICC values than the current study. While this may indicate greater variability in the current study,

caution should be taken when comparing ICC values between separate investigations, as this statistic is largely influenced by the range of data scores and the population studied (Atkinson & Nevill, 1998).

Past studies have been limited to isokinetic dynamometry of single-joint exercises (Larsson et al., 1999a; Larsson et al., 1999b). As such, a back squat variation was employed in the current study to analyse multi-joint isoinertial resistance exercise. As the HBS exercise has more degrees of freedom than single-joint isokinetic dynamometry, there is greater potential for variability in exercise technique both within and between testing sessions. For example, it is possible that the position of the knees in reference to the toes during the HBS might have altered slightly between sets and/or testing sessions. Such changes in technique would likely influence segmental orientation, and subsequent motor recruitment patterns. However, as the current investigation did not extend to a kinematic analysis of the HBS exercise, it is unclear whether alterations in technique did in fact affect muscle activation patterns.

As expected, the reported inter-test reliability of EMG variables was lower than the intra-test reliability. Further, the CV and ICC reliability measures both varied greatly across the exercise loads and repetition schemes assessed. Varied reliability has been previously reported by Larsson et al. (2003) for non-normalised RMS (ICC = 0.05-0.95), and median frequency (ICC = 0.48-0.88) variables, between two trials of 100 maximum concentric knee extensions at $90°\cdot s^{-1}$ separated by 7-8 days (CV values not reported). Interestingly, the

current MDF data displays moderate to strong levels of reliability between sessions for the GM, VL and VM. However, the inter-test reliability of MDF from BF was poor. Although it is unclear why MDF from BF is unreliable from the current data, it is possible that these findings relate to the antagonistic role of BF during the HBS. During knee extension, the hamstrings stabilise the knee and hip joints (Schwanbeck, Chilibeck, & Binsted, 2009). During heavy dynamic resistance exercise, the recruitment of antagonist muscles is dependent upon proprioceptive balance and stability feedback, and the higher variability in BF recruitment may reflect small adjustments made in segmental orientation in order to maintain balance and stability during exercise.

The increased inter-test variability may also be explained by small alterations in the re-application of electrodes prior to the second trial. Despite subjects being instructed to re-mark electrode locations between trials, some marks were not visible at the second trial and were subsequently re-measured. While this is likely to occur in applied exercise science and clinical settings that employ EMG assessment (Larsson et al., 2003), changes in electrode placement may explain the decreased reliability of inter-test values. Nonetheless, as the intra-test reliability of EMG values also varied, factors other than electrode placement must influence reliability. At present, it is difficult to determine the cause of the varied reliability within certain sets, as no clear relationships were evident between the level of reliability and either the number of repetitions performed in a set, nor the load of the set. It is possible that the small sample size employed, although similar to previous investigations that have assessed EMG reliability

(Larsson et al., 1999a; Larsson et al., 1999b), may have contributed to these findings.

While previous research has established the test-retest reliability of NIRS to monitor muscle oxygenation status during various exercise protocols (Celie et al., 2012; Kell et al., 2004; Pereira et al., 2005; Tanimoto & Ishii, 2006), this research is the first to assess the reproducibility of NIRS during isoinertial multi-joint resistance exercise. The current data demonstrate that at most loads and set volumes, HbO_{2min} and HHb_{max} values display acceptable intra-test reliability. Further, TSI_{min} values were found to be highly reliable across each set. An intriguing finding was that the reliability of HbO_{2min} and HHb_{max} appeared to be affected by the volume of each set (i.e. number of repetitions) across all intensities. HbO_{2min} displayed greater reliability during single-repetition sets, whereas the reliability of HHb_{max} was increased during multiple-repetition sets. While these results might reflect increased variability in factors known to affect muscular oxygen kinetics, such as mitochondrial efficiency and total blood delivery to the working muscles, the exact reason for these findings is unclear at this time.

The current investigation suggests that the inter-test reliability of NIRS during multi-joint resistance exercise is lower than reported in previous investigations using isokinetic and isometric exercise (Celie et al., 2012; Kell et al., 2004; Pereira et al., 2005; Tanimoto & Ishii, 2006). While great attention was paid to standardise experimental conditions between exercise trials, several factors

may have contributed to the day-to-day variability observed. Differences in the range of scores and the subject population are likely to have influenced the reliability statistics (Atkinson & Nevill, 1998). Small differences in ambient and muscular temperature (Ferrari, Mottola, & Quaresima, 2004), as well as slight changes in posture (Bringard, Denis, Belluye, & Perrey, 2006), can affect NIRS-derived measurements of muscle oxygenation. It is possible that increased intramuscular temperature, as well as changes in posture across the duration of the testing protocol due to muscular fatigue, may have accounted for some of the variation between trials, as previously discussed for EMG variables. Furthermore, while care was taken to ensure consistent placement of the NIRS device between testing sessions, small differences in its positioning could have contributed to variation in measurements, similarly to EMG.

Many of the inter-test CV values observed in the current study are greater than the 5-10% threshold that is generally recommended to consider a measurement reliable (Hopkins, 2000). However, this does not necessarily imply that NIRS-derived measures during resistance exercise are unusable. Indeed, moderate and strong intra-test ICC values were observed when monitoring HbO_{2min} and TSI_{min} during most single-repetition sets at each exercise intensity. The easy-to-use non-invasive nature of NIRS, and its practicality during tests in the laboratory or the field, make it an attractive method to monitor muscle oxygenation status during and following exercise (Buchheit, Ufland, Haydar, Laursen, & Ahmaidi, 2011). Moreover, the results of the current investigation are similar to those reported for the reliability of NIRS to quantify muscle re-

oxygenation and oxygen uptake recovery kinetics following intermittent running activity (Buchheit et al., 2011).

Conclusions

The current study highlights that the reliability of EMG and NIRS variables is higher between repeated sets within the same exercise session than between separate test-retest trials. This suggests that these technologies may have inherent practical limitations. Despite efforts being made to ensure consistent electrode and device placement, the increased variability between separate testing sessions is likely to be accounted for by small changes in the location of sensors. While the reliability of EMG and NIRS variables observed in the current study is lower than in some previous investigations, these studies have generally employed single-joint, isometric or isokinetic exercises. As such, greater physiological variability would be expected in the current study, which was reflected in our data. Furthermore, differences in statistical methods may affect the comparison of reliability results between studies. Importantly, the non-invasive and user-friendly nature of telemetric EMG and NIRS devices provides important information pertaining to the function of contracting muscle during resistance exercise. To improve the reliability of these devices, consistent device placement, exercise technique and testing conditions are vital, particularly when monitoring dynamic multi-joint resistance exercises.

Practical Applications

- Given that intra-test reliability was higher than inter-test reliability, strategies should be developed to ensure consistent device placement during test-retest examinations.
- The reliability of EMG and NIRS devices in this study was lower than in previous investigations using single-joint or isometric tasks. This could result from small changes in exercise technique during high-load dynamic resistance exercise. Care should therefore be taken to ensure consistent technique between test-retest trials.

Chapter 4

Study 2

Physical Performance during High-load Resistance Exercise in Normoxic and Hypoxic Conditions

As per the peer-reviewed paper **Accepted and Published** in the *Journal of Strength and Conditioning Research*:

Scott, B. R., Slattery, K. M., Sculley, D. V., Hodson, J. A., & Dascombe, B. J. (2015). Physical performance during high-intensity resistance exercise in normoxic and hypoxic conditions. *Journal of Strength and Conditioning Research,* 29(3), 807-815.

Abstract

This study aimed to determine whether different levels of hypoxia affect physical performance during high-load resistance exercise, or subsequent cardiovascular and perceptual responses. Twelve resistance-trained young men (age: 25.3 ± 4.3 yr; height: 179.0 ± 4.5 cm; body mass: 83.4 ± 9.1 kg) were tested for 1RM in the back squat and deadlift. Following this, subjects completed three separate randomised trials of 5 sets of 5 repetitions at 80% 1RM with 180 s rest between sets, in either normoxia (NORM; F_IO_2 = 21%), moderate-level hypoxia (MH; F_IO_2 = 16%), or high-level hypoxia (HH; F_IO_2 = 13%) via a portable hypoxic unit. Peak and mean force and power variables were monitored during exercise. SpO_2, heart rate (HR), and RPE were assessed immediately following each set. No differences in force or power variables were evident between conditions. Similar trends were evident in these variables across each set and across the exercise session in each condition. SpO_2 was lower in hypoxic conditions than in NORM ($p < 0.001$), whereas HR was higher following sets performed in hypoxia ($p < 0.001$). There were no differences between conditions in RPE. These results indicate that a hypoxic stimulus during high-load resistance exercise does not alter physical performance during repetitions and sets, or affect how strenuous exercise is perceived to be. While further research is needed, this novel training strategy can be used without adversely affecting the physical training dose experienced.

Introduction

Resistance training using loads of 20-50% of 1RM combined with BFR to working muscles has been demonstrated to rapidly increase muscle size and strength in athletic populations (Cook et al., 2014; Yamanaka et al., 2012). These responses are likely related to an increase in localised hypoxia during BFR, which effects beneficial acute changes in the intramuscular environment (Takarada et al., 2000a). For example, limited oxygen availability increases the reliance on anaerobic metabolism during exercise, augmenting intramuscular metabolic stress (Suga et al., 2012). The resultant accumulation of metabolic by-products is proposed to increase muscle cell swelling (Yasuda et al., 2012), hypertrophic signalling (Fry et al., 2010), and motor unit recruitment (Takarada et al., 2000a), which are all likely to benefit muscular development. Furthermore, localised hypoxia may increase the activation and proliferation of satellite cells, further enhancing muscular hypertrophy (Nielsen et al., 2012). Increased concentrations of systemic hormones have also been observed (Takarada et al., 2000a), though the role of exercise-induced hormonal responses in resistance training adaptation has been recently questioned (West & Phillips, 2010).

Recently, researchers have begun to investigate whether resistance exercise performed while breathing normobaric hypoxic air can augment muscular development, similarly to BFR exercise (Kon et al., 2010; Kon et al., 2012; Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b; Nishimura et al., 2010). Manimmanakorn et al. (2013a) investigated the adaptive responses to

five weeks of low-load IHRT (20% 1RM), with the F_IO_2 being adjusted to maintain SpO_2 at ~80%. The IHRT elicited similar increases in the combined CSA of the knee extensor and flexor muscles to work-matched BFR training, and greater increases than the equivalent training in normoxia in netball athletes. Substantially greater increases in strength and muscular endurance were also reported following the IHRT and BFR training when compared to the control group. Additionally, Nishimura et al. (2010) reported that six weeks of moderate-load IHRT (70% 1RM; F_IO_2 = 16%) resulted in significant hypertrophy in untrained males, despite no change in muscle CSA after matched training in normoxia. Muscle strength was also found to increase significantly after only three weeks of IHRT, whereas significant strength increases in the normoxia group required 6 weeks (Nishimura et al., 2010).

Evidence indicates that improvements in muscular strength following BFR training result primarily from increases in muscular size (Yasuda et al., 2010b). This suggests that neuromuscular adaptations do not contribute strongly to augmented strength levels following BFR training, which is likely related to the low-load of training. It is widely accepted that optimal training for maximal strength requires high-load resistance exercise (1-6-repetition maximum) to facilitate substantial neuromuscular development (Tan, 1999). Therefore, an interesting question that remains unanswered is whether the addition of systemic hypoxia to high-load training can enhance the hypertrophic response to a form of training which largely targets neuromuscular development. As many athletes are required to develop both muscular size and strength, the potential

to optimise the development of these characteristics together via IHRT would be of great interest.

During low- and moderate-load resistance training, the primarily goal is to elicit physiological responses conducive to muscular adaptation. Therefore, relatively brief inter-set rest periods are often employed to elicit substantial metabolic stress (Kraemer & Ratamess, 2004), meaning that the actual weight lifted and the concentric power produced during training may be compromised. In contrast, high-load training places greater emphasis upon lifting heavy loads and attempting to complete the concentric phase of each repetition as powerfully as possible to provide a potent neuromuscular stimulus. It stands to reason that if high-load IHRT is to be beneficial for enhanced hypertrophic and strength development, it is important that the addition of hypoxia does not adversely affect physical performance during training. If resistance exercise performance is impaired by additional hypoxia, it is likely that the training dose experienced will be substantially decreased, limiting the practical applications of high-load IHRT.

Evidence suggests that supplemental hypoxia during repeated sprints (10 s cycling sprints with 30 s recovery between efforts) can profoundly affect cerebral oxygenation, which may result in decreased central nervous system motor output (Smith & Billaut, 2010). Therefore, it is plausible that hypoxia-related central fatigue might impact on performance of high-load resistance exercise under systemic hypoxic conditions. Furthermore, hypoxia has been

demonstrated to impair anaerobic performance during 15 s repeated sprints (15-45 s recovery between efforts) in elite female road cyclists (Brosnan, Martin, Hahn, Gore, & Hawley, 2000), and to decrease peak speed during 6 s repeated sprints on a non-motorised treadmill (30 s recovery between efforts) in male athletes (Bowtell, Cooke, Turner, Mileva, & Sumners, 2014). Both repeat sprint activities and resistance exercise are characterised by repeated short-duration high-intensity efforts that are fuelled largely by anaerobic metabolism. However, these forms of exercise are inherently different and thus may produce dissimilar responses with the addition of hypoxia.

While research suggests that low- and moderate-load IHRT can offer hypertrophic and strength benefits above the equivalent normoxic training (Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b; Nishimura et al., 2010), the efficacy of high-load IHRT has not yet been examined. However, due to the demanding nature of high-load resistance exercise and the importance placed on physical performance during training, it is important to first consider whether additional hypoxia might impact negatively on participants' ability to perform adequately during training. Despite the potential anabolic benefits of IHRT, if force and power output is decreased during training, or if the exercise is perceived to be much more difficult by participants, the applications of such protocols may be limited. It is also not known whether the degree of hypoxia can influence the magnitude of these physical performance indicators. Therefore, the purpose of this study was primarily to assess whether physical performance (measured via concentric force and power variables) during high-

load resistance exercise is affected by the addition of moderate or high levels of hypoxia. Secondly, the project aimed to examine whether common cardiovascular and perceptual measures of intensity are altered during high-load IHRT under different levels of hypoxia.

Methods

Experimental Design

Subjects visited the laboratory on four occasions, each separated by one week. Session one involved 1RM testing for the back squat and deadlift exercises. Using a single-blind, randomised crossover design, subjects then visited the laboratory on three additional occasions, each separated by at least one week. During these trials, subject's performed a high-load resistance training protocol while breathing air via a hypoxic generator (ATS-HP-Hyperoxic, Altitude Training Systems, Lidcombe NSW, Australia), under one of three conditions; NORM (F_IO_2 = 21%); MH (F_IO_2 = 16%); and HH (F_IO_2 = 13%). Force and power variables were monitored during each set via a linear position transducer (GymAware, Kinetic Performance Technology, Canberra, Australia) that was attached to the bar. SpO_2, HR and RPE scores were also obtained immediately following each set to quantify the cardiovascular demands and perception of exercise intensity.

Subjects

Twelve healthy male subjects (age: 25.3 ± 4.3 yr; height: 179.0 ± 4.5 cm; body mass: 83.4 ± 9.1 kg; back squat 1RM: 135.5 ± 28.1 kg; deadlift 1RM: 158.1 ±

29.2 kg) volunteered to participate in this study. All subjects had at least two years resistance training experience and were free of any musculoskeletal disorders. Prior to the commencement of the study, subjects were informed of the purpose and requirements of the research, provided written consent and were screened for medical contraindications. The study and its methods were approved by the University of Newcastle Human Ethics Committee.

Maximal Strength Testing

During their first visit to the laboratory, subjects performed 1RM testing of the back squat and deadlift exercises. Subjects completed a general warm-up (five minutes on a cycle ergometer at a moderate intensity), before performing three specific warm-up sets of the back squat. These were comprised of 10 repetitions at 50% of predicted 1RM weight (as estimated by the subject), 5 repetitions at 70%, and 1 repetition at 90%. Following this, the weight was increased by ~5% and subjects performed a single repetition. This process continued until the subjects were unable to successfully perform a lift, with 180 s rest between attempts. Subjects' 1RM was defined as their heaviest completed repetition, and was determined within 3-6 sets. Following the back squat 1RM assessment, subjects rested for 10 minutes before completing specific warm-up sets and progressing through the same 1RM testing protocol for the deadlift.

During the back squat, subjects supported the bar on the superior trapezius at the base of the neck, and flexed at the knees and hips to descend until the front

of the thighs were parallel with the ground. A customised elastic stringline was set so that subjects' superior hamstrings came in contact with the stringline at this bottom position to signal that appropriate depth had been reached (Cotterman et al., 2005; Scott et al., 2014). This was visually confirmed by members of the research team positioned adjacent to the participant. Subjects were also given verbal cues on when they were to halt the down phase, and begin the up phase, of the squat (Cronin & Hansen, 2005). For the deadlift, conventional technique was used (rather than sumo or Romanian variations), and subjects were required to maintain the spine in a neutral position throughout the lift. A successful repetition was completed once the subject was standing with their shoulders positioned behind the vertical orientation of the bar, which was assessed by a member of the research team positioned adjacent to the subject. If the subject failed to adhere to these performance criteria during a repetition, the lift was deemed unsuccessful. This 1RM testing protocol has previously demonstrated high reliability (CV = 2.6%, ICC = 0.98) (Scott et al., 2014).

Experimental Trials

Experimental trials entailed a high-load resistance exercise protocol performed in either NORM, MH or HH. The exercise protocol was comprised of 5 sets of 5 repetitions of both back squats and deadlifts at 80% 1RM, with 180 s recovery between sets. All sessions were conducted at the same time of day do account for diurnal variations in exercise performance, and subjects were required to abstain from alcohol and caffeine for at least 24 h prior to each testing session,

and strenuous activity for 48 h. Subjects were also instructed to replicate their dietary consumption for each testing day.

For experimental trials, subjects were fitted with a face mask connected to a hypoxic generator, and afforded 10 minutes to acclimate to the oxygenation condition. During this time, subjects were encouraged to perform any specific mobility or flexibility activities that they use prior to high-load squatting and deadlifting exercise. Subjects then performed two warm-up sets of the back squat (10 repetitions at 50% 1RM and 7 repetitions at 65% 1RM), which were separated by 90 s. After 180 s rest, subjects commenced the first of 5 sets of 5 repetitions at 80% 1RM, which were each separated by 180 s recovery. Following the final set of back squats, subjects again rested for 180 s before beginning the same warm-up and exercise protocol for the deadlift exercise. Immediately following each set, SpO_2 and HR were monitored via a pulse oximeter (Rossmax Innotek Corp. Taipei, Taiwan), and Category Ratio-10 (CR-10) RPE (Borg, Hassmen, & Lagerstrom, 1987) scores were obtained, to reflect the blood oxygenation status and cardiovascular demand across each condition. During each visit, subjects were blinded to both oxygenation condition and SpO_2 measures at all times.

Physical Performance Monitoring

During each 5-repetition set, the displacement of the bar and time between data points was recorded via a linear position transducer, sampling at up to 50 Hz. This device is a valid and reliable tool to quantify force and power variables

during resistance exercise (Drinkwater, Galna, McKenna, Hunt, & Pyne, 2007; Scott, Delaney, Elsworthy, & Dascombe, 2013). Data were collected and stored on an iPad device (Apple Inc., Cupertino, USA), before being uploaded to an online database for analysis following each experimental trial. The concentric phase of each repetition was automatically identified by the linear position transducer. *Post hoc* analysis determined measures of peak and mean force and power across the concentric phase of each repetition. To assess changes in performance across a set, mean values of force and power variables were calculated for repetitions 1-5. Furthermore, to assess performance across the exercise protocol, force and power variables were calculated for the first and fifth set of each exercise, to report the percentage change across the five sets.

Statistical Analyses

Data were tested using a Shapiro-Wilk test, and were found to be normally distributed. All data were analysed using a 2-way analysis of variance (ANOVA) with repeated measures. If significant differences were noted, a Bonferroni *post hoc* analysis was performed to assess where differences existed. All analyses were performed using Statistical Package for the Social Sciences (v20.0, IBM Corporation, Somers, New York, USA). The level of statistical significance was set at $p \leq 0.05$. Data are expressed as mean ± SD.

Results

Mean and peak values of force and power variables during each repetition of the back squat exercise are in Figure 4.1. No significant differences were

observed between conditions for any force or power variable. Similarly, no significant differences were observed in mean peak force between repetitions across the exercise protocol. A significant main effect was observed for mean force between repetitions ($F_{4,44}$ = 22.66; p < 0.001; η^2 = 0.67), specifically with repetition 5 being significantly lower than repetition 1 (p < 0.001). A significant main effect was also observed for peak power between repetitions ($F_{4,44}$ = 12.65; p < 0.001; η^2 = 0.54), specifically, with repetition 5 being significantly lower than repetition 1 (p = 0.023). A significant main effect was observed for mean power between repetitions ($F_{4,44)}$ = 129.00; p < 0.001; η^2 = 0.92), specifically, with repetition 1 being significantly greater than repetitions 3-5 ($p \leq 0.001$).

Mean and peak values of force and power variables during the deadlift exercise are presented in Figure 4.2. No significant differences were observed between conditions for any force or power variable. A significant main effect was observed for peak force between repetitions ($F_{4,44}$ = 31.05; p < 0.001; η^2 = 0.74), with repetition 1 being significantly lower than repetitions 2-5 (p = 0.001). There were no significant differences between repetitions for mean force values. A significant main effect was observed for peak power between repetitions ($F_{4,44}$ = 7.22; p < 0.001; η^2 = 0.40), specifically with repetition 1 being significantly lower than repetition 2 (p = 0.022). A significant main effect was observed for mean power between repetitions ($F_{4,44}$ = 18.13; p < 0.001; η^2 = 0.62), with repetition 1 being significantly lower than repetitions 2, 3 and 4 (p = 0.001, 0.003 and 0.027, respectively).

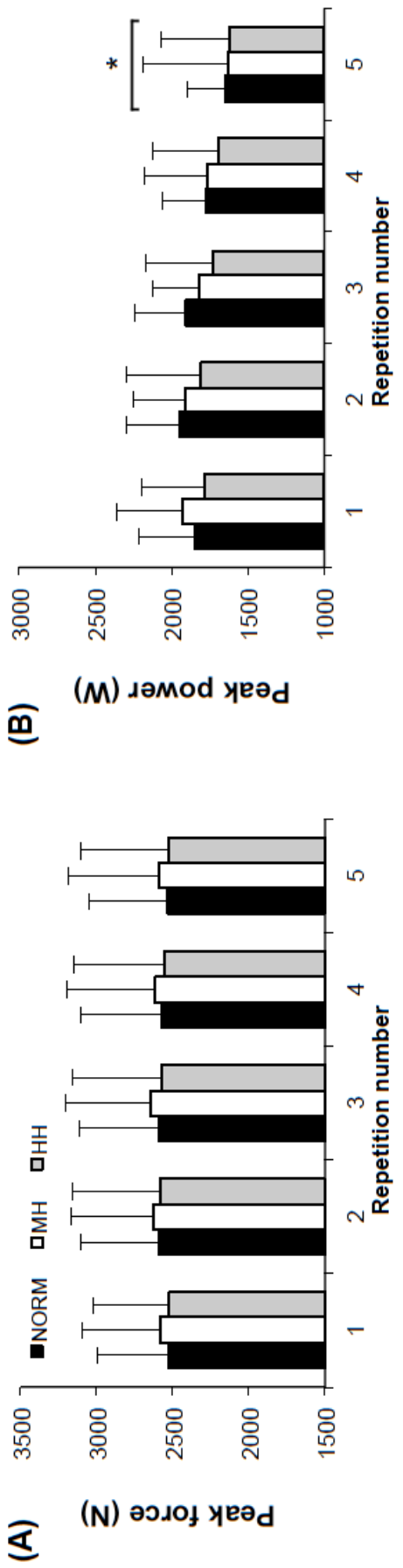

Figure 4.1. Pooled data for (A) peak and (C) mean force as well as (B) peak and (D) mean power during the concentric phase of each repetition across five sets of the back squat. Data are mean ± SD.
NORM normoxia, *MH* moderate-level hypoxia, *HH* high-level hypoxia.
*Significantly different from repetition 1 across all conditions ($p < 0.05$).

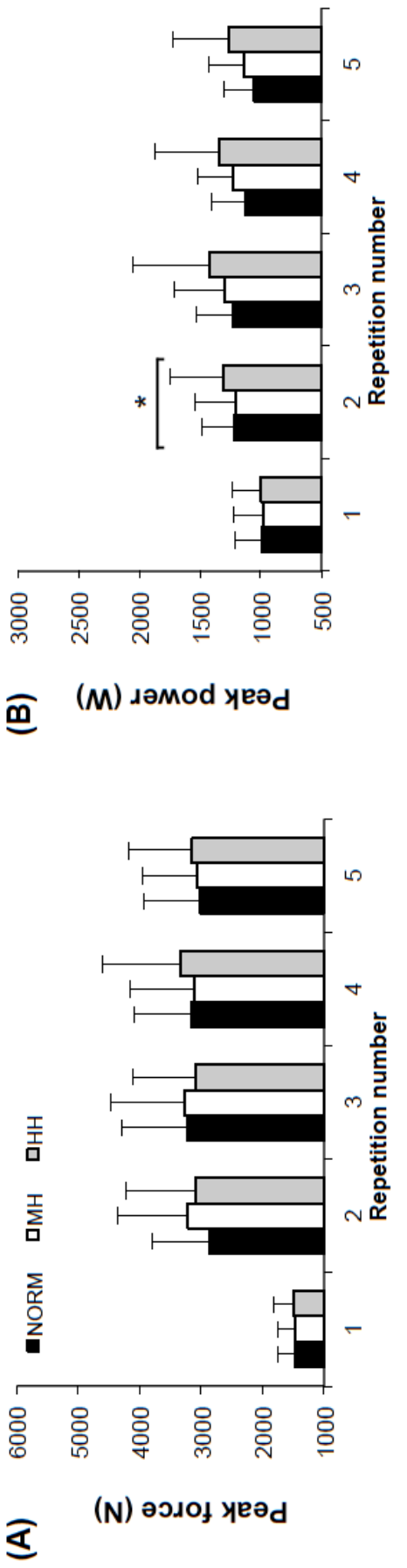

Figure 4.2. Pooled data for (A) peak and (C) mean force as well as (B) peak and (D) mean power during the concentric phase of each repetition across five sets of the deadlift. Data are mean ± SD.
NORM normoxia, *MH* moderate-level hypoxia, *HH* high-level hypoxia.
*Significantly different from repetition 1 across all conditions ($p < 0.05$).

The change in force and power variables for the back squat and deadlift exercises across the 5 sets of the exercise protocol are represented in Figure 4.3. There were no significant main effects for between-condition differences. Figure 4.4 represents the SpO_2, HR and RPE responses following each 5-repetition set for both exercises. There was a significant and strong main effect for SpO_2 between conditions in the back squat ($F_{2,22}$ = 400.73; p < 0.001; η^2 = 0.97) and deadlift ($F_{2,22}$ = 212.64; p < 0.001; η^2 = 0.95). More specifically, SpO_2 in NORM was significantly greater (p < 0.001) in both exercises than in MH and HH, while MH was greater than HH (p < 0.001). There was no difference in SpO_2 between sets in either exercise in any condition.

The HR responses during the back squat exercise demonstrated significant main effects between both conditions ($F_{2,22}$ = 7.14; p = 0.004; η^2 = 0.39) and sets ($F_{4,44}$ = 18.30; p < 0.001; η^2 = 0.63). Specifically, HR was greater in HH than in NORM (p = 0.009), and the HR following set 1 was lower than sets 2-5 (p ≤ 0.003). For the deadlift, there was only a significant main effect for HR between conditions ($F_{2,20}$ = 10.43; p = 0.001; η^2 = 0.51). Again, HR during HH was greater than for NORM (p < 0.001). No differences were found in RPE scores between conditions in either exercise. There was a significant main effect for RPE between sets in the back squat ($F_{4,44}$ = 23.28; p < 0.001; η^2 = 0.68), with RPE scores being greater than set 1 following sets 4 and 5 (p ≤ 0.002). There was a significant main effect for RPE between sets in the deadlift ($F_{4,44}$ = 13.94; p < 0.001; η^2 = 0.56), with RPE scores being greater following set 5 than set 1 (p = 0.001).

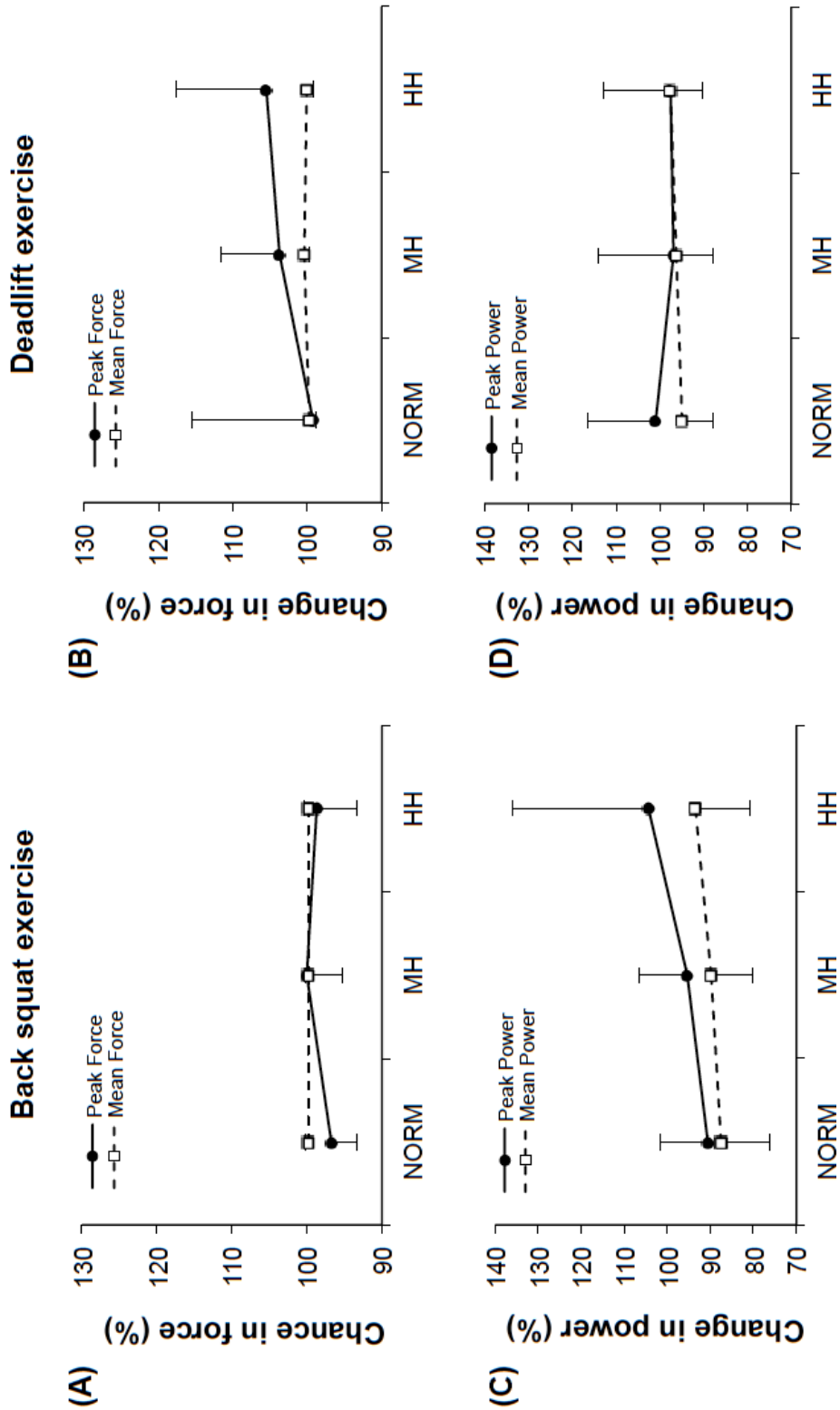

Figure 4.3. Percentage change in concentric force and power from the first to the fifth set of the back squat (A and C) and the deadlift (B and D) in NORM, MH and HH. Data are mean ± SD.
NORM normoxia, *MH* moderate-level hypoxia, *HH* high-level hypoxia.

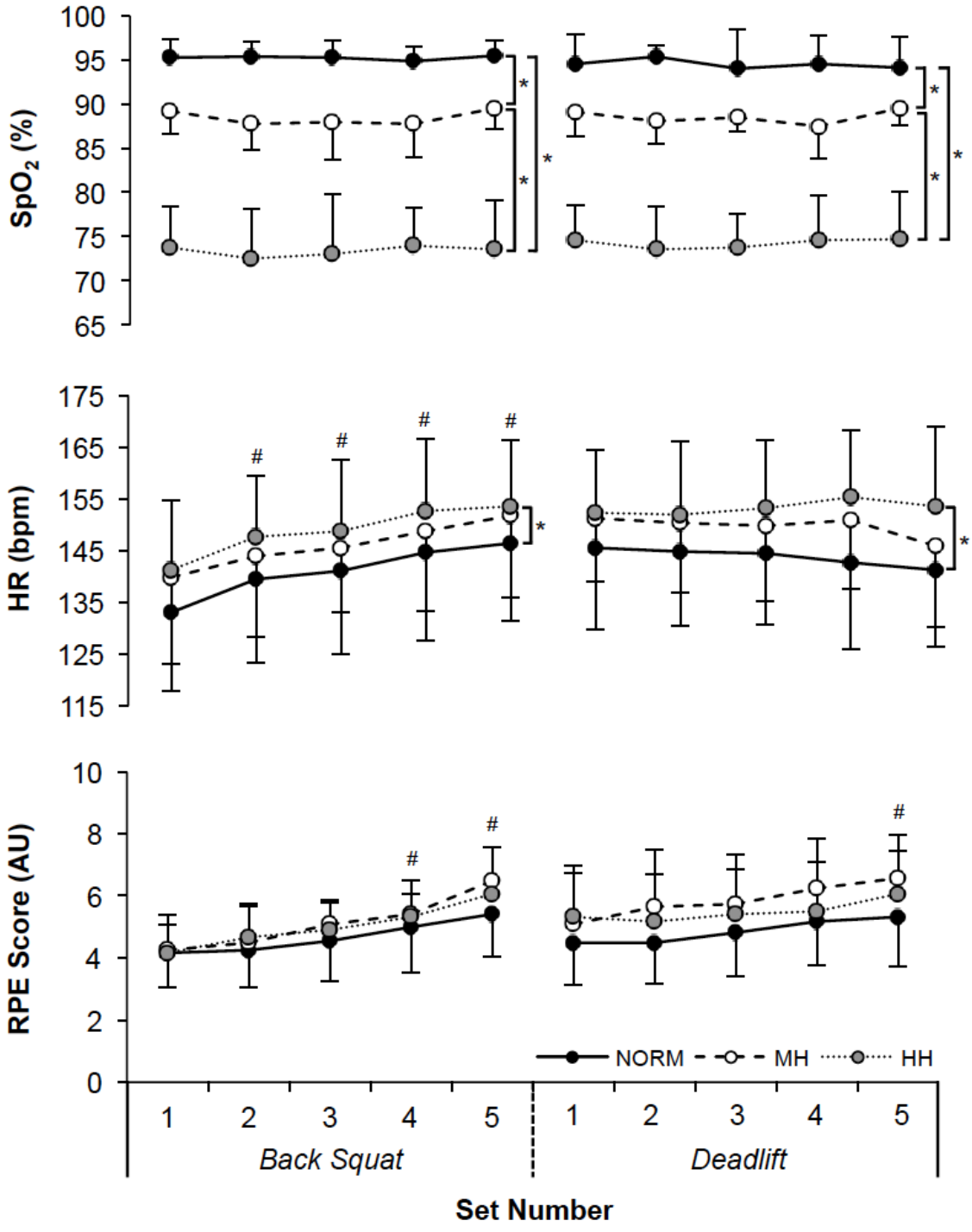

Figure 4.4. Pooled data for SpO_2, HR and RPE immediately following each set of 5 repetitions for the back squat and deadlift exercises. Data are mean ± SD.
SpO_2 arterial oxygen saturation, *HR* heart rate, *RPE* rating of perceived exertion, *NORM* normoxia, *MH* moderate-level hypoxia, *HH* high-level hypoxia.
*Significantly different between conditions ($p < 0.05$), #Significantly different from set 1 across all conditions ($p < 0.05$).

Discussion

This study aimed to quantify physical performance, as well the cardiovascular and perceptual responses, during high-load resistance exercise under hypoxic conditions. The main findings of this investigation demonstrate that force and power variables were not affected by a hypoxic stimulus during high-load resistance exercise, despite an increased cardiovascular demand. While further research is required to fully elucidate the anabolic responses to high-load IHRT, these data highlight that the physical training stimulus is not compromised by the addition of hypoxia.

Although hypoxia has previously caused performance decrements during high-intensity anaerobic exercise (Bowtell et al., 2014; Brosnan et al., 2000), the current data demonstrated no differences in performance during high-load resistance exercises in either MH or HH. Furthermore, trends in force and power values across each set were consistent in all experimental conditions. For the back squat, there was a trend for both mean and peak power values to decline across the set when compared to the first repetition. This may be explained by an accumulation of neuromuscular and metabolic fatigue with each repetition across a set. Indeed, similar trends in resistance exercise sets have been previously noted, with researchers hypothesising that performance decrements result from decreases in adenosine triphosphate (ATP) and PCr concentrations, in concert with increased metabolic stress (Haff et al., 2008). Interestingly, augmented metabolic stress has been noted in recent IHRT research, with higher BLa^- concentrations following hypoxic resistance exercise

compared to the equivalent exercise in normoxia (Kon et al., 2010; Kon et al., 2012). While this might suggest that the onset of fatigue could occur more readily during resistance exercise under hypoxic conditions due to increased metabolic stress, the current data do not support this inference.

This is most likely due to the high-load protocol used, which employed fewer repetitions per set than previous low- and moderate-load IHRT research (5 versus 10-14) (Kon et al., 2010; Kon et al., 2012), and as such decreased the total time-under-tension during which fatiguing intramuscular metabolites could accumulate. Also, the longer inter-set recovery periods used in the current study (180 s versus 60 s) (Kon et al., 2010; Kon et al., 2012) are likely to have facilitated greater removal of these intramuscular metabolites, further lessening metabolic stress. Additionally, these increased recovery periods may have allowed for greater resynthesis of PCr stores. Although hypoxia has been shown to slow the rate of PCr recovery following repeated submaximal plantar flexion exercise when compared to normoxic and hyperoxic conditions, PCr levels returned close to resting levels within 120 s (Haseler, Hogan, & Richardson, 1999). It is therefore likely that the 180 s inter-set recovery employed in the current study was sufficient to allow near-complete PCr resynthesis. To this point, a recent investigation using moderate-load IHRT (10-repetition maximum; 10RM) with longer inter-set rest periods than typically used at that load (120 s) has demonstrated no added hypertrophic or strength benefit for IHRT (Ho et al., 2014b). These results contrast those of Nishimura et al. (2010) and could indicate that longer inter-set rest intervals attenuate any

hypoxia-mediated increases in metabolic stress, and subsequent downstream anabolic responses. It is therefore important to acknowledge that while additional hypoxia does not appear to affect physical performance during high-load resistance exercise in hypoxia, these findings may not translate to low- or moderate-load exercise using more repetitions per set and shorter inter-set recovery periods. Future research should aim to clarify the impact of hypoxia on low- and moderate-load resistance exercise performance, as well as the acute metabolic and potential anabolic responses to high-load resistance exercise with hypoxia.

Similar trends were also evident in each condition for force and power variables for the deadlift exercise, though these were somewhat disparate from those observed for the back squat. For peak force and power, as well as mean power, the first repetition of each set demonstrated lower physical performance than each subsequent repetition. There are two likely explanations for this. Firstly, deadlifts commence with the barbell on the floor, and as such the exercise is initiated with a concentric contraction. In contrast, the back squat is initiated with an eccentric contraction where the subject lowers the weight to the bottom position. As such, the first repetition of a set of deadlifts is unable to take advantage of a preceding eccentric phase, and thus does not benefit from the stretch-shortening cycle, which has previously been shown to enhance contractile force in a subsequent concentric contraction (Komi, 2000). Secondly, subjects were instructed to perform the deadlift in a ‘touch-and-go’ fashion, meaning that each eccentric contraction ended when the barbell came in

contact with the floor, and subjects were instructed to immediately initiate the concentric phase of the next repetition. In doing this, it is likely that the barbell itself underwent a positive change in momentum, which decreased the inertial forces that subjects were required to overcome in the subsequent concentric phase.

As expected, the HH condition resulted in the lowest SpO_2 values, whereas NORM resulted in the highest SpO_2 values. The inverse of this trend was observed when examining the HR values following each set, with HH producing significantly greater responses than NORM. Furthermore, HR values for MH tended to lie between NORM and HH values following each set. These findings likely reflect an increased cardiac output which occurs in response to hypoxia, and may mitigate muscular oxygen deprivation (Gore, McSharry, Hewitt, & Saunders, 2008). Importantly, increased cardiac output, and resultant oxygen delivery, can benefit the aerobic resynthesis of PCr (Glaister, 2005), which is crucial to the production of power during subsequent bouts (McMahon & Jenkins, 2002). However, despite the increase in cardiovascular demand during MH and HH, subjects did not perceive the exercise to be any more difficult than the NORM condition. While RPE scores following sets 4 and 5 of the back squat and set 5 of the deadlift were significantly higher than after the first set of each exercise, there were no differences observed between conditions.

Studies have shown that RPE values provide an effective method of measuring exertion during resistance training (Day, McGuigan, Brice, & Foster, 2004;

Gearhart et al., 2002), and are related to physiological measures of intensity including metabolic stress markers and the magnitude of muscle activation (Lagally et al., 2002), Furthermore, RPE is sensitive to the inverse relationship between resistance exercise intensity and set volume, with heavier sets using fewer repetitions being perceived as more difficult than lifting comparatively lighter weight for more repetitions when external work is held constant (Gearhart et al., 2002). Considering that RPE values are easily obtained without the need for additional equipment and are non-invasive in nature, this tool would provide a practical method to monitor the intensity of resistance exercise in conjunction with a hypoxic stimulus.

Conclusions

In conclusion, the current study demonstrates that the addition of a hypoxic stimulus during high-load resistance exercise does not affect measures of physical performance. Furthermore, despite the increased cardiovascular demands associated with MH and HH, there was no perception that the resistance exercise was any more strenuous than in NORM. Taken together, these findings highlight that a matched physical training dose can be applied to individuals performing resistance exercise in either normoxia or hypoxia. While future research should examine whether the acute responses to high-load resistance exercise in hypoxia promote a favourable anabolic environment, the current data highlight that the training dose is not adversely affected by this novel practice.

Practical Applications

- High-load resistance exercise can be performed in systemic hypoxia without adversely impacting on the imposed physical training dose. This strategy could be employed without negative impacts on their force or power output during training sessions.
- As set RPE values were not affected by the addition of hypoxia to resistance exercise, they may used to easily and non-invasively monitor exercise intensity.
- Athletes who regularly perform intermittent hypoxic training or exposure for haematological or peripheral adaptations may be able to incorporate IHRT into their training to increase their total hypoxic dose in a given week, without sacrificing the physical training stimulus experienced.

Chapter 5

Study 3

Systemic Hypoxia does not Enhance Acute Responses to High-Load Resistance Exercise

As per the peer-reviewed paper **Under Review** in *the Scandinavian Journal of Medicine and Science in Sports*:

Scott, B. R., Slattery, K. M., Sculley, D. V., Smith, S. M., & Dascombe, B. J. (Under Review). Systemic hypoxia does not enhance acute responses to high-load resistance exercise. *Scandinavian Journal of Medicine and Science in Sports*.

Abstract

This study assessed whether hypoxia during high-load resistance exercise could enhance acute physiological responses related to muscular development. Using a randomised single blind cross-over design, 12 trained males (age: 25.3 ± 4.3 yr: height: 179.0 ± 4.5 cm: body mass: 83.4 ± 9.1 kg) performed high-load resistance exercise in three conditions; NORM (F_IO_2 = 21%), MH (F_IO_2 = 16%) and HH (F_IO_2 = 13%). Exercise comprised 5 sets of 5 repetitions of back squats and deadlifts at 80% of 1RM, with 180 s inter-set rest. Muscle oxygenation status and activation were monitored via NIRS and surface EMG, respectively. Metabolic stress was estimated via capillary blood sampling. Perceived fatigue and soreness were also quantified following the exercise. While there appeared to be a trend for HH to cause the lowest levels of muscle oxygenation during exercise, significant differences between conditions were only noted in for HHb_{max} in the deadlift (p = 0.009, ES = 0.82). Although markers of metabolic stress were increased from baseline following exercise ($p \leq 0.004$, ES = 1.10-1.83), there were no differences between conditions. Muscle activation, and perceived fatigue and soreness also did not differ between conditions. While metabolic stress is thought to be a primary moderator of muscle activation and subsequent enhanced muscular development during IHRT, this study suggests that it is not augmented during high-load resistance exercise. This is likely due to the inherent nature of high-load strength training, particularly the relatively low number of repetitions performed and the long inter-set rest periods.

Introduction

Resistance training performed while inspiring hypoxic air has resulted in larger increases in muscular strength and/or hypertrophy using both low (Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b) and moderate (Kurobe et al., 2015; Nishimura et al., 2010) loads. While this novel strategy is a promising new method to enhance muscular development following resistance training, a paucity of research has examined the underlying physiological responses that may underpin these adaptations. In theory, a fundamental physiological response to IHRT is a more hypoxic intramuscular environment. Tissue hypoxia is postulated as a key stressor of cellular metabolism during resistance exercise (Kacin & Strazar, 2011). However, it is not yet clear whether brief hypoxic exposures during IHRT can acutely alter the oxygenation status of contracting muscle.

Hypoxic exposure is known to cause a compensatory increase in vasodilation to match an increased oxygen demand at the muscular level (Casey & Joyner, 2012), and it is possible that this response may hinder any large decreases in oxygen availability from reaching the muscle. Nonetheless, Kon et al. (2010) have demonstrated that moderate-load resistance exercise in hypoxia (5 sets of 10 repetitions for the bench press and leg press using 70% of 1RM) resulted in lower relative oxygen content in the VL than the equivalent exercise in normoxia. These results suggest that the oxygenation status of the muscular environment is acutely altered during IHRT, though further data are required to support these findings.

Theoretically, a more hypoxic intramuscular environment would increase the reliance on anaerobic metabolism during resistance exercise, resulting in heightened markers of metabolic stress. To this end, studies have demonstrated increased BLa^- concentrations during low- and moderate-load resistance exercise when combined with systemic hypoxia (Kon et al., 2010; Kon et al., 2012). The degree of metabolic stress associated with exercise is thought to moderate, at least in part, several downstream processes involved in muscular hypertrophy. An important consequence of metabolic stress during resistance training may be changes in the recruitment of muscle fibres. This has been noted during low-load exercise with BFR (Yasuda, Brechue, Fujita, Sato, & Abe, 2008; Yasuda et al., 2009), which is known to cause increased metabolic stress (Suga et al., 2012). Previous research has also observed significantly greater motor unit recruitment (measured via iEMG) during submaximal isometric contractions when breathing hypoxic compared to normoxic air (Katayama, Amann, Pegelow, Jacques, & Dempsey, 2007). However, it is not yet known whether resistance exercise under systemic hypoxia does in fact augment motor unit recruitment.

While research has examined IHRT using low (Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b) and moderate (Kurobe et al., 2015; Nishimura et al., 2010) loads, limited data are available regarding the acute responses to high-load IHRT. Study 2 has demonstrated that various levels of systemic hypoxia do not adversely impact on physical performance during high-load resistance exercise, or on the perception of effort in well-trained subjects,

meaning that the physical training dose is not affected by the addition of hypoxia. This is critical, considering the importance of concentric performance during high-load strength training. However, it is not yet known whether beneficial muscular responses to high-load resistance exercise can be augmented by a hypoxic stimulus. This is of particular importance for athletic cohorts who often undertake high-load resistance exercise. Many athletes are required to train for maximal strength in concert with numerous other physical and physiological qualities, and therefore cannot dedicate large portions of training solely to high-load resistance exercise. Methods to enhance the adaptive potential of muscle to high-load resistance training are of great interest for these populations, as they may allow for increased muscular development following a given training stimulus. Therefore, this study aimed to examine the effects of different levels of hypoxia on acute muscle oxygenation, metabolite accumulation and muscle activation during high-load resistance exercise.

Methods

Experimental Design

Subjects reported to the laboratory on four occasions, each separated by at least one week. During their first visit, they were familiarised with the research procedures and performed 1RM testing for the back squat and deadlift as described previously (Study 2). Subjects' 1RM was defined as their heaviest completed repetition, and was determined within 3-6 sets. Using a single-blinded, randomised crossover design, subjects then visited the laboratory on three additional occasions to complete a high-load resistance exercise protocol

using the back squat and deadlift. During these trials, subject's breathed air via a hypoxic generator (ATS-HP-Hyperoxic, Altitude Training Systems, Lidcombe NSW, Australia), under one of three conditions; NORM (F_IO_2 = 21%); MH (F_IO_2 = 16%); and HH (F_IO_2 = 13%). All trials took place at the same time of day for each subject to avoid diurnal variations in metabolism and performance.

Subjects

Twelve healthy male subjects (age: 25.3 ± 4.3 yr; height: 179.0 ± 4.5 cm; body mass: 83.4 ± 9.1 kg; back squat 1RM: 135.5 ± 28.1 kg; deadlift 1RM: 158.1 ± 29.2 kg) volunteered to participate in this study. All subjects had at least two years resistance training experience and were free of any musculoskeletal disorders. All subjects reported no exposure to an altitude of greater than 2000 m within six months prior to experimental trials, had no history of severe acute mountain sickness, and were taking no medications that could affect the results of the study (i.e. anabolic steroids, creatine, sympathoadrenal drugs). Prior to the study, subjects were provided with information detailing the purpose and requirements of the research, provided informed consent and were screened for medical contraindications. The study and its methods were approved by the University of Newcastle Human Ethics Committee.

Experimental Trials

Upon arriving for experimental trials, subjects rested for 30 minutes before being fitted with a face mask connected to the hypoxic generator, and afforded 10 minutes to acclimate to the assigned condition. During this acclimation,

subjects were encouraged to perform any specific mobility or flexibility activities that they use prior to high-load resistance exercise. Subjects then performed two warm-up sets of the back squat (10 repetitions at 50% 1RM and 7 repetitions at 65% 1RM) that were separated by 90 s. Subjects then rested for 180 s before commencing the first of 5 sets of 5 repetitions at 80% 1RM, with 180 s recovery between each set. Following the final set of back squats, subjects again rested for 180 s before beginning the same warm-up and exercise protocol for the deadlift. At the conclusion of the protocol, subjects removed the face mask and rested for 40 minutes while breathing ambient air.

Near-Infrared Spectroscopy Monitoring

Muscle oxygenation of the right VL was monitored continuously during trials using a NIRS device (Portamon, Atrinis Medical System, BV, The Netherlands), positioned on the belly of the VL, following SENIAM recommendations (Hermens & Freriks, 1997). To ensure consistent placement, clear plastic sheeting was used during the first trial to mark the device's position in reference to surrounding landmarks on the skin (e.g. sun spots and moles). During subsequent trials, the plastic sheeting was realigned with these landmarks to determine correct placement site for re-application. The device was wrapped in transparent plastic to eliminate direct contact with the skin, and was affixed to the skin with dark tape to prevent contamination from ambient light.

Data were sampled at 10 Hz and transferred via Bluetooth connection to a personal computer for analogue-to-digital conversion, storage, and analysis

using Oxysoft software (Oxysoft, Artinis Medical Systems, BV, The Netherlands). Changes in tissue concentrations HbO_2 and HHb were measured using their chromophoric properties at 750 and 860 nm. Cuff ischemia was performed after five minutes of passive recovery following each experimental trial to obtain maximal HHb and minimum HbO_2 values. This process involved a thigh cuff placed proximal to the NIRS probe, which was rapidly inflated to 250 mmHg for eight minutes or until a nadir in HHb was reached. Cuff ischemia was administered with subjects lying supine and the instrumented leg extended horizontally. Following cuff ischemia, the cuff was deflated and subjects rested for five minutes. Data were smoothed using a 0.5 s moving average, before relative changes in HHb and HbO_2 for each resistance exercise set were calculated by taking the difference between baseline at rest (i.e. after cuff ischemia) and the maximal HHb or minimum HbO_2 obtained during exercise, and dividing by the maximal HHb or minimum HbO_2 obtained during cuff ischemia (Hoffman et al., 2003). In order to report on changes in peripheral oxygenation state during resistance exercise, data are reported as relative HbO_{2min} and HHb_{max} values during each exercise set.

Blood Sampling and Analyses

Capillary blood samples were obtained from a hyperaemic fingertip using capillary tubes (100 µL) prior to subjects being fitted with the hypoxic face mask (i.e. while breathing ambient air), and immediately following the final set of both back squat and deadlift exercises, under the assigned experimental condition. Capillary blood samples were immediately expelled from the capillary tubes into

the sample well of a $CG4^+$ i-STAT cartridge, and analysed for BLa^- concentration, pH, oxygen saturation (sO_2), and partial pressure of oxygen (PO_2), using an i-STAT portable analyser (i-STAT Corporation, East Windsor, USA). This analysis has previously been shown to exhibit high levels of reliability (Dascombe et al., 2007).

Electromyography Monitoring

Muscle activation during each set was monitored via EMG recordings from the left GM, BF, VM and VL muscles. Prior to electrode placement, the skin was shaved, lightly abraded and cleaned with alcohol to ensure optimal electrical conductance. Telemetric surface electrodes (Trigno™ Wireless, Delsys Inc., Boston, USA) were positioned on the belly of the muscle, following the guidelines of SENIAM (Hermens & Freriks, 1997). Clear plastic sheeting was used to identify landmarks on the skin for re-application, as described previously for the NIRS device.

Data were sampled at 2000 Hz and passed through a differential amplifier at a gain of 1000. A band pass filter (fourth order Butterworth filter) was applied at 16-500 Hz. All EMG data were analysed across the concentric phase of movement. The iEMG was calculated for the concentric phase of each set. iEMG values during experimental trials are reported relative to the control NORM condition as per Takarada et al. (2000b). All EMG analyses were performed using EMGworks software (v4.01, Delsys Inc, Boston, USA).

Perceptual Measures

Visual analogue scales were used to subjectively assess physical fatigue and muscle soreness. Subjects were asked to rate their degree of physical fatigue and muscle soreness by marking a 100 mm line with a pen at a point between 0 (no fatigue/soreness) and 100 (maximum fatigue/soreness) (Kon et al., 2012). Physical fatigue was recorded prior to exercise, and at 0, 20 and 40 minutes following the protocol, whereas muscle soreness was recorded prior to exercise (with the initial fatigue score), and at 24 h following exercise.

Statistical Analyses

All data approximated a normal distribution and are represented as mean ± SD. Data were analysed using ANOVA with repeated measures. Where a significant main effect was observed, paired sample *t*-tests were employed to assess where differences existed using Statistical Package for the Social Sciences (version 22.0; IBM Corp., Somers, NY, USA). ES were calculated as the difference in group means divided by the standard deviation of the pooled data to quantify the magnitude of difference in measures between conditions, and were classified as trivial (≤ 0.19), small (0.20-0.49), moderate (0.50-0.79) or large (≥ 0.80) (Cohen, 1988). Statistical significance was set at $p \leq 0.05$.

Results

Muscle Oxygenation

Figure 5.1 shows the mean values of HbO_{2min} and HHb_{max} of the VL during the back squat and deadlift exercises, reported relative to the range in oxygenation

between CI and passive rest. There appeared to be a trend for the lowest levels of HbO_{2min} in HH and the highest levels in NORM, while the inverse relationship for HHb_{max}. However, there were no significant differences observed between conditions for HbO_{2min} in either exercise, or for HHb_{max} in the back squat. A significant main effect was noted between conditions for HHb_{max} in the deadlift ($F_{2,20}$ = 3.976; p = 0.035; η^2 = 0.284), with further analysis confirming HHb_{max} was increased in HH compared with NORM (p = 0.009, ES = 0.82).

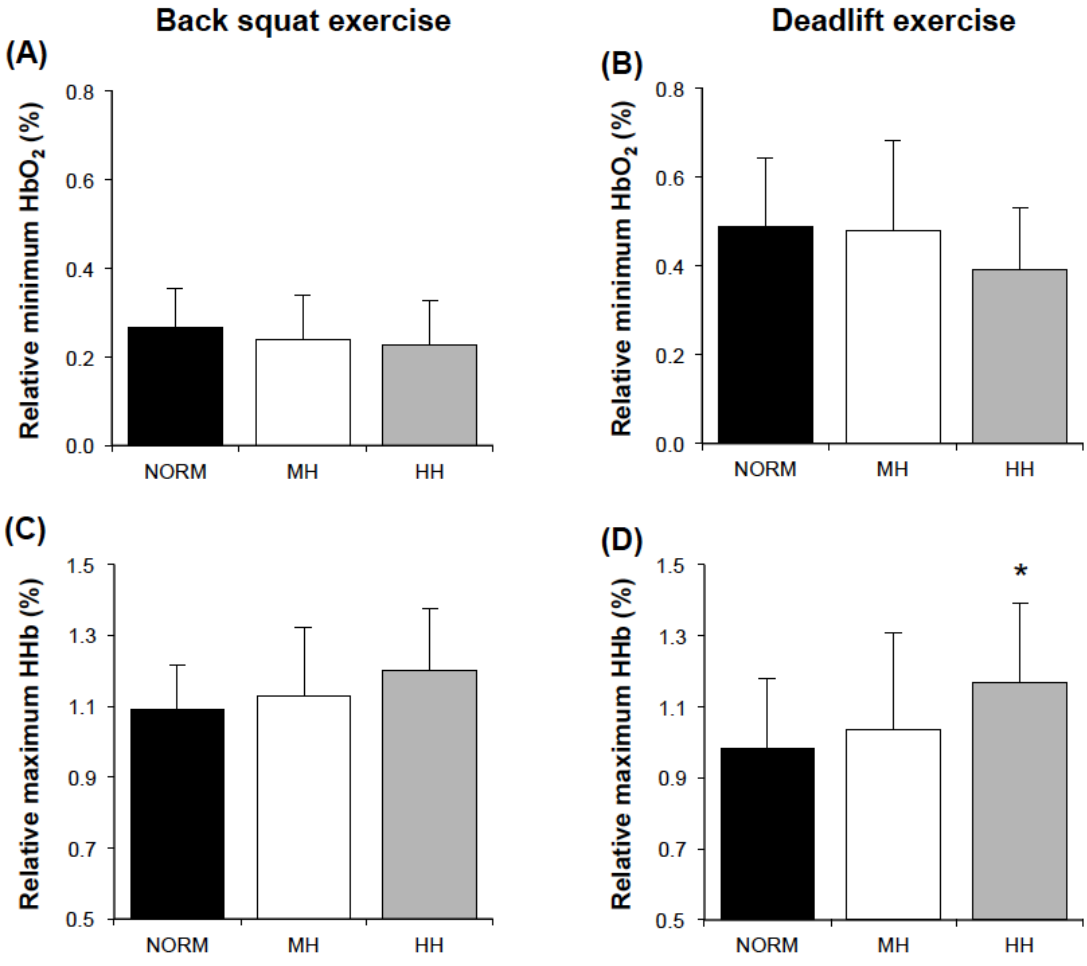

Figure 5.1. Mean ± SD relative values for minimum HbO_2 (A and B) and maximum HHb (C and D), during high-load back squat exercise (A and C) and deadlift exercise (B and D).
NORM normoxia, *MH* moderate-level hypoxia, *HH* high-level hypoxia, *HbO_2* oxyhaemoglobin, *HHb* deoxyhaemoglobin
*Significantly different to NORM.

Metabolic Responses and Blood Oxygenation

BLa^- was increased across all conditions from pre-exercise values following back squats ($p < 0.001$, ES = 1.76-1.83) and deadlifts ($p < 0.001$, ES = 1.70-1.76) in all conditions (Figure 5.2). There were no significant differences between conditions at any time. Blood pH was decreased in all conditions from pre-exercise following back squats ($p \leq 0.004$, ES = 1.28-1.67) and deadlifts ($p \leq 0.004$, ES = 1.10-1.41) in all conditions. A significant main effect was observed between conditions for pH ($F_{2,14} = 5.525$; $p = 0.017$; $\eta^2 = 0.441$), with *post hoc* analyses confirming a significant difference between NORM and HH following back squats ($p < 0.001$, ES = 0.58).

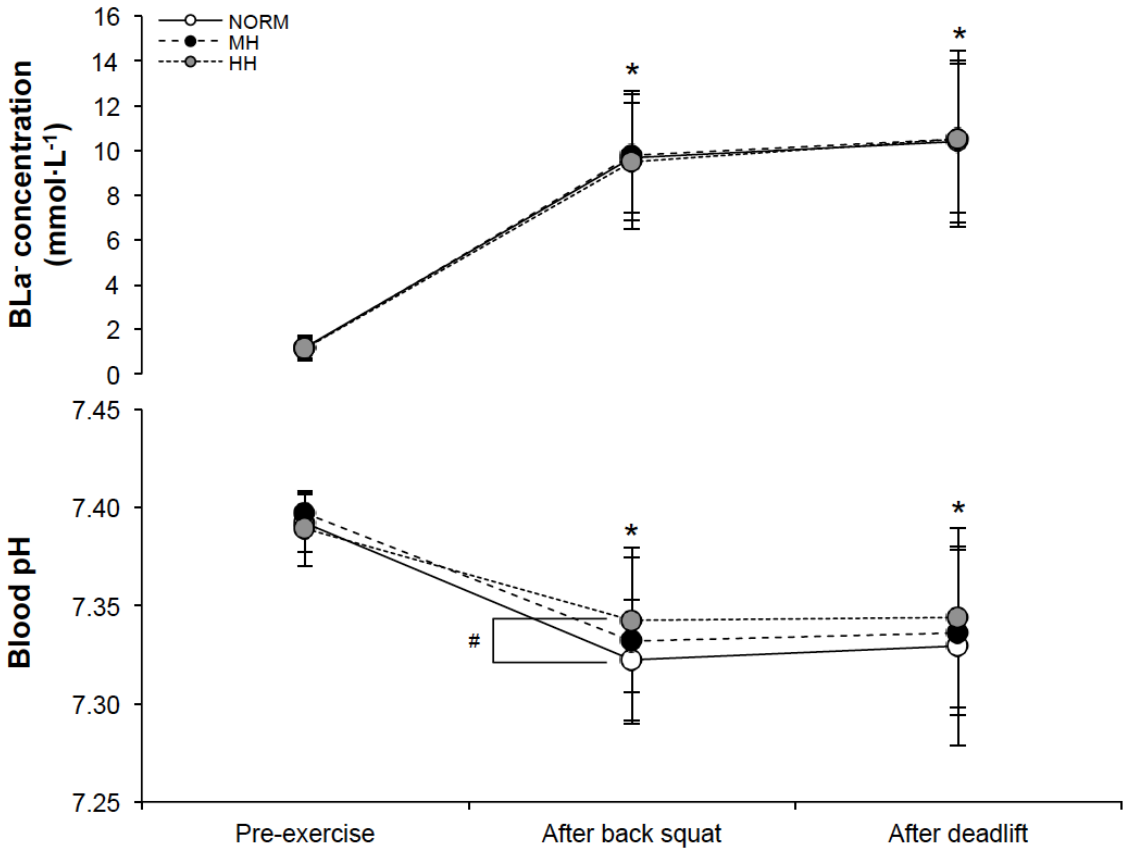

Figure 5.2. Blood lactate (BLa^-) concentrations and pH levels prior to exercise, and following the final sets of back squat and deadlift exercises (mean ± SD).
BLa^- blood lactate concentration, *NORM* normoxia, *MH* moderate-level hypoxia, *HH* high-level hypoxia.
*Significantly different from Pre-exercise. #Significantly different between conditions.

For sO_2 and PO_2 values, there were no significant differences between conditions pre-exercise (Figure 5.3). Significant main effects were observed between conditions for sO_2 ($F_{2,14}$ = 95.446; p < 0.001; η^2 = 0.932), with *post hoc* analyses confirming significant differences between all three conditions following back squats (p < 0.001, ES = 1.62-1.84) and deadlifts (p ≤ 0.001, ES = 1.32-1.77). Significant main effects were observed between conditions for PO_2 ($F_{2,16}$ = 79.913; p < 0.001; η^2 = 0.909), with *post hoc* analyses confirming significant differences between all three conditions following back squats (p < 0.001, ES = 1.71-1.88) and deadlifts (p ≤ 0.001, ES = 1.54-1.83).

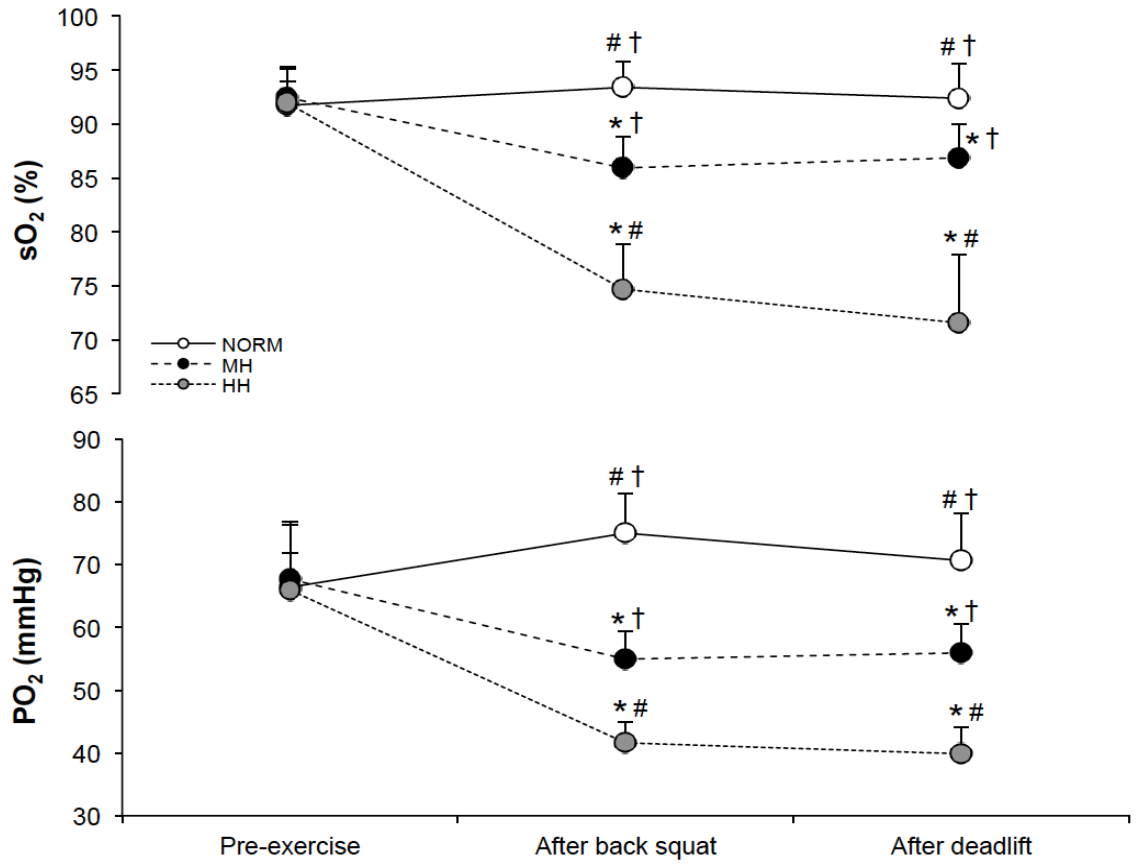

Figure 5.3. Blood oxygen saturation (sO_2) and partial pressure of oxygen (PO_2) prior to exercise, and following the final sets of back squats and deadlifts (mean ± SD).
NORM normoxia, *MH* moderate-level hypoxia, *HH* high-level hypoxia.
*Significantly different to NORM. #Significantly different to MH. †Significantly different to HH.

Muscle Activation

The iEMG values for each selected muscle during the back squat and deadlift exercises in each condition are displayed in Figure 5.4. For the back squat, MH tended to result in higher relative iEMG than both NORM and HH for all muscles assessed. However, statistical analyses observed no significant differences between conditions for iEMG vales. Due to the close proximity of the bar to the anterior thigh during the deadlift exercise, the VM electrode was removed, and was therefore excluded from the analysis. At the remaining electrode sites for this exercise, there were significant differences observed between conditions.

Perceptual Responses

Physical fatigue and muscle soreness responses are shown in Figure 5.5. For physical fatigue, a significant main effect was observed for time ($F_{3,33}$ = 29.287; p < 0.001; η^2 = 0.713), with *post hoc* analysis confirming that scores were significantly increased from pre-exercise levels immediately following exercise in all conditions ($p \leq 0.001$, ES = 1.38-1.55). For muscle soreness, a significant main effect was also observed for time ($F_{1,11}$ = 42.231; p < 0.001; η^2 = 0.793), with *post hoc* analysis confirming that scores were significantly increased from pre-exercise levels immediately following exercise in all conditions ($p \leq 0.004$, ES = 1.19-1.51).

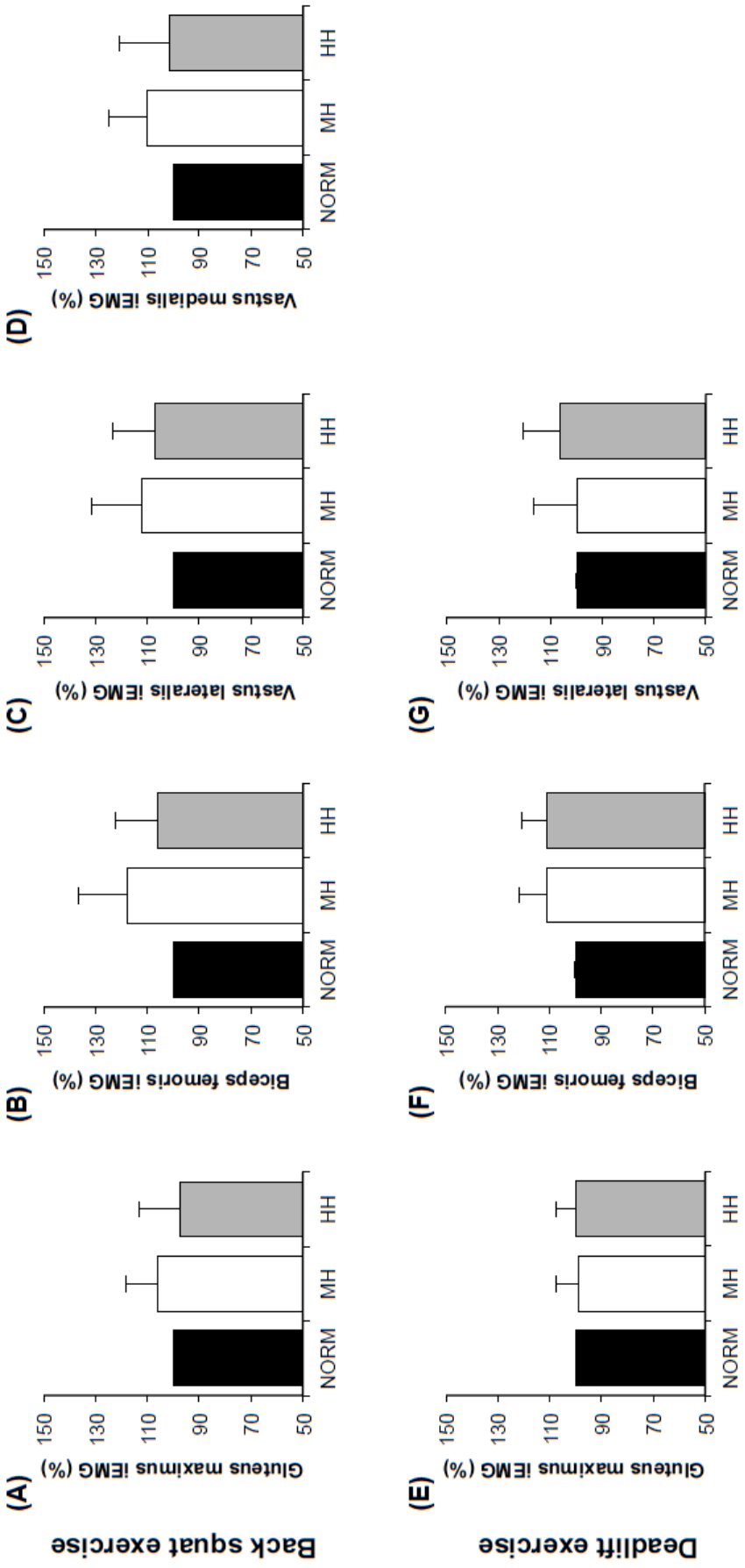

Figure 5.4. Mean iEMG during the concentric phase of the back squat (A-D) and deadlift (E-G) exercises in normoxia (NORM), moderate-level hypoxia (MH) and high-level hypoxia (HH) conditions. Data are presented relative to the NORM condition (mean ± SD).
NORM normoxia, *MH* moderate-level hypoxia, *HH* high-level hypoxia, *iEMG* integrated electromyography.

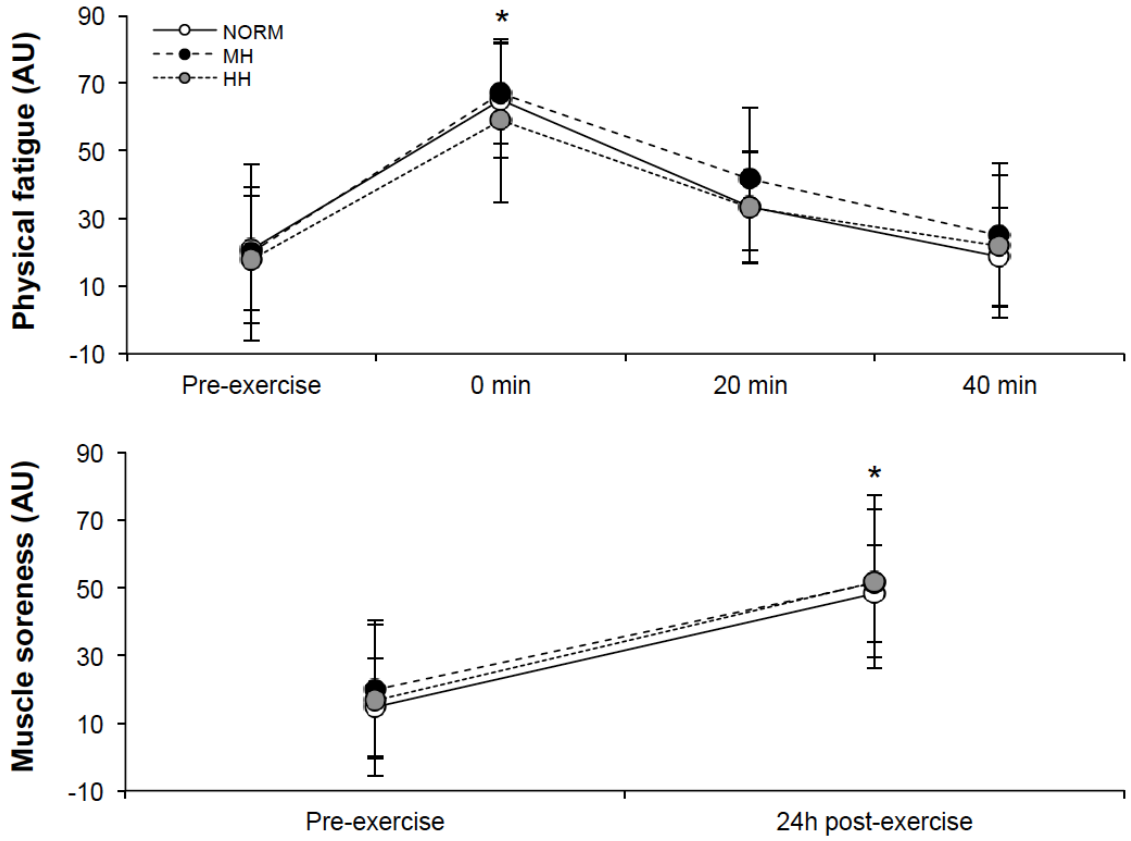

Figure 5.5. Physical fatigue and muscle soreness scores prior to and for up to 40 minutes (fatigue) or 24 h (soreness) post-exercise. Data are mean ± SD.
NORM normoxia, *MH* moderate-level hypoxia, *HH* high-level hypoxia.
*Significantly different to pre-exercise levels.

Discussion

This study is the first to examine the acute physiological responses to high-load resistance exercise under hypoxic conditions. Although a hypoxic dose was evident in blood markers of sO_2 and PO_2 during resistance exercise, large differences in muscular oxygenation were not observed between conditions. Furthermore, while increased metabolic stress and motor unit recruitment are proposed as moderators of IHRT adaptation, the current data do not provide evidence for heightened metabolite accumulation or neuromuscular responses during high-load resistance exercise in hypoxia.

In the current data, there does appear to be a trend for the lowest HbO_{2min} values in the HH condition, and the highest values in NORM. The inverse of this trend was noted for HHb_{max}, with the lowest values observed for the NORM condition, and the highest for the HH condition. However, the only significant difference between conditions for muscle oxygenation data was for HHb_{max} between NORM and HH. This is somewhat different to previous research, which has reported lower levels of minimum oxygenation in working muscle during moderate-load resistance exercise in hypoxia compared to normoxia (Kon et al., 2010). It is possible that biological variations in the muscular oxygen flux may affect the reliability of NIRS variables during high-load dynamic resistance exercise (Study 1), which may have accounted for these results not reaching significance. Additionally, an important consideration is also the level of hypoxia used. Research that has observed significantly lower levels of muscular oxygenation in hypoxia has employed long exposures to moderate terrestrial altitude (3 h at 1800 m) during resistance exercise (Oguri et al., 2004), or a more severe level of hypoxia (F_IO_2 = 10-12%) at rest (Richardson et al., 2006; Rupp et al., 2013). It is also possible that the volume of resistance exercise performed during each set in the current study was not sufficient to facilitate large oxygenation changes in the muscular environment. Indeed, Kon et al. (2010) employed 5 sets of 10 repetitions (70% 1RM; F_IO_2 = 13%) in their investigation. Further research is therefore required to fully elucidate how best a hypoxic stimulus should be combined with resistance exercise to beneficially alter muscular oxygenation.

Previous research has demonstrated that additional hypoxia can increase the degree of metabolic stress (estimated via BLa^- concentration) associated with low-load (Kon et al., 2012) and moderate-load (Kon et al., 2010) resistance exercise. However, the current data provide conflicting results, with no differences between conditions observed for BLa^- concentration. While pH was found to be lower in NORM than HH following back squats, the magnitude of these changes is within the SD of the data and may be a product of the very small absolute changes observed in biological pH levels. It has recently been proposed that metabolic stress plays an integral role in the hypertrophic response of skeletal muscle to resistance exercise (Schoenfeld, 2013). The degree of metabolic stress associated with resistance exercise is likely to moderate several downstream factors, which may contribute to muscular adaptation. Importantly, it is thought that metabolic acidosis has an affect on increasing muscle fibre recruitment during exercise to protect against conduction failure (Yasuda et al., 2010a). The similar BLa^- and pH responses between conditions in this study is likely due to the inherent design of high-load strength training, which uses fewer repetitions per set and longer inter-set recovery periods than low- and moderate-load exercise. Indeed, while protocols similar to that used in the current study are often employed to develop maximal strength in athletic populations, the number of repetitions within each set was markedly lower than in previous low- and moderate-load IHRT research (5 versus 10-14 repetitions) (Kon et al., 2010; Kon et al., 2012), meaning that the time-under-tension during which metabolites could accumulate was decreased.

Furthermore, while high-load strength training generally uses inter-set recovery periods of at least 180 s to allow for adequate neuromuscular recovery between sets, this duration of rest probably allowed for intramuscular metabolites to be largely removed, further lessening their accumulation. In addition, while hypoxia has been shown to slow the rate of PCr recovery following repeated submaximal plantar flexion contractions when compared to normoxic and hyperoxic conditions, PCr levels returned close to resting levels within 120 s (Haseler et al., 1999). It is therefore likely that the 180 s inter-set recovery employed in the current study was sufficient to allow near-complete PCr resynthesis. To this point, while research using moderate-load IHRT with brief inter-set rest periods (60 s) has reported enhanced hypertrophic and strength responses (Nishimura et al., 2010), a recent investigation using similar training loads (10RM) but with longer inter-set rest periods (120 s) has demonstrated no added benefit for IHRT (Ho et al., 2014b). These results could indicate that longer inter-set rest intervals attenuated any hypoxia-mediated increases in metabolic stress, and subsequent downstream anabolic responses. Although further research is required to examine the effects of manipulating inter-set rest periods during hypoxic resistance exercise, it is likely that hypoxia-mediated increases in metabolic stress may only be possible when using relatively brief recovery intervals (Appendix G and Appendix H).

While a primary mechanism by which IHRT may enhance muscular development is increased motor unit recruitment, the current study did not observe any significant differences in iEMG between conditions. However,

considering the BLa^- and pH data presented in this investigation, these findings are not surprising. It is thought that the primary mechanism by which hypoxia may enhance muscle recruitment is increased metabolic stress and the subsequent fatigue of motor units (Schoenfeld, 2013). As BLa^- and pH values were not largely affected by the hypoxic conditions in this study, hypoxia-mediated increases in motor unit recruitment would not be expected. Furthermore, the findings of no differences in perceptual responses between conditions may be explained by these metabolic and neuromuscular data. It could be expected that fatigue responses may be heightened if greater accumulation of metabolites had occurred in hypoxic conditions, and that higher ratings of soreness could result from more muscle being recruited during exercise if motor unit recruitment was increased.

Conclusions

The current study has shown that while muscle oxygenation was somewhat impacted by hypoxia during resistance exercise, large differences were not observed between conditions. Furthermore, markers of metabolic stress and motor unit recruitment were not augmented by hypoxia. Finally, additional hypoxia did not have an effect on the degree of perceived fatigue or muscle soreness following high-load resistance exercise. Taken together, these data suggest that combining hypoxia with high-load resistance exercise does not provide added benefit for acute muscular responses, possibly due to the inherent structure of high-load training (relatively short time under tension with long rest periods).

Practical Applications

- Results from this study do not provide evidence for the efficacy of IHRT to improve muscular development during high-load strength training.
- It is possible that for IHRT to provide beneficial acute responses, exercise should be structured to elicit a potent metabolic stimulus. An important factor in this may be implementing sufficient repetition volume and relatively brief inter-set rest periods.
- Further research should aim to investigate the use of moderate-load resistance exercise in hypoxia using short recovery periods between sets to assess whether this may result in greater accumulation of metabolites and motor unit recruitment.

Chapter 6

Study 4

Acute Physiological Responses to Moderate-Load Resistance Exercise in Systemic Hypoxia

As per the peer-reviewed paper **To Be Submitted** to *Medicine and Science in Sports and Exercise*:

Scott, B. R., Slattery, K. M., Sculley, D. V., Lockhart, C., & Dascombe, B. J. (Under Construction). Acute physiological responses to moderate-load resistance exercise in systemic hypoxia. *Medicine and Science in Sports and Exercise.*

Abstract

This study aimed to assess whether hypoxia could augment acute anabolic responses to moderate-load resistance exercise. Fourteen trained males (age: 24.6 ± 2.7 yr; height: 179.7 ± 5.9 cm; body mass: 84.6 ± 11.6 kg) performed moderate-load resistance exercise in two conditions; NORM (F_IO_2 = 21%) and MH (F_IO_2 = 16%). The resistance exercise comprised 3 sets of 10 repetitions of back squats and deadlifts at 60% of 1RM, with 60 s inter-set rest. BLa^- was quantified after each exercise, while SpO_2 and HR were assessed following each set. Thigh circumference was measured before and following exercise. Muscle activation and oxygenation status were monitored via surface EMG and NIRS, respectively. Relative BLa^- concentrations were significantly higher following squats (p = 0.041) and deadlifts (p = 0.002) in MH. SpO_2 was lower following each set in MH (p < 0.001), though there were no between-condition differences for HR or thigh circumference. iEMG was higher in the MH trial at several time points for the back squat (p ≤ 0.32). Muscle oxygenation did not differ between conditions. This investigation illustrates for the first time that hypoxia during moderate-load resistance exercise can augment acute physiological responses in well-trained subjects. Increased accumulation of metabolites is thought to be important for downstream processes related to hypertrophy, particularly for an increased recruitment of motor units. These findings suggest that adding systemic hypoxia to moderate-load resistance training may provide benefit over the equivalent training in normoxia, with additional motor units being engaged, which in turn may facilitate greater adaptation.

Introduction

The magnitude of adaptation to resistance training is typically explained by alterations to acute training variables, including muscle action, exercise loading and volume, exercise selection and order, inter-set rest periods, repetition velocity and training frequency (Bird et al., 2005). Interestingly, some recent research has suggested that performing resistance exercise under hypoxic conditions may also influence subsequent muscular development, using both low- (Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b) and moderate-loads (Kurobe et al., 2015; Nishimura et al., 2010). Known as IHRT, this strategy may therefore provide a means to augment the adaptive potential of muscle for a given physical training dose, which has obvious implications for moderating the external loads applied to a participant. However, the mechanisms underpinning adaptation to IHRT remain largely unknown.

An important mechanism by which hypoxia may augment muscle hypertrophy is through an elevation of metabolic stress during resistance training (Schoenfeld, 2010). This response is likely associated with the more hypoxic intramuscular environment that results from IHRT (Kon et al., 2010), which essentially increases the reliance on anaerobic processes and thus results in greater production of metabolic byproducts such as BLa^- and H^+. Higher accumulation of BLa^- has been reported in untrained subjects following resistance exercise in hypoxia (F_IO_2 = 13%) compared to normoxia, using both low- (Kon et al., 2012) and moderate-load protocols (Kon et al., 2010). This heightened accumulation of metabolites is thought to moderate downstream biological events related

hypertrophic processes, including increased motor unit recruitment and cellular swelling (Schoenfeld, 2013).

While greater levels of metabolic stress have been reported following low- and moderate-load resistance exercise in hypoxia, conflicting results were obtained in Study 3 using high-load back squat and deadlift exercise (5 sets of 5 repetitions using 80% 1RM with 180 s inter-set rest). Whilst the data demonstrated that a systemic hypoxic stimulus was delivered (estimated via blood sO_2 and PO_2), there was no difference in BLa^- and blood pH levels between conditions. Furthermore, there were no significant differences in the mean iEMG during exercise between conditions for any muscle assessed. Although this may suggest limited benefit for performing resistance exercise under systemic hypoxia, these results are likely affected by the design of high-load strength training, most importantly the 180 s inter-set rest periods used.

Extended inter-set recovery periods during IHRT may attenuate any hypoxia-mediated increase in metabolic stress, as there is sufficient time for PCr stores to be resynthesised and for intramuscular metabolites to be removed from the muscles between sets (Appendix G and Appendix H). Therefore, for IHRT to enhance metabolic stress and result in greater muscular development than the equivalent normoxic training, it is likely that exercise should be structured using brief rest periods between sets. While the application of systemic hypoxia during resistance exercise structured with brief rest periods appears to be a promising new exercise strategy (Kurobe et al., 2015; Nishimura et al., 2010),

understanding of the mechanisms that underpin these adaptations is limited. In addition, moderate-load IHRT research has so far only employed untrained subjects. It is possible that trained subjects who are more accustomed to resistance exercise may not benefit from the additional peripheral stress of hypoxia. Therefore, the primary aims of this study were to investigate the effects of systemic hypoxia during moderate-load resistance exercise on acute metabolic, muscle activation, limb swelling, and muscular oxygenation responses, using well-trained subjects. It was hypothesised that moderate-load IHRT would cause a more hypoxic intramuscular environment, and translate into greater metabolite production, motor unit recruitment and limb swelling.

Methods

Experimental Design

Subjects reported to the laboratory on three occasions, each separated by at least one week. During their first visit, subjects performed 1RM testing of the back squat and deadlift following protocols described previously (Study 2). Subjects' 1RM was defined as their heaviest completed repetition, and was determined within 3-6 sets. Using a single blinded, counterbalanced crossover design, subjects then visited the laboratory on two additional occasions to complete a moderate-load resistance exercise protocol using these exercises. During these trials, subject's breathed air via hypoxic generators (ATS-BASE KIT, Altitude Training Systems, Lidcombe, Australia), under NORM (F_IO_2 = 21%) or MH (F_IO_2 = 16%) conditions. All trials took place at the same

time of day for each subject to avoid diurnal variations in metabolism and exercise performance.

Subjects

Fourteen healthy male subjects (age: 24.6 ± 2.7 yr; height: 179.7 ± 5.9 cm; body mass: 84.6 ± 11.6 kg; back squat 1RM: 138.7 ± 32.6 kg; deadlift 1RM: 161.3 ± 34.8 kg) volunteered to participate in this study. All subjects had at least two years of resistance training experience (mean training age of 4.4 ± 1.5 yr), and were free of any musculoskeletal disorders. All subjects reported no exposure to an altitude of greater than 2000 m within six months prior to experimental trials, had no history of severe acute mountain sickness, and were taking no substances that could affect the study's results (i.e. anabolic steroids, creatine, sympathoadrenal drugs). Prior to commencement of the study, all subjects were provided with information detailing the purpose and requirements of the research, provided informed consent and were screened for medical contraindications. The study and its methods were approved by the University of Newcastle Human Ethics Committee.

Experimental Trials

Upon arriving at the laboratory for experimental trials, subjects were assessed for baseline mid-thigh circumference and BLa^- concentration. Surface EMG electrodes and a NIRS device were affixed to subjects to monitor muscle activation and oxygenation status, respectively. Subjects were then fitted with a face mask connected to the hypoxic generator, and afforded 10 minutes to

acclimate to the assigned condition. During this time, subjects were encouraged to perform any specific mobility or flexibility activities that they use prior to resistance exercise. Subjects then performed two warm-up sets of back squats (10 repetitions at 40% and 50% 1RM), before commencing the first of 3 sets of 10 repetitions at 60% 1RM, with 60 s recovery between all sets. Following the final set of back squats, subjects rested for eight minutes before performing the same warm-up and exercise protocol for the deadlift. This protocol was devised during pilot testing to elicit substantial metabolite accumulation whilst still being achievable for the vast majority of subjects. Immediately following the completion of each working set, SpO_2 and HR were assessed by a pulse oximeter (Rossmax, InnoTek Corp., Taipei, Taiwan) and a HR monitor (Polar FT1, Polar Electro, Kempele, Finland), respectively.

Blood Sampling and Analyses

Capillary blood samples (0.2 µL) were obtained from a hyperaemic earlobe at baseline (before exposure to the assigned condition), and immediately following the final set of each exercise (under the experimental condition). Capillary blood samples were analysed for lactate concentration using a Lactate Scout portable analyser (SensLab GmbH, Leipzig, Germany). This analyser has previously been shown to exhibit acceptable reliability (Bonaventura et al., 2015).

Thigh Circumference

To provide an estimation of muscle swelling, circumference of the left thigh was assessed prior to exercise, and immediately following the conclusion of exercise

trials under ambient conditions. Circumference was assessed at the midpoint between the *trochanterion* and the *tibiale laterale*. Two measurements taken prior to exercise and the mean of these assessments was calculated. If the measured values differed by more than 1% a third measurement was taken. The same process was undertaken again immediately following the protocol, to determine changes in thigh circumference resulting from exercise. During pre-exercise circumference measurements, a line was made on the subject's skin using a marker pen to ensure that post-exercise measurements were taken at the same position. The assessment of mid-thigh circumference was very reliable between repeated measurements at each time point (CV = 0.3%, ICC = 1.00) and between testing sessions (CV = 1.3%, ICC = 0.98).

Electromyography Monitoring

Muscle activation during each set was monitored via surface EMG recordings taken from the left GM, VL, VM and BF muscles. However, due to the close proximity of the bar to the VM electrode during the deadlift, this electrode was removed during this exercise. Prior to electrode placement, the skin was shaved, lightly abraded and cleaned with alcohol to ensure optimal electrical conductance. Telemetric surface electrodes (Trigno™ Wireless, Delsys Inc., Boston, USA) were positioned on the belly of the muscle, and according to the recommendations of SENIAM (Hermens & Freriks, 1997). To ensure consistent electrode placement, clear plastic sheeting was used during the first trial to mark the placement of each electrode in reference to surrounding landmarks on the skin (e.g. sun spots and moles). During subsequent trials, the plastic

sheeting was realigned with these landmarks to determine correct electrode placement sites.

Data were sampled at 2000 Hz and passed through a differential amplifier at a gain of 1000. A band pass filter (fourth order Butterworth) was applied at 16-500 Hz. All EMG data were analyzed across the concentric phase of movement. The iEMG was calculated for the concentric phase of each set. iEMG values during the three main sets for each exercise were summed in groups of 2 repetitions to provide an indication of muscle activation across the protocol. Taking a similar approach to Yasuda et al. (2008), these data were normalised to the first 2 repetitions of the warm-up set at 50% 1RM. All EMG analyses were performed using EMGworks software (v4.01, Delsys Inc, Boston, USA).

Near-Infrared Spectroscopy Monitoring

Muscle oxygenation of the right VL was monitored continuously during experimental trials using a NIRS device (Portamon, Atrinis Medical System, BV, The Netherlands), which was positioned on the belly of the VL as per the SENIAM guidelines (Hermens & Freriks, 1997). Clear plastic sheeting was used to identify landmarks on the skin for re-application, as described for EMG electrodes. The device was wrapped in transparent plastic to eliminate direct contact with the skin, before the device was affixed to the skin with dark tape to prevent contamination from ambient light.

Data were sampled at 10 Hz and transferred via Bluetooth connection to a personal computer for analogue-to-digital conversion, storage, and analysis using Oxysoft software (Oxysoft, Artinis Medical Systems, BV, The Netherlands). Cuff ischemia was performed after five minutes of passive recovery following each experimental trial to obtain maximal HHb and minimum HbO_2 values. Further information regarding this process has been previously detailed in Study 3.

Statistical Analyses

All data approximated a normal distribution and are represented as mean ± SD. Data were analysed using a 2-way ANOVA with repeated measures. Where a significant main effect was observed, paired sample *t*-tests were employed to assess where differences existed. These analyses were completed using Statistical Package for the Social Sciences (version 22.0; IBM Corp., Somers, NY, USA). The ES was also calculated as the difference in group means divided by the standard deviation of the pooled data to quantify the magnitude of difference in measures between conditions, and were classified as trivial (≤ 0.19), small (0.20-0.49), moderate (0.50-0.79) or large (≥ 0.80) (Cohen, 1988). The level of statistical significance was set at $p \leq 0.05$.

Results

Blood Lactate, Arterial Oxygen Saturation and Heart Rate

Figure 6.1 shows changes in relative BLa^- concentration following the final sets of back squats and deadlifts relative to pre-exercise values. A significant main

effect was observed for relative BLa^- concentration between conditions ($F_{1,13}$ = 14.878; p = 0.002; η^2 = 0.534), with paired sample t-tests confirming higher relative BLa^- values in MH following both back squats (p = 0.041; ES = 0.58) and deadlifts (p = 0.002; ES = 0.58).

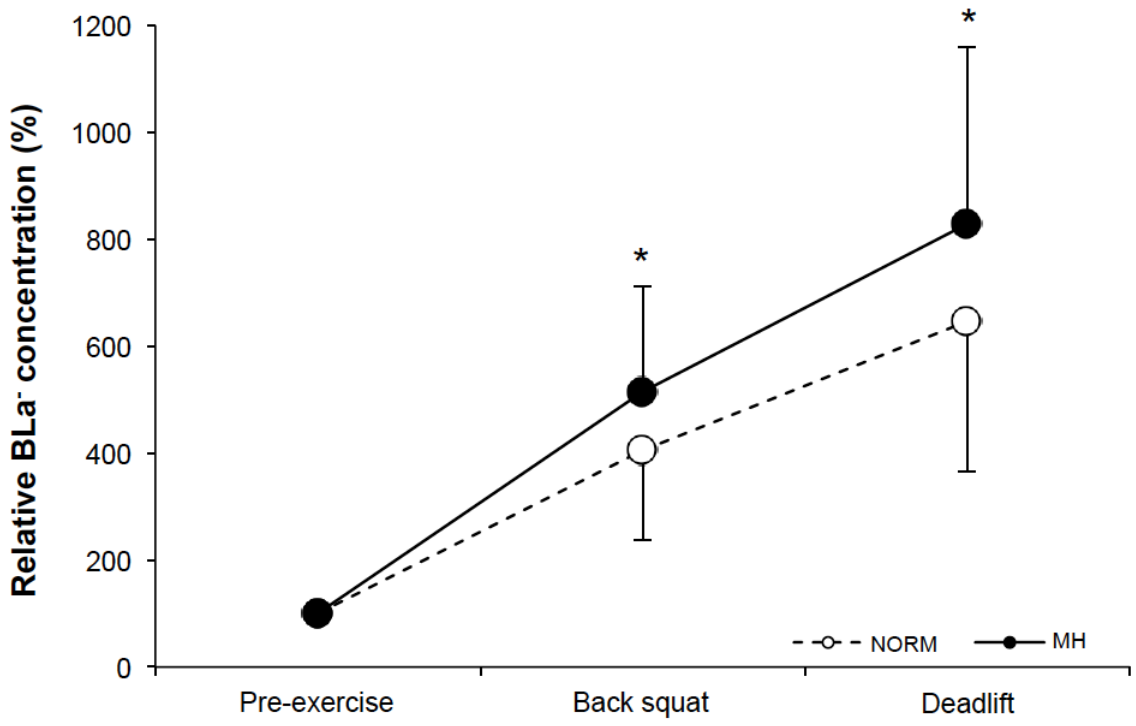

Figure 6.1. Blood lactate (BLa^-) concentrations expressed relative to pre-exercise values immediately following the final set of back squats and deadlifts. Data are mean ± SD.
BLa^- blood lactate, *NORM* normoxia, *MH* moderate-level hypoxia.
*Significant difference between conditions.

Figure 6.2 illustrates the SpO_2 and HR responses to each trial. Significant main effects were observed for SpO_2 between conditions for back squats ($F_{1,13}$ = 238.076; p < 0.001; η^2 = 0.948) and deadlifts ($F_{1,13}$ = 167.745; p < 0.001; η^2 = 0.928). Paired sample t-tests confirmed higher SpO_2 values following sets in NORM for back squats (p < 0.001; ES = 1.63-1.78) and

deadlifts (p < 0.001; ES = 1.63-1.69). Conversely, there were no significant differences between conditions for HR following working sets of both exercises.

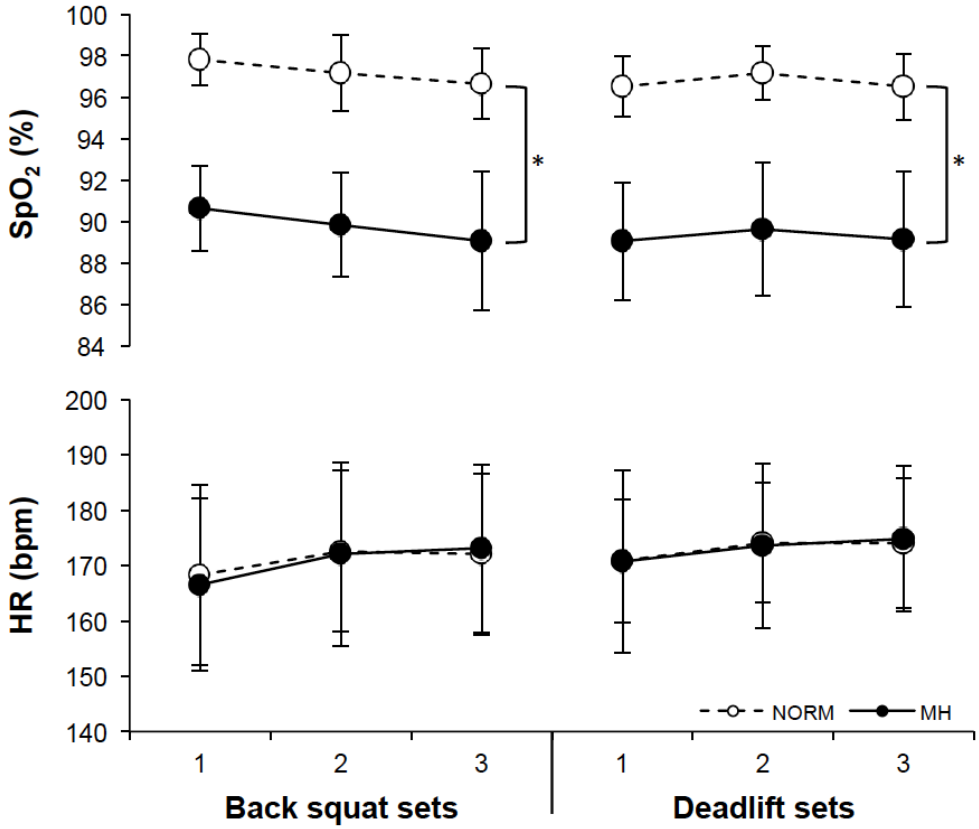

Figure 6.2. Arterial oxygen saturation (SpO_2) and heart rate (HR) immediately following each set of back squats and deadlifts. Data are mean ± SD.
NORM normoxia, *MH* moderate-level hypoxia.
*Significant difference between conditions for all sets.

Thigh Circumference

Following the resistance exercise protocol, mid-thigh circumference was increased from pre-exercise values by 1.5 ± 0.8% in the NORM trial and 2.5 ± 2.4% in the MH trial. While thigh circumference was increased by a larger magnitude in MH, this increase was not significantly greater than that observed in the NORM condition.

Muscle Activation

Figure 6.3 shows the concentric iEMG for each muscle assessed during the back squat. For the GM, no significant main effects were observed between conditions in the back squat. For the BF, a significant main effect was observed between conditions for set 3 of back squats ($F_{1,12}$ = 6.536; p = 0.025; η^2 = 0.353). However, *post hoc* analysis did not identify significant differences between conditions for any repetitions within the set (p = 0.191 – 0.649). For the VL, a significant main effect was observed between conditions for set 2 ($F_{1,12}$ = 14.557 p = 0.002; η^2 = 0.548) and set 3 ($F_{1,12}$ = 19.951; p = 0.001; η^2 = 0.624) of back squats. Paired sample *t*-tests confirmed higher relative iEMG values in MH during set 2 for repetitions 5-6 (p = 0.015; ES = 0.46) and 9-10 (p = 0.003; ES = 0.59). For set 3, higher iEMG values were reported in MH for repetitions 1-2 (p = 0.007; ES = 0.48), 3-4 (p = 0.014; ES = 0.58), 5-6 (p = 0.032; ES = 0.44), 7-8 (p = 0.014; ES = 0.53) and 9-10 (p = 0.003; ES = 0.79). For the VM, a significant main effect was observed between conditions for set 1 ($F_{1,10}$ = 4.992; p = 0.049; η^2 = 0.333), with higher relative iEMG values observed in MH during repetitions 7-8 (p = 0.008; ES = 0.99). A significant main effect was also observed between conditions for set 3 ($F_{1,10}$ = 14.991;p = 0.003; η^2 = 0.600), with *t*-tests demonstrating relative iEMG values were higher in MH during repetitions 1-2 (p = 0.001; ES = 1.06), 3-4 (p = 0.015; ES = 0.69) and 9-10 (p < 0.001; ES = 1.01). For the deadlift, while there appeared to be a trend for higher relative iEMG values in MH compared to NORM (Figure 6.4), there were no significant between-condition differences for any set in the three muscles assessed.

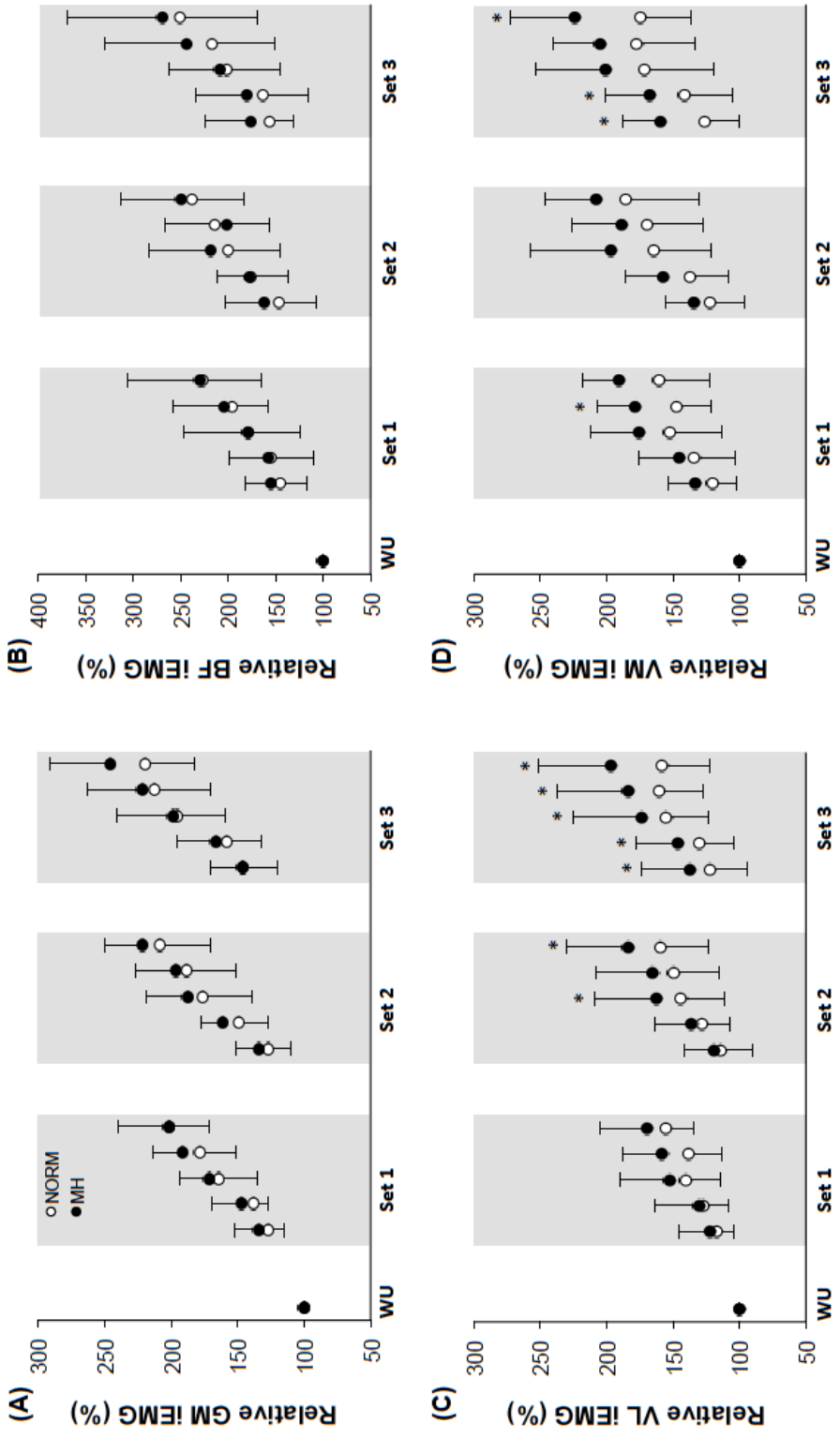

Figure 6.3. Mean ± SD integrated electromyography (iEMG) values during the back squat for the *gluteus maximus* (GM; A), *biceps femoris* (BF; B), *vastus lateralis* (VL; C) and *vastus medialis* (VM; D). Each data point represents the summed iEMG for 2 successive repetitions, normalised to the first 2 repetitions of a warm-up set at 50% 1RM (WU).
*Significant difference between conditions

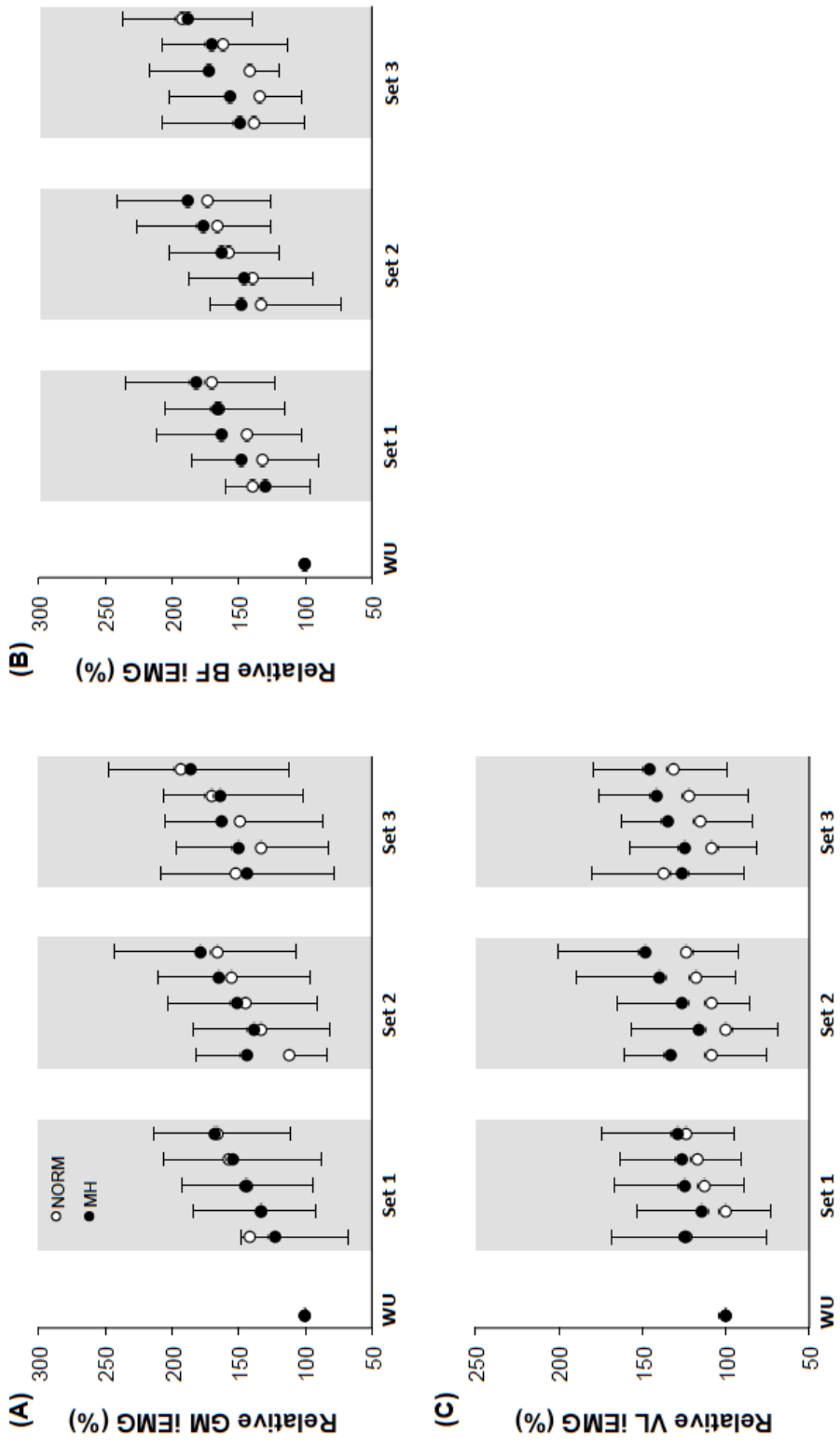

Figure 6.4. Mean ± SD integrated electromyography (iEMG) values during the deadlift for the *gluteus maximus* (GM; A), *biceps femoris* (BF; B) and *vastus lateralis* (VL; C). Each data point represents the summed iEMG for 2 successive repetitions, normalised to the first 2 repetitions of a warm-up set at 50% 1RM (WU).

Muscle Oxygenation

The mean relative changes in HbO_{2min} and HHb_{max} values for back squats and deadlifts are shown in Figure 6.5. There were no significant differences between conditions for any NIRS-derived variable.

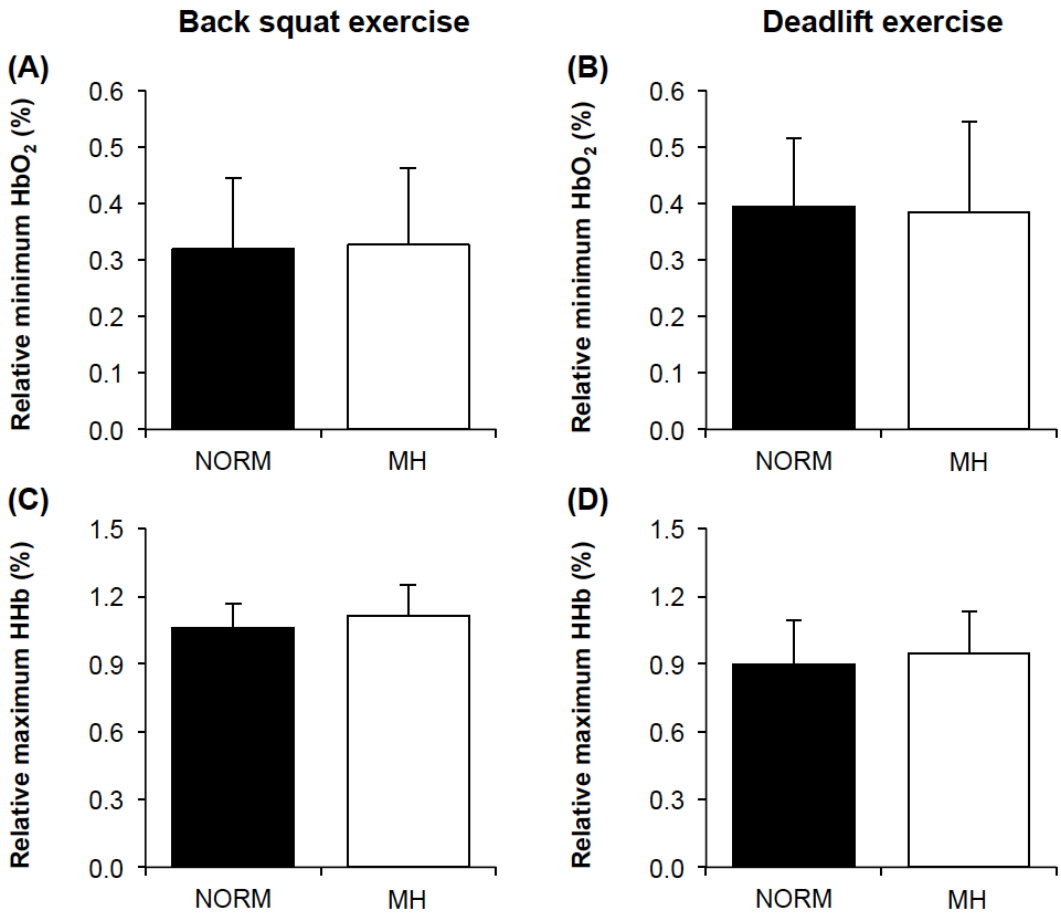

Figure 6.5. Mean ± SD relative values for minimum HbO_2 (A and B) and maximum HHb (C and D), during back squat (A and C) and deadlift (B and D) exercise.
NORM normoxia, *MH* moderate-level hypoxia, *HbO_2* oxyhaemoglobin, *HHb* deoxyhaemoglobin

Discussion

The main findings of this study demonstrated that moderate-load resistance exercise performed in hypoxia can enhance markers of metabolic stress and

muscle activation in well-trained subjects. These acute responses are thought to be important for subsequent muscular adaptation to resistance exercise. Interestingly, while IHRT also resulted in lower levels of SpO_2 than the equivalent exercise in normoxia, muscular oxygenation status did not differ between conditions.

The current study is the first to report that performing resistance exercise in systemic hypoxia can enhance the metabolic stress response in well-trained subjects. These findings are in agreement with previous research that has observed enhanced BLa^- concentrations following low-load (Kon et al., 2012) and moderate-load (Kon et al., 2010) resistance exercise in hypoxia for untrained subjects. Importantly, a common methodological feature of this study and those conducted by Kon et al. (2010; 2012) is the relatively brief inter-set rest periods employed (60 s). In contrast, Study 3 used longer rest periods (180 s) with high-load exercise, and did not observe any effect of hypoxia on BLa^- or pH. In addition, other research which has not observed hypoxia to enhance metabolic stress has prescribed low-load exercise combined with relatively long rest intervals (Ho et al., 2014a), or has not employed work-matched exercise between hypoxic and normoxic conditions (Yan et al., In Press). Furthermore, Kurobe et al. (2015) prescribed exercise protocols using low exercise volume and small muscles (3 sets of 10RM elbow extensions), which resulted in relatively low post-exercise BLa^- concentrations (~2.5-3.5 $mmol \cdot L^{-1}$) and therefore small magnitudes of difference between conditions. While further research is needed to directly examine the impact of inter-set rest periods and

exercise volume on acute responses to hypoxic resistance exercise, it is likely that these variables should be appropriately manipulated to maximise the effects of a hypoxic stimulus (see Appendix G and Appendix H).

While an increased recruitment of muscle fibres is proposed as an important mechanism for enhanced adaptive responses to IHRT, research into this area is limited. The present study is the first to demonstrate that IHRT can enhance muscle activation during resistance exercise. These findings are dissimilar to Study 3, where no differences between conditions were observed for mean relative iEMG during back squats and deadlifts. However, as discussed previously, an important difference between the current study and Study 3 is the exercise protocol employed, and resultant dissimilar levels of BLa^- observed. Given that metabolic acidosis may inhibit muscle contractility and subsequently promote the recruitment of additional high-threshold motor units (Debold, 2012; Takarada et al., 2000b), it is likely that these results are indicative of the higher levels of BLa^- noted in the hypoxic condition of the current investigation.

Additionally, this investigation assessed changes in muscle activation across the exercise protocol, using similar methods as in previous BFR research (Yasuda et al., 2008; Yasuda et al., 2012). From the current data, it appears that the largest differences in iEMG values between conditions occurred in the VL and VM towards the end of the back squat protocol. This could indicate that as the concentration of metabolic by-products increased, so too did the levels of muscular fatigue and consequently additional motor units were recruited.

However, there were no significant differences between conditions for iEMG in the GM or BF. In addition, while the hypoxic trial appears to cause increased iEMG during the deadlift, particularly for the VL (Figure 6.4), these values were not significantly greater than the normoxic condition. This is despite a significantly greater BLa^- concentration following the deadlift. A possible explanation is the inherent variability in test-retest results for EMG variables during multi-joint resistance exercise. Study 1 has reported that the inter-test typical error (expressed relatively as a CV) for iEMG during a back squat exercise at 70% 1RM varies greatly across different repetition schemes from 21.2-52.8%. This may be due to small changes in segmental orientation of the limbs and torso during dynamic exercises such as squats and deadlifts, which would influence the degree of muscle activation measured via surface EMG.

As expected, SpO_2 was found to be significantly lower following sets in hypoxia compared to normoxia. These values are similar to previous work investigating high-load IHRT (Study 2). Interestingly, HR was not different between conditions in the current investigation. While it may be expected that the hypoxic condition would result in higher HR values, representing increased cardiac output (Gore et al., 2008), this was not observed. This could be related to the moderate level of hypoxia employed in the current study. Study 2 has previously shown that while HR is significantly higher in HH (F_IO_2 = 13%) than NORM following sets of high-load resistance exercise, HR following sets in MH (F_IO_2 = 16%) was not different to either other condition.

It is proposed that muscle cell swelling may increase during exercise that promotes high levels of metabolite accumulation, and subsequently initiate anabolic and anti-catabolic processes (Schoenfeld & Contreras, 2014). Fry et al. (2010) have previously demonstrated that thigh circumference was elevated for up to 30 minutes following low-load BFR resistance exercise, and that this increase was significantly greater than the non-BFR control condition. Importantly, BFR exercise is well known to induce higher BLa^- concentrations than the equivalent exercise without BFR even after the cuff was removed (Suga et al., 2012), which may moderate these post-exercise increases in estimates of cell swelling. However, in the current study there was no difference between conditions for increases in mid-thigh circumference following exercise despite higher relative BLa^- concentrations. It is possible that the circumference measurement technique used was not sensitive enough to detect very small differences between conditions. While this assessment was found to be highly reliable, any differences in cell swelling between conditions may have been smaller than the error associated with the measurement. Indeed, the thigh circumference increases following the resistance exercise in hypoxia and normoxia (1.4 ± 1.3 cm and 0.8 ± 0.4 cm, respectively) were smaller than those reported by Fry et al. (2010) after exercise with or without BFR (2.5 ± 0.6 cm and 1.3 ± 0.3 cm, respectively). Additional research using more sensitive assessments of muscle circumference or thickness is therefore required to elucidate whether transient increases in muscle size do occur during hypoxic resistance exercise.

An unexpected finding from this study was the lack of difference in muscular oxygenation responses between conditions. Previous research has observed lower levels of minimum oxygenation in contracting muscles when performing moderate-load resistance exercise in hypoxia compared to normoxia (Kon et al., 2010). A potential explanation for these divergent findings may be the level of hypoxia employed. While this study employed hypoxia with a F_IO_2 of 16%, Kon et al. (2010) used a F_IO_2 of 13%. Additionally in Study 3, a general trend was observed for the greatest disturbance to muscular oxygenation during resistance exercise in HH (F_IO_2 = 13%), though there were no differences between the NORM and MH (F_IO_2 = 16%) conditions. In addition, other research that has observed significantly lower levels of muscular oxygenation in hypoxia has employed long exposures to moderate terrestrial altitude (3 h at 1800 m) during resistance exercise (Oguri et al., 2004), or a more severe level of hypoxia (F_IO_2 = 10-12%) at rest (Richardson et al., 2006; Rupp et al., 2013). While these data suggest that increases in relative BLa^- concentration and iEMG activity in the MH condition were not related to intramuscular hypoxia, this is difficult to reconcile. Theoretically, increased BLa^- concentration would result from a greater reliance on anaerobic muscular processes, presumably caused by a degree of muscular hypoxia. Further research to this point is required.

Conclusions

The current investigation is the first to demonstrate that moderate-load IHRT can enhance metabolite accumulation in conjunction with increased muscle activation in well-trained subjects. These findings provide insight regarding the

mechanisms by which IHRT may benefit muscular development. While further research is required to fully elucidate these mechanisms and to determine how best to combine resistance training with hypoxia to benefit adaptive responses, the current data suggest that supplemental hypoxia during resistance exercise may be a beneficial training strategy.

Practical Applications

- Well-trained individuals can employ MH during moderate-load resistance exercise with relatively brief inter-set rest periods (60 s) to enhance metabolite accumulation and muscle activation, which are proposed to increase hypertrophic and strength adaptations.
- Practical circumference measurements used in this study do not provide evidence for increased muscle swelling during IHRT. Further research is required using more sensitive measures such as magnetic resonance imaging to investigate muscle swelling responses to IHRT.
- Despite altered SpO_2 and metabolic responses between conditions, muscle oxygenation does not appear to be affected by moderate hypoxia (F_IO_2 = 16%). Further research is required to determine the impact of systemic hypoxia during resistance exercise on muscle oxygenation.

Chapter 7

Study 5

Resistance Exercise in Hypoxia does not Affect Markers of Physical Performance, Training Stress or Neuromuscular Recovery

As per the peer-reviewed paper **To Be Submitted** in the *International Journal of Sports Physiology and Performance:*

Scott, B. R., Slattery, K. M., Sculley, D. V., & Dascombe, B. J. (Under Construction). Resistance exercise in hypoxia does not affect markers of physical performance, training stress or neuromuscular recovery. *International Journal of Sports Physiology and Performance*

Abstract

This study aimed to determine whether performing resistance exercise in hypoxia affects markers of physical performance, training stress and neuromuscular function. Fourteen male subjects (age: 24.6 ± 2.7 yr; height: 179.7 ± 5.9 cm; body mass: 84.6 ± 11.6 kg) with at least two years resistance training experience performed moderate-load resistance exercise in two conditions; NORM (F_IO_2 = 21%) and MH (F_IO_2 = 16%). Resistance exercise comprised 3 sets of 10 repetitions of back squats and deadlifts at 60% of 1RM, with 60 s inter-set rest. Physical performance was assessed by quantifying velocity and power variables during all repetitions. Perceptual ratings of perceived exertion, physical fatigue, muscle soreness and overall wellbeing were obtained during and following exercise. Neuromuscular performance was assessed via vertical jump and isometric mid-thigh pull (MTP) tasks for up to 48 h following exercise. While physical performance declined across sets, there were no differences between conditions. Similarly, perceived exertion and fatigue scores were not different between conditions. Muscle soreness was increased similarly from baseline at 24-48 h following exercise in both conditions ($p \leq 0.001$). Jump height and MTP peak force were decreased from baseline immediately after exercise ($p \leq 0.026$), but returned to pre-exercise values after 24 h. These findings corroborate previous research suggesting that IHRT does not affect exercise performance or perceived exercise intensity. Additionally, practical markers of training stress and neuromuscular recovery were not affected by hypoxia, suggesting that IHRT may not add substantially to the training dose experienced.

Introduction

Breathing hypoxic air during resistance training, also known as IHRT, can significantly augment hypertrophic responses to both low-load (Manimmanakorn et al., 2013a) and moderate-load (Kurobe et al., 2015; Nishimura et al., 2010) resistance training. However, an important consideration for those looking to implement IHRT strategies is the potential impact of hypoxia on the applied training dose. Performance decrements across a set or during a session of resistance exercise are thought to result from decreases in ATP and PCr concentrations, in concert with increased metabolic stress (Haff et al., 2008). Given that PCr resynthesis kinetics are affected by oxygen availability (Haseler et al., 1999), and that breathing hypoxic air during resistance exercise has caused elevated BLa^- levels (Kon et al., 2010; Kon et al., 2012; Study 4), it is plausible that physical performance across repeated efforts could be adversely affected. Study 2 has demonstrated that physical performance during high-load resistance exercise is not affected by hypoxic conditions (F_IO_2 = 16% and 13%). However, the markers of metabolic stress (BLa^- and pH) were also not different between conditions (Study 3), possibly because the long rest periods associated with high-load training mitigated any hypoxia-mediated increases in metabolic stress (Appendix H). Therefore, the observation that systemic hypoxia did not affect physical performance during repetitions was not surprising. Therefore, while increased metabolic stress may potentially moderate muscular development during IHRT, it is not yet clear whether physical performance during such exercise may be compromised.

The reduced oxygen availability during hypoxic exercise may produce a greater peripheral physiological stimulus than exercise in normoxia, despite the same absolute external stimulus (McLean, Gore, & Kemp, 2014). It could therefore be argued that the internal training dose would be exaggerated by adding hypoxia during resistance training. This is important, as sudden increases in training load above the normal training limits can cause performance decrements and lead to injury or illness (Foster, 1998). The most practical method to quantify overall stress associated with resistance exercise and the degree of fatigue resulting from training is via perceptual responses. The session RPE (sRPE) method allows individuals to provide a single global rating for how difficult an entire resistance training session was using an RPE scale, and has been reported as a valid (Sweet, Foster, McGuigan, & Brice, 2004) and reliable (Day et al., 2004) indicator of resistance training intensity. However, sRPE has not been compared between normoxic and hypoxic sessions of resistance exercise. Furthermore, it is unknown whether the additional stress of hypoxia during resistance training may impact on other perceptual markers of wellbeing (McLean, Coutts, Kelly, McGuigan, & Cormack, 2010) and physical fatigue (Kon et al., 2012).

In addition, perceived muscle soreness can indicate the magnitude of exercise-induced muscle damage (Clarkson & Hubal, 2002), though this has not yet been assessed following resistance exercise in hypoxia. Muscle damage is known to cause prolonged decrements in force production (Clarkson & Hubal, 2002), which would have obvious detrimental effects on subsequent exercise

performance if it is increased during IHRT. As such, practical tests of neuromuscular function such as vertical jumps and isometric MTP tasks may also reflect the degree of muscle damage following resistance exercise (Byrne & Eston, 2002). However, research that investigates whether performing IHRT exaggerates the degree of muscle soreness and affect neuromuscular performance is currently lacking. Therefore, the purpose of this study was to quantify the effects of moderate-load resistance exercise in hypoxia on markers of physical performance, training stress, and neuromuscular recovery using practical methods common in athlete monitoring contexts (Taylor, Chapman, Cronin, Newton, & Gill, 2012). As hypoxia has been demonstrated to increase concentrations of metabolites during moderate-load resistance exercise (Kon et al., 2010; Kon et al., 2012; Study 4), it was hypothesised that hypoxia would cause decreased physical performance during exercise, in conjunction with increasing the perceived intensity of exercise.

Methods

Experimental Design

Subjects were assessed for 1RM using the back squat and deadlift exercises following protocols described previously (Study 2). Subjects' 1RM was defined as their heaviest completed repetition, which was determined within 3-6 attempts. During this session, subjects were also familiarised with the perceptual scales and tests of neuromuscular function to be used throughout the study. Using a single blinded, counterbalanced crossover design, subjects then visited the laboratory to perform two experimental trials, separated by one

week. These trials comprised a moderate-load resistance exercise protocol, during which subject's breathed air via hypoxic generators (ATS-BASE KIT, Altitude Training Systems, Lidcombe, Australia) under NORM (F_IO_2 = 21%) or MH (F_IO_2 = 16%) conditions. All trials took place at the same time of day for each subject to avoid diurnal variations in metabolism and exercise performance.

Subjects

Fourteen healthy male subjects (24.6 ± 2.7 yr; 179.7 ± 5.9 cm; 84.6 ± 11.6 kg; back squat 1RM: 138.7 ± 32.6 kg; deadlift 1RM: 161.3 ± 34.8 kg) participated in this study. All subjects had at least two years of resistance training experience (mean training age of 4.4 ± 1.5 yr), and were free of any musculoskeletal disorders. All subjects reported no exposure to an altitude of greater than 2000 m within six months prior to experimental trials, had no history of acute mountain sickness, and were taking no substances that could affect the study's results (i.e. anabolic steroids, creatine, sympathoadrenal drugs). Prior to the study, all subjects were provided with information detailing the purpose and requirements of the research, provided informed consent and were screened for medical contraindications. The study and its methods were approved by the University of Newcastle Human Ethics Committee.

Experimental Trials

Before each trial, subjects completed an assessment of neuromuscular function comprised of countermovement jump (CMJ), squat jump (SJ) and isometric

MTP tasks. These assessments were also completed immediately after and at 24 and 48 h following experimental trials. Following this, subjects were fitted with a face mask connected to the hypoxic generators, and afforded 10 minutes to acclimate to the assigned condition. During this time, they performed any specific mobility or flexibility activities that they use prior to resistance exercise. Two warm-up sets of the back squat (10 repetitions at 40% and 50% 1RM) were then performed, before subjects commenced the first of 3 sets of 10 repetitions at 60% 1RM, with 60 s recovery between all sets. Following the final set of back squats, subjects rested for eight minutes before performing the same warm-up and exercise protocol for the deadlift. During each repetition of the experimental trials, mean concentric velocity and power were calculated to monitor the impact of hypoxia on physical performance. Following each set and at 20 minutes after concluding the protocol, CR-10 RPE values were recorded to quantify the perceived difficulty of the exercise stimulus. Perceived levels of muscle soreness, physical fatigue and wellbeing were also assessed prior to and for up to 48 h following trials.

Physical Performance

During each working set (60% 1RM), the displacement of the bar and time between data points was recorded via a linear position transducer, sampling at up to 50 Hz (GymAware, Kinetic Performance Technology, Canberra, Australia). Data were collected and stored on an iPad device (Apple Inc., Cupertino, USA), before being uploaded to an online database for analysis following each experimental trial. The concentric phase of each repetition was

automatically identified by the linear position transducer. *Post hoc* analysis determined measures of mean velocity and power across the concentric phase of each repetition. To assess changes in performance across sets in both conditions, mean repetition values of velocity and power were calculated for each subject, and these values were pooled to determine mean velocity and power values across repetitions 1-10 in each condition. To ensure consistent effort between conditions, subjects were instructed to perform the eccentric phase of each repetition under control, and the concentric phase as explosively as possible. The use of a linear position transducer to quantify resistance exercise performance has previously exhibited a high level of reliability (CV = 0.9-11.8%, ICC = 0.8-1.0) (Scott et al., 2013).

Perceptual Responses

Visual analogue scales were used to assess physical fatigue and muscle soreness. Subjects rated their physical fatigue and muscle soreness by marking a 100 mm line at a point between 0 (no fatigue/soreness) and 100 (maximum fatigue/soreness) (Kon et al., 2012). Physical fatigue was recorded prior to, immediately after and 20 minutes after exercise. Muscle soreness was recorded prior to exercise (with the initial fatigue score) and at 24 and 48 h following trials. In addition, an RPE score was obtained immediately following each set, and a sRPE score was collected at 20 minutes following the conclusion of the exercise trial to reflect the intensity of each set and the entire trial, respectively. Prior to the commencement of each training session, also subjects completed a psychological wellbeing questionnaire (McLean et al., 2010), which assesses

exercise-related factors (fatigue and general muscle soreness) in conjunction with lifestyle factors (sleep quality, stress and mood) on a 5-point scale. Overall wellbeing was subsequently determined by summing the scores from the 5 categories (McLean et al., 2010).

Neuromuscular Function Assessment

At the beginning of each experimental trial, subjects performed a brief warm-up of 120 s cycling (self-selected intensity) before completing a dynamic stretching protocol. This protocol designed to target the lower body musculature used during jumping and MTP tasks (Table 7.1). Twenty repetitions of each exercise were completed while moving across a 10 m distance.

Table 7.1. Dynamic stretching protocol used prior to assessment of neuromuscular function.

Exercise	Description	Repetitions
Heel kicks	Kicking heels toward buttocks while moving forward	10 x each leg
High knees	Lifting knees up to ~90˚ hip flexion while moving forward	10 x each leg
Walking hamstrings	Actively swinging a straight leg forward until a stretch is felt in the hamstrings while moving forward	10 x each leg
Walking quadriceps	Holding a food behind the buttocks until a stretch is felt in the quadriceps while moving forward (2 s per leg, alternating legs)	10 x each leg
Dynamic calves	Slowly taking the ankle through complete range of dorsiflexion and plantar flexion	10 x each leg
Broomstick squats	Back squats to full depth with a broomstick held across the upper back	10 x
Banded deadlifts	Deadlifts performed against light tension from elastic exercise bands held under the feet	10 x
Practice CMJ 50% effort 70% effort 90% effort	Performed with 10 s rest between attempts with gradual increases in the intensity of efforts	2 x at each intensity

Following the warm-up, subjects completed two trials each of the CMJ, SJ and MTP, all separated by 120 s. For the CMJ, subjects started from an erect standing position, before making a downward countermovement to a self-selected depth and rapidly jumping in one continuous movement. For the SJ, subjects squatted down until knees were flexed at ~90° and held for 3 s, before jumping. During all jumps, jump height and relative peak concentric power were assessed via a linear position transducer attached to a broomstick held across the upper back. The better of the two trials was assessed as that with the highest relative peak concentric power. These variables were calculated automatically by the linear position transducer, considering each subject's body weight and the mass of the broomstick (0.7 kg). For each jump, subjects were instructed to jump as high as possible (Cormack, Newton, McGuigan, & Doyle, 2008).

MTP trials were conducted with subjects standing on a portable force platform (Quattro Jump, Kistler, Winterthur, Switzerland) which was positioned below a bar within a customised rack (Figure 7.1). Vertical ground reaction forces were recorded at 500 Hz by Quattro Jump software (V1.1.1.4), and stored prior to further analysis. Subjects were positioned so that they assumed a body position similar to that during the second pull of a clean. Knee and hip angles (139 ± 6˚ and 117 ± 8˚, respectively) were measured with goniometry to ensure that the subjects' position was similar to that previously used in MTP research (Kawamori et al., 2006). Subjects' hands were attached to the bar using weightlifting straps, and they were instructed to pull the bar as hard and fast as

possible (Sahaly, Vandewalle, Driss, & Monod, 2001) for 5 s. Peak force and rate of force development (RFD) were calculated in accordance with Khamoui et al. (2011), as the highest force value registered and the slope of the force-time curve (i.e. Δforce / Δtime), respectively. Data are reported as the average of these values from the two trials (Haff et al., 2005). These methods have previously demonstrated acceptable levels of reliability for quantifying MTP variables (ICC ≥ 0.96) (Kawamori et al., 2006).

Figure 7.1. Isometric mid-thigh pull using a customised power rack and force platform.

Statistical Analyses

All data approximated a normal distribution and are represented as mean ± SD. Data were analysed using a 2-way ANOVA with repeated measures. Where a significant main effect was observed, paired sample *t*-tests were employed to assess where differences existed. These analyses were completed using Statistical Package for the Social Sciences (version 22.0; IBM Corp., Somers, NY, USA). The ES was also calculated as the difference in group means divided by the standard deviation of the pooled data to quantify the magnitude of difference in measures between conditions, and were classified as trivial (≤ 0.19), small (0.20-0.49), moderate (0.50-0.79) or large (≥ 0.80) (Cohen, 1988). The level of statistical significance was set at $p \leq 0.05$.

Results

Physical Performance

Twelve subjects completed all repetitions in the both experimental trials. Two subjects could not complete the final set of deadlifts during their first trial (MH for both), achieving 9 and 6 repetitions in that set. To ensure that experimental trials were work matched for these two subjects, they were instructed to complete the same number of repetitions in their final set of deadlifts during the subsequent normoxia trial.

The physical performance of subjects during repetitions of the exercise protocol is shown in Figure 7.2. No significant differences were observed between conditions for any velocity or power variable during back squats and deadlifts. A

significant main effect was observed during back squats between repetitions for mean concentric velocity ($F_{9,117}$ = 64.488; p < 0.001; η^2 = 0.832) and for mean concentric power ($F_{9,117}$ = 64.684; p < 0.001; η^2 = 0.833). Paired sample *t*-tests confirmed that repetitions 6-10 were lower than repetition 1 for mean concentric velocity ($p \leq 0.010$; ES = 0.49-1.32) and mean concentric power ($p \leq 0.008$; ES = 0.32-0.88). A significant main effect was observed during deadlifts between repetitions for mean concentric velocity ($F_{9,117}$ = 29.528; p < 0.001; η^2 = 0.694) and for mean concentric power ($F_{9,117}$ = 25.095; p < 0.001; η^2 = 0.659). Paired sample *t*-tests confirmed that repetitions 2-8 were higher than repetition 1 for mean concentric velocity ($p \leq 0.001$; ES = 0.17-0.61) and mean concentric power ($p \leq 0.002$; ES = 0.63-0.91).

Perceptual Responses

At 20 minutes following exercise trials, sRPE scores of 5.9 ± 1.7 AU and 6.1 ± 1.8 were reported for NORM and MH trials, respectively. There were no significant differences between conditions for sRPE.

Figure 7.3 illustrates the RPE responses for each main set during trials. There was a significant main effect for set number for the back squat ($F_{2,26}$ = 76.279; p < 0.001; η^2 = 0.854) and the deadlift ($F_{2,26}$ = 22.763; p < 0.001; η^2 = 0.636). Paired sample *t*-tests confirmed significant increases in set RPE values across the three sets for back squats (p < 0.001, ES = 0.50-1.27) and deadlifts ($p \leq 0.017$, ES = 0.29-0.79). There were no significant differences between conditions for set RPE.

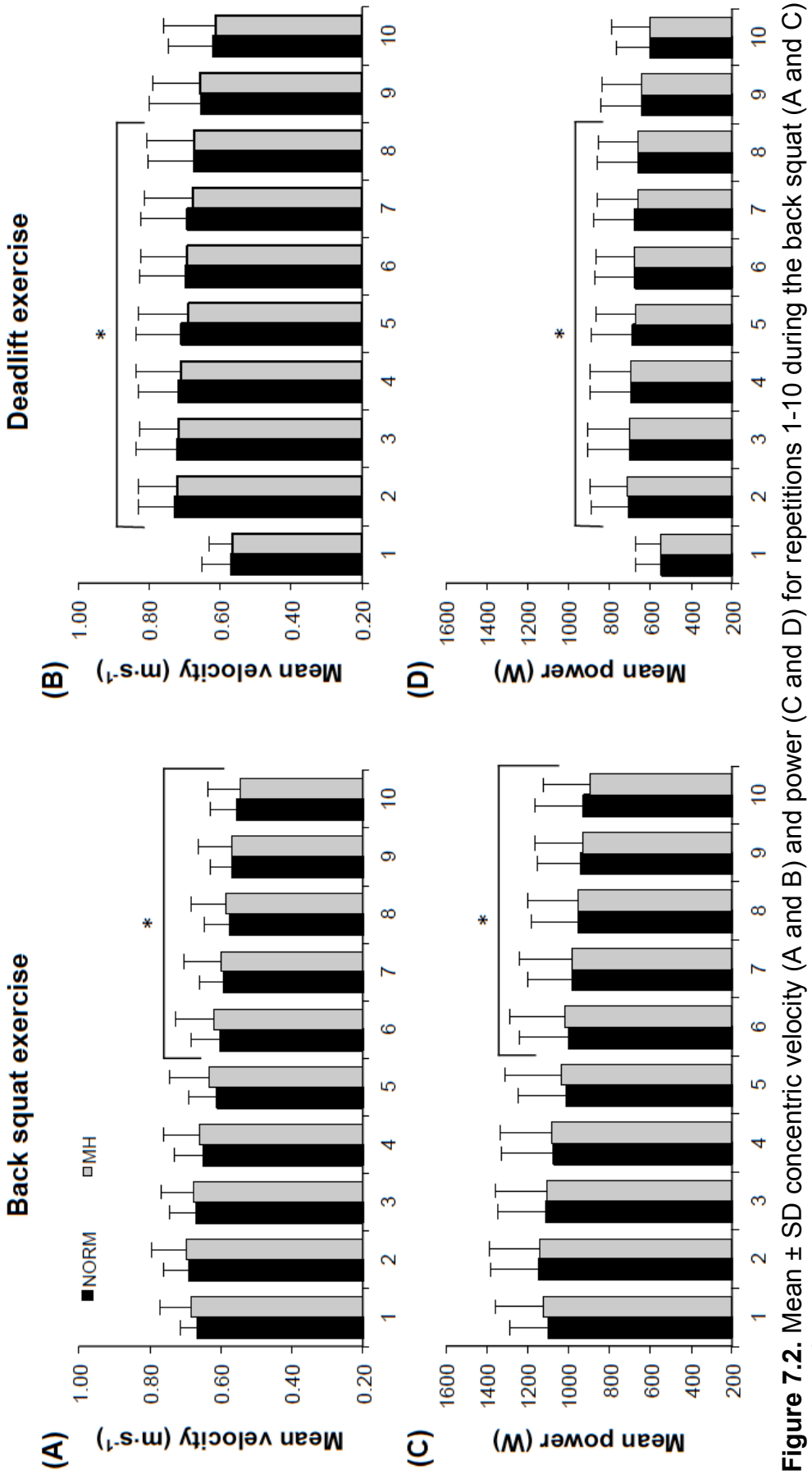

Figure 7.2. Mean ± SD concentric velocity (A and B) and power (C and D) for repetitions 1-10 during the back squat (A and C) and deadlift (B and D).
NORM normoxia, *MH* moderate level hypoxia.
*Significantly different to repetition 1.

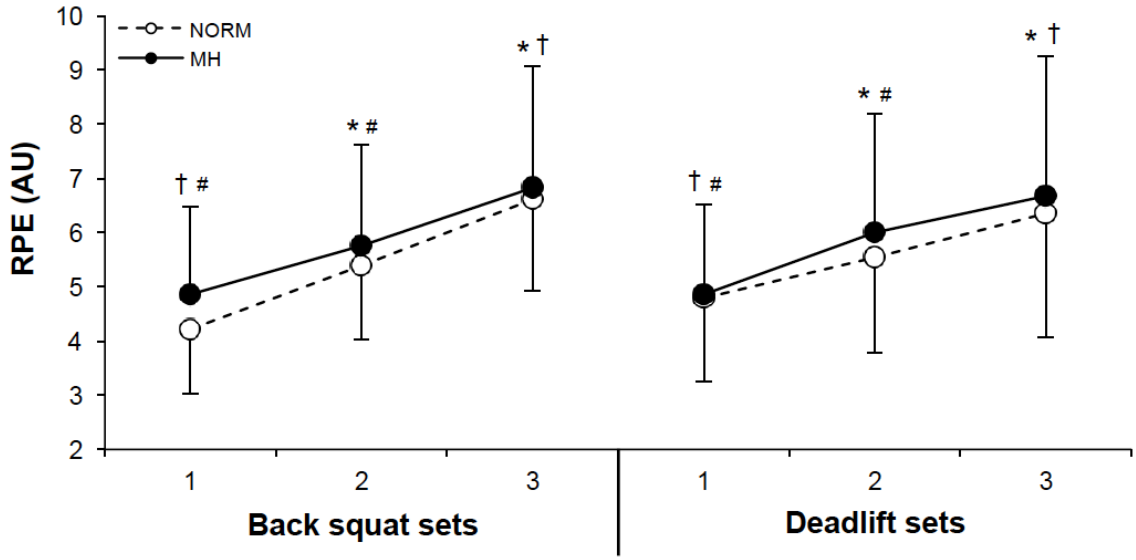

Figure 7.3. RPE values for sets of back squats and deadlifts in normoxia and hypoxia. Data are mean ± SD.
NORM normoxia, *MH* moderate level hypoxia, *RPE* rating of perceived exertion.
*Significantly different to set 1, †Significantly different to set 2, #Significantly different to set 3.

Perceived levels of physical fatigue and muscle soreness are shown in Figure 7.4. For both variables, there were no significant differences observed between conditions. For physical fatigue, a significant main effect was observed for time ($F_{2,26}$ = 33.426; p < 0.001; η^2 = 0.720). Paired sample *t*-tests confirmed significant differences between all time points ($p \leq 0.005$, ES = 0.78-1.56). For muscle soreness, a significant main effect was observed for time ($F_{2,26}$ = 40.489; p < 0.001; η^2 = 0.757), with *t*-tests confirming that pre-exercise values were significantly lower than at 24 h ($p \leq 0.001$, ES = 1.42-1.50) and 48 h ($p \leq 0.001$, ES = 1.23-1.40).

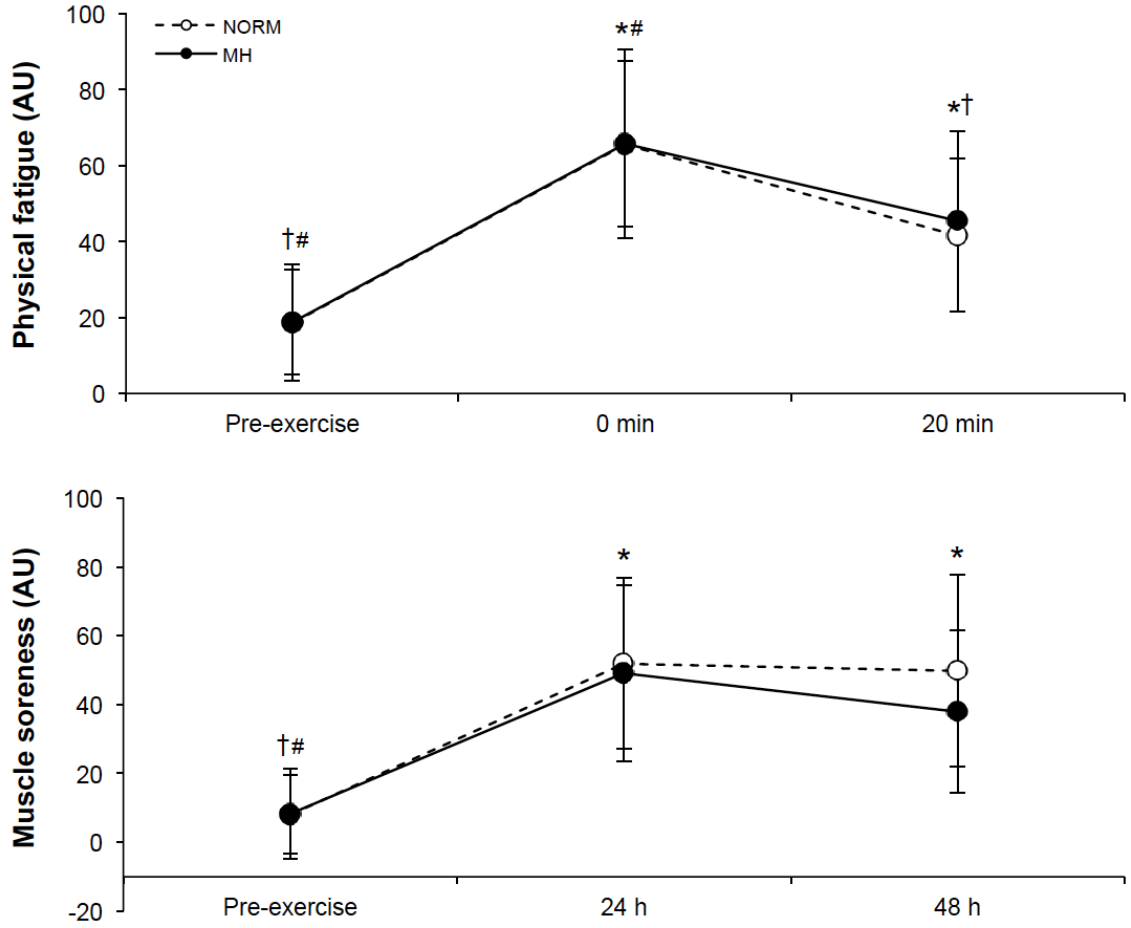

Figure 7.4. Perceived levels of physical fatigue and muscle soreness prior to and following experimental trials measured via visual analogue scales. Data are mean ± SD.
*Significantly different to set pre-exercise, †Significantly different to 0 min/24 h, #Significantly different to 20 min/48 h.

Overall wellbeing scores for up to 48 h following trials are shown in Table 7.2. There were no differences between conditions. A significant main effect was observed for time ($F_{2,26}$ = 10.953; p < 0.001; η^2 = 0.457). Paired sample *t*-tests confirmed that wellbeing was decreased from baseline significantly both conditions at 24 h ($p \leq 0.015$, ES = 0.76-0.82) and 48 h ($p \leq 0.047$, ES = 0.51-0.73) time points.

Table 7.2. Overall wellbeing scores prior to exercise and at 24 and 48 h following trials. Data are mean ± SD.

	Pre-exercise	24 h	48 h
NORM	18.6 ± 2.0	16.4 ± 2.8 *	16.7 ± 2.8 *
MH	19.3 ± 3.0	16.9 ± 3.0 *	17.8 ± 2.7 *

*Significantly different to pre-exercise.

Neuromuscular Performance

Figure 7.5 illustrates changes in performance during the CMJ and SJ trials prior to exercise, and immediately, 24 h and 48 h following trials. There were no differences between conditions at any time point. For the CMJ height, there was a significant main effect observed for time ($F_{3,39}$ = 7.968; p < 0.001; η^2 = 0.380). Paired sample *t*-tests confirmed that jump height was significantly decreased from pre-exercise values immediately following exercise in MH (p = 0.008, ES = 0.64), but not in NORM. For the SJ height, there was a significant main effect observed for time ($F_{3,39}$ = 6.845; p = 0.001; η^2 = 0.345). Paired sample *t*-tests confirmed that jump height was significantly decreased from the pre-exercise trial immediately following exercise in both conditions (p = 0.009-0.047, ES = 0.52-0.57). There were no differences observed between conditions or time points for relative peak power during either the CMJ or the SJ.

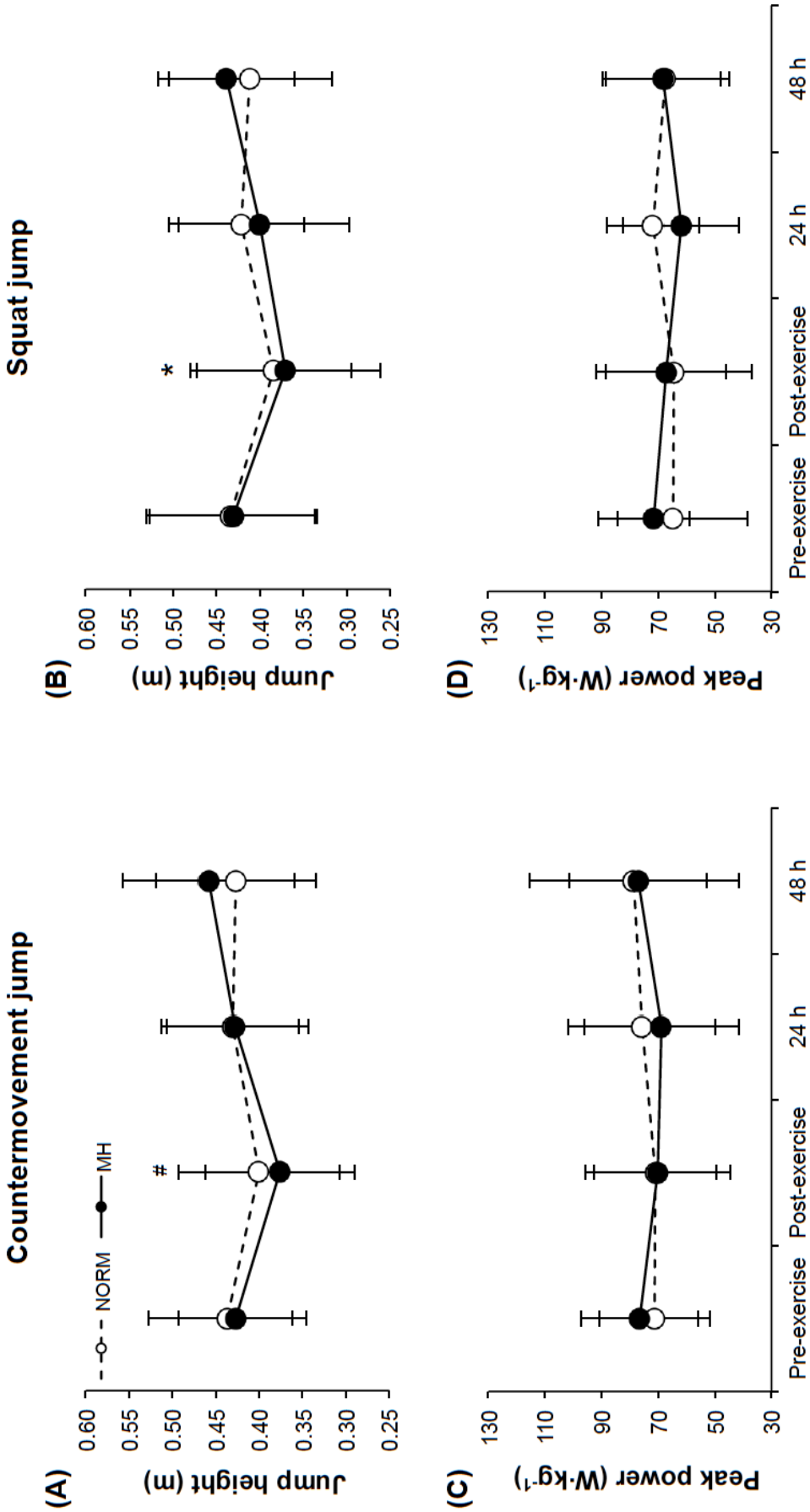

Figure 7.5. Jump height (A and B) and peak relative concentric power (C and D) prior to, immediately following and at 24 h and 48 h after experimental trials during the countermovement jump (A and C) and squat jump (B and D) (mean ± SD).
NORM normoxia, *MH* moderate-level hypoxia.
#Significantly different from pre-exercise in MH. *Significantly different to pre-exercise in both conditions.

Figure 7.6 shows changes in performance during the MTP trials prior to exercise, and for up to 48 h following trials. For peak force, a significant main effect was observed for time ($F_{3,39}$ = 4.706; p = 0.007; η^2 = 0.266). Paired sample *t*-tests confirmed that peak force was significantly decreased from pre-exercise values immediately following exercise in both conditions ($p \leq 0.026$, ES = 0.65). There were no differences observed in RFD between conditions or time points.

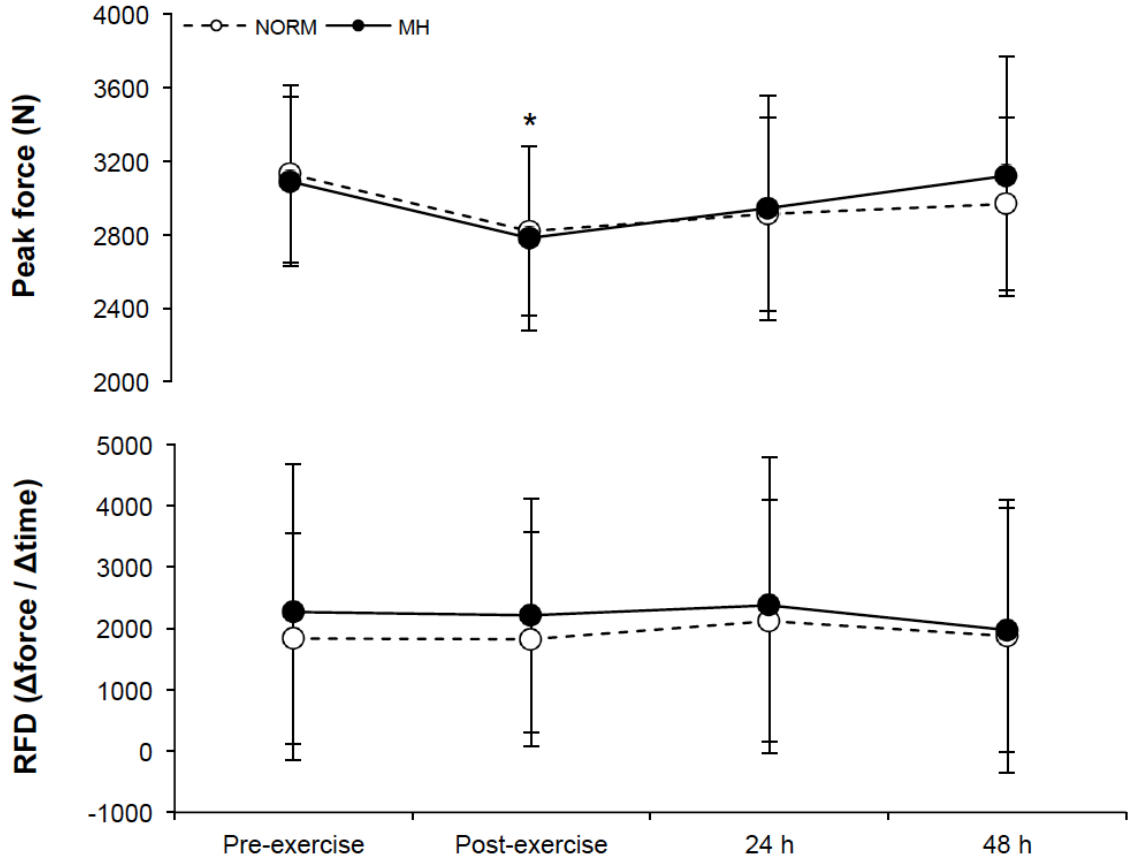

Figure 7.6. Peak force and rate of force development (RFD) prior to, immediately following and at 24 h and 48 h after experimental trials. Data are mean ± SD.
NORM normoxia, *MH* moderate-level hypoxia, *RFD* rate of force development.
*Significantly different to pre-exercise in both conditions.

Discussion

This investigation is the first to quantify common practical markers of exercise performance, training stress and neuromuscular function during and following moderate-load hypoxic resistance exercise. The main findings indicate that hypoxia does not affect physical performance during repetitions, or result in the exercise stimulus being perceived as any more difficult. Additionally, the hypoxic condition did not appear to affect levels of overall wellbeing, muscle soreness or neuromuscular function across 48 h following trials.

The mechanical stimulus associated with resistance training is pertinent to muscular adaptation (Crewther, Cronin, & Keogh, 2005). As such, even if IHRT could enhance biological processes related to hypertrophy (Kon et al., 2010; Kon et al., 2012; Study 4), large hypoxia-related decrements in exercise performance could attenuate the long-term efficacy of this training strategy. Results from this study suggest that hypoxia does not impact on physical performance during resistance exercise, and confer with previous results from this thesis (Study 2). However, an important difference between Study 2 and the current investigation is the structure of exercise and the resultant metabolic responses; the high-load protocol used previously did not result in significantly elevated markers of metabolic stress in hypoxic conditions (Study 3), whereas the moderate-load protocol used in this investigation did (Study 4). The physical performance data from this study are therefore somewhat perplexing, given that declines in force generating capacity during resistance exercise are thought to result from increases in metabolite accumulation in concert with decreased ATP

and PCr concentrations (Haff et al., 2008). While we have not measured PCr concentration in this investigation, PCr resynthesis kinetics known to be affected by oxygen availability (Haseler et al., 1999). Research has also demonstrated that decreases in repetition velocity across sets of different load and repetition schemes was very strongly related to BLa^- concentration for the squat (r = 0.93-0.97) (Sánchez-Medina & González-Badillo, 2011). As such, monitoring the velocity and power of repetitions during resistance training is therefore proposed as a valid method to monitor fatigue and performance (Jovanović & Flanagan, 2014).

Nonetheless, while MH did not affect velocity and power measurements, two subjects could not complete the final set of deadlifts in this condition, which could indicate hypoxia-mediated fatigue. To ensure that the exercise protocol was work matched between conditions for these subjects, they were instructed to compete the same number of repetitions in the NORM trial. It is therefore not known whether these subjects could have completed the entire exercise protocol under normoxic conditions. An alternative explanation for these subjects not completing the protocol is that both undertook their first experimental trial in MH. Research investigating the effects of repeated sessions of resistance exercise has demonstrated that high magnitudes of muscle damage are experienced following the first bout of exercise, but this is significantly lower following a second session of the same exercise, even in well-trained participants (Meneghel et al., 2014). This was a primary reason for the counterbalanced designation of subjects into NORM and MH conditions in

this study. Further research is therefore required to understand the impact of hypoxia on resistance exercise performance, particularly regarding the number of repetitions at a given load to concentric failure.

An important finding from the current study was that hypoxia did not affect perceptual ratings of exercise intensity or physical fatigue during trials, or muscle soreness and overall wellbeing for up to 48 h following exercise. Perceptual responses to exercise are easy to collect, and are becoming commonplace in high-performance sport (Taylor et al., 2012). The RPE approach has become popular to quantify the intensity of resistance exercise, and is proposed as a simple strategy to determine the perceived stress associated with bouts of resistance exercise (McGuigan & Foster, 2004). Furthermore, the wellbeing questionnaire used in this study (McLean et al., 2010) has been shown it to corroborate neuromuscular and muscle damage responses during intensified periods of competition for team sport athletes (Johnston et al., 2013). Consequently, the comprehensive subjective monitoring approach employed in this study indicates that resistance exercise is not perceived as more demanding when performed in hypoxia.

Typically, muscle soreness is heightened at 24-48 h following resistance exercise (Clarkson & Hubal, 2002; Meneghel et al., 2014). The current data corroborate these results, with perceived muscle soreness being higher than pre-exercise levels at 24 and 48 h after exercise in both conditions. Muscle soreness is often used as a practical indirect marker of exercise-induced

muscle damage (Clarkson & Hubal, 2002). Therefore, while muscle damage appears present following exercise in this investigation, it was not affected by hypoxia. This makes sense, given that greater muscle damage appears to be related to mechanical disruption on account of the contractions performed rather than metabolic fatigue (Teague & Schwane, 1995), which was consistent between trials.

Decreased force generating capacity is also considered a valid and reliable indirect measure of muscle damage (Clarkson & Hubal, 2002). Dynamic jump and isometric MTP tasks are often employed to measure neuromuscular and force generating capabilities in athletic contexts (Cormack et al., 2008; Kawamori et al., 2006; Khamoui et al., 2011). However, the time-course of changes in neuromuscular performance in the current study was dissimilar to alterations in muscle soreness, suggesting some form of disconnect between these indirect markers of muscle damage. While some jumping and MTP variables showed neuromuscular function to decline immediately following exercise, performance at 24 h and 48 h following exercise had returned to pre-exercise levels. In contrast, other research has shown that CMJ and SJ performance is decreased significantly for three days following back squat exercise (Byrne & Eston, 2002). A possible explanation for these dissimilar findings is that the exercise performed in the current study was not designed to cause exaggerated muscle damage like that prescribed by Byrne and Eston (2002) (10 sets of 10 repetitions using 70% body mass). Therefore, smaller magnitudes of muscle damage in the current study may not have been

detectable by the practical tests of neuromuscular function employed. Nevertheless, acceptable levels of reliability have been previously reported for vertical jump (Cormack et al., 2008) and MTP (Khamoui et al., 2011) tasks. It is possible that the sampling rate of the force platform used in this research (500 Hz) was not high enough to detect very subtle changes in force generation during MTP trials. Therefore, the effect of resistance exercise on practical tests of neuromuscular function therefore warrants further research attention.

Conclusions

The current study indicates that performing moderate-load resistance exercise in hypoxia does not impact on physical performance during repetitions or cause exercise to be perceived as more difficult in well-trained subjects. Additionally, hypoxia did not affect indirect estimates of muscle damage, and the recovery of neuromuscular function was similar between conditions for up to 48 h following resistance exercise. These data suggest that acute bouts of IHRT do not exaggerate practical markers of training stress or affect force generating capacity. However, it must be acknowledged that the findings of this study are specific to the level of hypoxia employed and further research is required to examine the impact of a more severe hypoxic stimulus during similar exercise.

Practical Applications

- Velocity and power variables are not affected by additional hypoxia during moderate-load resistance exercise. However, it is possible that

hypoxia may result in some subjects completing fewer repetitions before concentric failure due to the exacerbated metabolic stress.

- Supplemental hypoxia during resistance exercise does not affect common perceptual markers of exercise intensity, physical fatigue or psychological wellness, indicating that the internal stress associated with IHRT may not be largely increased from normoxic training.
- Hypoxia does not appear to affect indirect assessments of muscle damage (muscle soreness and neuromuscular function) or the time course for recovery of jumping performance and force generating capacity, meaning that subsequent training bouts will not likely be impacted.

Chapter 8

General Discussion

Overview of Thesis

This thesis examined the acute responses to IHRT in order to elucidate whether hypoxia can up-regulate anabolic processes during resistance exercise for well-trained individuals. More specifically, this thesis first investigated the reliability of non-invasive methods to monitor contracting muscle, before quantifying the acute performance-based, physiological and perceptual responses to high-load and moderate-load IHRT. Finally, the body of work assessed whether the addition of hypoxia during resistance exercise resulted in heightened markers of training stress. These investigations have been presented in this thesis as five separate studies:

1. Reliability of telemetric EMG and NIRS during high-load resistance exercise;
2. Physical performance during high-load resistance exercise in hypoxia;
3. Acute physiological responses to high-load resistance exercise in hypoxia;
4. Acute physiological responses to moderate-load resistance exercise in hypoxia, and;
5. Impact of hypoxia during resistance exercise on markers of training stress and neuromuscular recovery.

The outcomes of each study will be discussed collectively to demonstrate the main findings of this research, and how these results can be applied to the resistance training practices of well-trained individuals.

Reliability of Methods to Monitor Muscle during Resistance Exercise

Alterations in muscle activation and oxygenation during IHRT are thought to be important variables that moderate subsequent muscular development. The results of Study 1 illustrated that intra-test reliability was higher than inter-test reliability for EMG and NIRS variables. These findings may be explained by small alterations in the re-application of the monitoring devices during separate trials. While this is likely to occur in applied exercise science and clinical settings that employ similar assessments (Larsson et al., 2003), changes in electrode placement may impact on the meaningfulness of test-retest data. Precautions were therefore taken throughout the subsequent studies in this thesis to ensure that re-application of EMG and NIRS devices was as consistent as possible between trials. Nevertheless, variation in test-retest EMG and NIRS data was also evident within a single testing session, indicating that factors other than device placement contribute to the reliability of these technologies.

For example, it is possible that small changes in exercise technique between repetitions would influence segmental orientation, and subsequent motor recruitment and oxygenation patterns. As multi-joint dynamic exercises have more degrees of freedom than single-joint tasks or isometric dynamometry, there is greater potential for variability in exercise technique both within and between testing sessions. These small alterations probably accounted for the EMG and NIRS variables assessed exhibiting lower levels of reliability than have previously been demonstrated during single-joint or isometric muscle

contractions (Celie et al., 2012; Kell et al., 2004; Larsson et al., 1999a; Larsson et al., 1999b; Pereira et al., 2005; Tanimoto & Ishii, 2006). Nevertheless, despite these limitations, the easy-to-use non-invasive nature of EMG and NIRS makes these technologies attractive methods to quantify the physiological responses of working muscles during exercise. However, it is important to ensure that device placement and exercise technique are as consistent as possible between trials.

Physical Performance and Neuromuscular Recovery during Hypoxic Resistance Training

Athletic cohorts regularly perform both high- and moderate-load resistance training in order to elicit improvements in maximal strength and muscle size. However, whether hypoxia can enhance the acute anabolic responses to these exercise stimuli had not yet been investigated in well-trained cohorts. Due to the importance placed on physical performance during resistance exercise, particularly during high-load training, it was essential to determine whether additional hypoxia might impact on participants' ability to perform adequately during training. Study 2 demonstrated that physical performance during repetitions did not differ between NORM, MH and HH conditions during high-load resistance exercise. Similar findings were also observed in Study 4 using moderate-load exercise. Furthermore, comparable trends were observed between conditions in both these studies for a fatigue-related decline in concentric performance across sets of resistance exercise.

The findings that physical performance was not affected by hypoxia are positive for individuals looking to implement IHRT strategies. However, these data are difficult to completely explain at present. For example, decreases in repetition velocity across sets of different load and repetition schemes are strongly related to increases in BLa^- concentration for the squat (r = 0.93-0.97) (Sánchez-Medina & González-Badillo, 2011). While high-load IHRT did not facilitate an increase in BLa^- concentrations compared to normoxic exercise (Study 3), moderate-load IHRT did result in significantly higher relative BLa^- concentrations (Study 4). Therefore, it could have been expected that repetition velocity during moderate-load IHRT would have reflected a greater decline in physical performance. Further research to this point is required.

Importantly, practical markers of neuromuscular function (jumping performance and MTP force generating capacity) were not different between NORM and MH conditions for up to 48 h following moderate-load trials (Study 5). Jump height and MTP peak force were decreased from pre-exercise levels immediately following trials ($p \leq 0.047$), but returned to baseline after 24 h in both conditions. These data suggest that a hypoxic stimulus during resistance exercise does not affect the time course of post-exercise neuromuscular recovery. For the strength and conditioning coach or athlete looking to implement IHRT, these findings are important and suggest that performance in subsequent training bouts will not be adversely affected by adding hypoxia during resistance exercise.

Physiological Responses to Resistance Exercise in Hypoxia

Tissue hypoxia is postulated as a key stressor of cellular metabolism during resistance exercise (Kacin & Strazar, 2011), and it is proposed that a more hypoxic intramuscular environment during resistance exercise may lead to increased metabolic stress, in conjunction with higher levels of muscle activation (Schoenfeld, 2013). A measureable hypoxic dose was observed during MH and HH trials in this thesis via changes in blood oxygen levels. In Studies 2 and 4, hypoxic conditions resulted in the lowest SpO_2 values, whereas the highest SpO_2 values were observed during NORM trials. These findings were corroborated by capillary blood analyses, which demonstrated significantly lower levels of oxygen saturation and pressure during MH and HH trials compared to the NORM condition (Study 3). However, the results from this thesis do not provide strong evidence for this hypoxic stimulus reaching the muscular level during IHRT. While there appeared to be a trend for HH to cause the lowest levels of muscle oxygenation during exercise in Study 3, significant differences between conditions were only noted between NORM and HH for HHb_{max} in the deadlift (p = 0.009). Similarly, there were no differences in NIRS-derived measures of muscle oxygenation between conditions in Study 4. A likely explanation was the magnitude of hypoxia used. Research which has observed significantly lower levels of muscular oxygenation in hypoxia has used long exposures to moderate terrestrial altitude (3 h at 1800 m) during resistance exercise (Oguri et al., 2004), or a more severe level of hypoxia (F_IO_2 = 10-12%) at rest (Richardson et al., 2006; Rupp et al., 2013). Furthermore, an

investigation that reported lower levels of minimum oxygenation in working muscle during moderate-load IHRT employed hypoxia using a F_IO_2 of 13% (Kon et al., 2010).

Increased metabolic stress is proposed as an important moderator for downstream hypertrophic processes following resistance training (Schoenfeld, 2013). Although markers of metabolic stress (BLa^- and pH) were increased from baseline following exercise in Study 3 ($p \leq 0.004$), there were no differences between conditions. The similar BLa^- and pH responses between conditions were likely due to the inherent design of high-load strength training. Indeed, the volume of training in this study was markedly lower than in previous low- and moderate-load IHRT research (5 versus 10-14 repetitions) (Kon et al., 2010; Kon et al., 2012), meaning that the time-under-tension during which metabolites could accumulate was decreased. Furthermore, the extended duration of inter-set recovery periods (180 s) likely allowed for intramuscular metabolites to be removed, further lessening their accumulation. In further support of this explanation, larger increases in relative BLa^- concentration were observed in MH compared with NORM in Study 4 ($p \leq 0.041$). These data are in agreement with the hypothesis that for IHRT to cause increased metabolic stress over the equivalent normoxic training, repetition volume should be sufficient (i.e. ≥ 10 repetitions) and the inter-set rest periods should be short enough (i.e. ≤ 60 s) to promote substantial accumulation of metabolites whilst not allowing complete removal between sets (see Appendix G and Appendix H).

An important consequence of metabolic stress during resistance training is thought to be earlier fatigue of motor units and a subsequent increase in the recruitment of muscle fibres (Schoenfeld, 2013). Research has observed significantly greater motor unit recruitment during submaximal isometric contractions when breathing hypoxic compared to normoxic air (Katayama et al., 2007). However in Study 3, iEMG values did not differ between conditions. These findings are not surprising though, considering that metabolic stress was also not enhanced by hypoxia during the high-load resistance exercise. On the other hand, iEMG values in Study 4 were significantly higher at several stages of the exercise protocol in MH during back squats, specifically for the VL and VM muscles. Given that relative BLa^- values were also higher during these trials, it appears that a hypoxia-mediated increase in metabolic stress can facilitate increases in muscle activation for a given exercise load. However, while iEMG did appear to be higher in MH than NORM during deadlifts, particularly for the VL, the observed differences were not statistically significant. A possible explanation for this may be small variations in exercise performance during dynamic multi-joint resistance exercise, which Study 1 suggested might affect the reliability of EMG assessment. Taken together, the findings from this thesis suggest that hypoxia would not likely provide benefit for muscle activation during high-load resistance training, though there may be benefits during moderate-load exercise designed to elicit increases in metabolic stress.

In addition, while it is proposed that exercise resulting in substantial metabolite accumulation would increase exercise-induced cell swelling and initiate anabolic

and anti-catabolic processes (Schoenfeld & Contreras, 2014), there was no difference between conditions for increases in mid-thigh circumference following exercise. It is possible that the circumference measurement technique used was not sensitive enough to detect very small differences between conditions, and further research is therefore required into this mechanism.

Perceptual Responses to Resistance Exercise in Hypoxia

Perceptual responses to exercise are easy to collect, and are commonly used in high-performance sport to monitor training responses (Taylor et al., 2012). The RPE approach is popular for quantifying the intensity of resistance exercise, and is proposed as a simple strategy to determine the perceived stress associated with bouts of resistance training (McGuigan & Foster, 2004). While RPE scores for individual sets were observed to increase across both high- and moderate-load protocols in the current studies, there were no differences between conditions. Similarly, sRPE values did not differ between NORM and MH trials following moderate-load exercise (Study 5). As RPE values provide an effective method of measuring exertion during resistance training (Day et al., 2004), these findings reflect that subjects observed no additional difficulty in completing each set or the entire exercise protocol in hypoxic compared with normoxic conditions. This was also reflected in physical fatigue and overall wellbeing scores during IHRT; despite increases in fatigue and decrements in wellbeing after exercise, hypoxia did not add to these responses. Taken

together, the current research indicates that participants do not perceive IHRT to be any more difficult than the equivalent training in normoxia.

Conclusions

The current findings provide important information regarding the acute responses to IHRT. Firstly, it was established that the reliability of telemetric EMG and NIRS technologies is affected by the re-application of devices when monitoring dynamic multi-joint exercises. Measures should be taken to ensure consistent device placement and exercise technique during test-retest assessments. Additional hypoxia during high- and moderate-load resistance exercise did not impact on physical performance or perceived exertion. However, further investigation revealed that hypoxia does not enhance metabolic stress or muscle activation during high-load resistance exercise. This is likely due to the inherent nature of high-load strength training, particularly the extended inter-set rest durations. Beyond these findings, resistance exercise structured to elicit a potent accumulation of metabolites (moderate-load exercise with relatively brief inter-set rest periods) was found to increase relative BLa^- concentrations and enhance muscle activation when performed in hypoxia. Finally, despite the apparent increase in physiological demands during moderate-load IHRT, practical markers of physical performance, training stress and neuromuscular recovery were not affected. Considering the findings of this thesis collectively, it is likely that IHRT can augment acute responses known to be important for muscular development in already well-trained subjects, providing that the exercise stimulus is structured appropriately.

Chapter 9

Summary and Practical Applications

Summary of the Major Findings

The mechanisms underpinning adaptation to IHRT have received increasing research attention within the past five years. However, until now there has not been a comprehensive assessment of the acute physiological, physical and perceptual responses to IHRT methods, particularly for well-trained participants. This thesis aimed to first determine the reliability of EMG and NIRS to monitor resistance exercise, before investigating the efficacy of high-load and moderate-load IHRT strategies to augment acute anabolic responses. For high- and moderate-load resistance exercise, additional hypoxia did not affect concentric performance, nor did it impact on perceptual markers of exercise intensity, fatigue and wellbeing. These findings provide evidence that IHRT strategies can be employed without negatively affecting the applied training dose. Furthermore, recovery of neuromuscular function and muscle soreness responses were not disturbed by supplementary hypoxia, indicating that subsequent training bouts are unlikely to be affected during IHRT. While hypoxia did not enhance markers of metabolic stress or muscle activation during high-load resistance exercise, these responses were augmented during moderate-load IHRT. Considering these findings it appears that to optimise acute responses to IHRT, the repetition volume must be sufficient to facilitate substantial metabolite accumulation and inter-set rest periods should be brief enough to limit metabolite removal. Theoretically, this will have a downstream effect on the recruitment of additional motor units to maintain contractile force and result in greater adaptive responses. A summary of the major findings from the investigations conducted as part of this thesis is shown in Table 9.1.

Table 9.1. Summary of the investigations conducted as part of this thesis.

Study	Subjects	Testing protocol				Physiological / physical / perceptual responses	Main findings
		Hypoxic dose	Exercise (load)	Sets x reps (inter-set rest; s)	Control group		
Study 1 (Chapter 3)	Well-trained males (n=12)	N/A	Harness back squat (70%, 80%, and 90% 1RM)	2 x 1 and 2 x 3/6/10 with increasing loads (120)	Repeated identical trials (ambient conditions)	EMG and NIRS were more reliable during the same testing session than between sessions Reliability varied, but was not affected by exercise loads Reliability was lower than previously reported for single-joint and isometric tasks	EMG and NIRS reliability during dynamic multi-joint resistance exercise appears to be affected by alterations in device placement and exercise technique.
Study 2 (Chapter 4)	Well-trained males (n=12)	Acute hypoxia (F_IO_2 = 16% and 13%); including 10 minutes pre-exercise	Back squat and deadlift (80% 1RM)	5 x 5 (180)	Normoxia (F_IO_2 = 21%)	↓ physical performance across sets and the session, but not different between conditions ↑ HR in HH compared to NORM ↓ SpO_2 with ↑ level of hypoxia ↑ RPE across sets, but not different between conditions	Physical performance during repetitions and perception of effort is not affected by hypoxia during high-load resistance exercise, despite increased cardiovascular demands.
Study 3 (Chapter 5)	Well-trained males (n=12)	Acute hypoxia (F_IO_2 = 16% and 13%); including 10 minutes pre-exercise	Back squat and deadlift (80% 1RM)	5 x 5 (180)	Normoxia (F_IO_2 = 21%)	↑ HHb_{max} in HH compared to NORM ↓ blood oxygen saturation and pressure in hypoxic conditions ↑ [BLa^-] and ↓ pH, but not different between conditions No difference in iEMG between conditions	While hypoxia affected blood oxygenation, consistent between-condition differences in muscle oxygenation were not observed. Hypoxia did not enhance metabolic stress or muscle activation, and may not provide anabolic benefit during high-load exercise.
Study 4 (Chapter 6)	Well-trained males (n=14)	Acute hypoxia (F_IO_2 = 16%); including 10 minutes pre-exercise	Back squat and deadlift (60% 1RM)	3 x 10 (60)	Normoxia (F_IO_2 = 21%)	↓ SpO_2 in MH compared to NORM, though no difference in HR or muscle oxygenation ↑ relative [BLa^-] in MH compared to NORM ↑ iEMG in MH compared to NORM at several time points for the squat No between-condition difference for thigh circumference	Metabolite accumulation and muscle activation were increased during moderate-load IHRT. However, further research is required to understand the effects of hypoxia on muscle oxygenation and muscle swelling.
Study 5 (Chapter 7)	Well-trained males (n=14)	Acute hypoxia (F_IO_2 = 16%); including 10 minutes pre-exercise	Back squat and deadlift (60% 1RM)	3 x 10 (60)	Normoxia (F_IO_2 = 21%)	↓ physical performance across sets, but not different between conditions ↑ RPE across sets, but not different between conditions ↑ fatigue and soreness, and ↓ overall wellbeing after exercise (no between-condition difference) ↓ jump height and peak force after exercise, (no between-condition difference)	Markers of physical performance, training stress and neuromuscular recovery were not affected by adding hypoxia during moderate-load resistance exercise. Detrimental effects associated with performance decrements or large increases in training stress are therefore unlikely during IHRT.

reps repetitions, *N/A* not applicable, *1RM* 1-repetition maximum, *EMG* electromyography, *NIRS* near-infrared spectroscopy, F_IO_2 fraction of inspired oxygen, *HR* heart rate, SpO_2 arterial oxygen saturation, *RPE* rating of perceived exertion, *NORM* normoxia, *MH* moderate-level hypoxia, *HH* high-level hypoxia, HHb_{max} maximal relative deoxygenated haemoglobin, *iEMG* integrated electromyography, *[BLa^-]* blood lactate concentration, *IHRT* intermittent hypoxic resistance training.

Practical Applications

This series of studies provides important practical information for strength and conditioning practitioners involved in prescribing resistance exercise for well-trained individuals, including:

- The reliability of EMG and NIRS variables during multi-joint resistance exercise may be affected by alterations in device placement and exercise technique. These variables should be controlled during test-retest assessments using EMG and NIRS technologies**.**
- Systemic hypoxia (F_IO_2 = 16-13%) does not impact on concentric performance during high-load strength training or moderate-load hypertrophy-focused resistance training.
- Additional hypoxia during high-load resistance exercise does not appear to enhance several acute responses related to muscular development, and is therefore not recommended to enhance hypertrophic and strength responses.
- Performing moderate-load IHRT with brief rest periods increases metabolite accumulation and muscle recruitment. This training strategy may augment muscular development for a given exercise stimulus.
- Hypoxia during resistance exercise does not affect several measures of training stress or neuromuscular recovery. As such, IHRT does not appear to cause measureable increases in the training dose applied.
- IHRT should be structured with sufficient repetition volume and brief rest periods (i.e. ≥ 10 repetitions and ≤ 60 s rest) to elicit accumulation of metabolites, and subsequent enhancement of muscle recruitment.

Recommendations for Future Research

The research presented in this thesis has broadened current understanding of the acute physiological, physical and perceptual responses to IHRT. However, several important questions remain to be answered regarding the definitive mechanisms underpinning adaptation to IHRT, and how training variables should be manipulated to optimise these responses. It is recommended that future research should examine the following:

- It appears that IHRT should be structured using sufficient volume to promote an increase in metabolic stress, but brief inter-set rest periods to limit complete removal of metabolites from the muscle. Research should examine how manipulating the inter-set rest periods affect the acute responses to IHRT using low- and moderate-load exercise schemes to test the hypothesis proposed in Appendix G and Appendix H.
- BFR exercise has been reported to increase proliferation and number of muscle satellite cells (Nielsen et al., 2012; Wernbom et al., 2013) and enhance stimulation of mTOR signalling (Fry et al., 2010; Fujita et al., 2007). A comprehensive assessment of the biochemical and signalling responses to IHRT should be conduced to elucidate the potential of hypoxia to enhance MPS and subsequent morphological adaptations.
- While previous research has observed hypoxia to decrease muscle oxygenation status during rest and exercise, these findings were not consistently replicated in the current thesis. This may be due to differences in the level of hypoxia employed. Research should determine the relationship between the level of hypoxia applied and the effects on

muscular oxygenation, in conjunction with metabolic, muscle activation, muscle swelling and biochemical signalling responses.

- If acute research can establish how best to manipulate exercise variables and the hypoxic stimulus to augment the anabolic responses, training interventions should follow to confirm whether IHRT can elicit muscular development over the equivalent training in hypoxia for a range of cohorts (clinical, elderly, untrained healthy and trained athletic populations).
- It is currently unknown whether long-term IHRT could have negative impacts on markers of training stress, given that physiological demands are increased during exercise. While acute data from this thesis suggest no negative effects, future research should assess whether extended periods of IHRT may impact on markers of training stress or adaptation.

Chapter 10

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Chapter 11

Appendices

Appendix A

Information Statement (Study 1)

FACULTY OF SCIENCE AND INFORMATION TECHNOLOGY

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

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Information Statement for the Research Project:

Association between Functional Movement Screen™ Scores and Team Sport-Specific Performance Tests

Document Version 2; dated 28/08/2012

You are invited to participate in the research project identified above which is being conducted by Dr Robert Lockie, Brendan Scott and Adrian Schultz from the School of Environmental and Life Sciences at the University of Newcastle.

Why is the research being done?
Strength coaches and athletic trainers often use functional movement screens as an assessment for team sport athletes. Functional movement involves the ability to perform basic locomotor, manipulative, and stabilizing actions, while maintaining control along the kinetic chain. The Functional Movement Screen™ (FMS™) has been developed to evaluate these capacities, and is comprised of 7 actions. These are the deep squat, hurdle step, in-line lunge, shoulder mobility, straight leg raise, push-up, and rotary stability. FMS™ scores may relate to performance in team sport-specific tests. If this is the case, than correcting movement deficiencies as assessed by the FMS™ should then translate to team sport-specific performance. This research will establish the strength of relationships between FMS™ scores and performance in team sport-specific tests.

Who can participate in the research? We are seeking males and females aged 18-40 years who are currently physically active in a team sport. To be eligible for this research you must:

- Be or have recently been physically active in a team sport (3 hours per week);

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- Have a history of team sport physical activity (≥2 times per week) extending over the previous six months;
- Be available for the entire duration of the study; and,
- Do not have any existing medical conditions that would compromise participation in the study.

What choice do you have?
Participation in this research is entirely your choice. Only those people who give their informed consent will be included in the project. Whether or not you decide to participate, your decision will not disadvantage you.

If you do decide to participate, you may withdraw from the project at any time without giving a reason and have the option of withdrawing any data which identifies you.

What would you be asked to do?
If you agree to participate, you will be asked to:

- Complete a consent form;
- Commit to time spent in the field for testing, for seven 30-60 minute sessions. Session 1 will involve the

  FMS™ assessment. Session 2 will involve 3 trials each of a 20-metre sprint, bilateral and unilateral vertical jump tests, and bilateral and unilateral sit-and-reach. Session 3 will incorporate the 505 change- of-direction speed test, bilateral and unilateral standing broad jump, unilateral lateral jump, and bilateral and unilateral seated medicine ball throws. Sessions 4 and 5 will involve one-repetition maximum (1RM) strength tests, involving variations of the squat exercise. Sessions 6 and 7 will involve completing squats at different intensities; specifically 70%, 80%, and 90% of 1RM. Subjects will also wear telemetric, non-invasive electromyography and near infrared spectroscopy units to determine muscle activity and oxygenation during the squats. Testing will be conducted in the Biomechanics laboratory and gymnasium at the University of Newcastle, Ourimbah campus, and will include specific warm-up and cool-down exercises.

- Wear appropriate clothing for exercise during testing.
- As a result of your participation, you will be tested in speed and agility performance (via a sit-and-reach box), and strength (via squats, telemetric electromyography, and near-infrared spectroscopy).

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How much time will it take?
Each testing session will take approximately 30-60 minutes. Completion of the seven testing sessions will be in accordance with you university timetable.

What are the risks and benefits of participating?
Participation in this study will expose participants to those risks associated with short-term maximal exercise, which are according to prior research, generally minimal. During exercise, certain physiological changes may occur, which could include muscle soreness, muscle strain, increased heart rate and general fatigue. Every effort is made to minimise these occurrences. Subjects will be pre-screened prior to their participation in this study, in order to ascertain whether they are physically capable of completing the study with encountering any undue harm. Should injury occur, first aid procedures will be followed.

The benefits of participating will be for individuals to learn about potential injury or performance risks as determined by the FMS™. Furthermore, participants will receive information about their linear and change- of-direction speed, lower and upper body power, and lower-limb flexibility, as determined by team sport-specific tests.

How will your privacy be protected?
Any information collected by the researchers which might identify you will be stored securely in a locked filing cabinet and/or a password protected computer in Robert Lockie's, Brendan Scott's and Adrian Schultz's office and only accessed by the researchers, unless you consent otherwise. Your confidentiality will be ensured by replacing your name with a numerical code. Per university guidelines, data will be stored for a minimum of five years.

How will the information collected be used?
The information collected will be reported in papers in scientific journals, and will be presented at conferences. Individual participants will not be identified in any reports or presentations arising from this research. You will be provided with a summary of the findings of the research and researchers will also be available to further explain your individual results to you. Your results will be sent to you via email; please ensure that you add your contact details to the consent form.

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What do you need to do to participate?

Please read this Information Statement and be sure you understand its contents before you consent to participate. If there is anything you do not understand, or you have questions, contact the researchers.

If you would like to participate, please complete the attached Consent Form and return it to Robert Lockie, Brendan Scott or to Adrian Schultz in the enclosed envelope. A member of the research team will then contact you to arrange a time convenient to you for the pre-testing sessions.

Further information

If you would like further information please contact Dr Robert Lockie, Brendan Scot and Adrian Schultz from whom potential participants can obtain further information about the project.

Thank you for considering this invitation.

Dr Robert Lockie
Lecturer in Exercise and Sport Science

Complaints about this research

This project has been approved by the University's Human Research Ethics Committee, Approval No. H- 2012-0216. Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308, Australia, telephone (02) 49216333, email Human-Ethics@newcastle.edu.au.

Appendix B

Consent Form (Study 1)

FACULTY OF SCIENCE AND INFORMATION TECHNOLOGY

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

Dr Robert Lockie
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Consent Form for the Research Project:

Association between Functional Movement Screen™ Scores and Team Sport-Specific Performance Tests

Robert Lockie, Brendan Scott and Adrian Schultz

Document Version 3; dated 28/08/2012

I agree participate in the above research project and give my consent freely.

I understand that the project will be conducted as described in the Information Statement, a copy of which I have retained.

I understand I can withdraw from the project at any time and do not have to give any reason for withdrawing.

I consent to:

- Commit to time spent in the field for testing, for seven 60-minute sessions, 48 hours apart. Session 1 will involve the FMS™ assessment. Session 2 will involve 3 trials each of a 20-metre sprint, 5-bound test, bilateral and unilateral vertical jump tests, and bilateral and unilateral sit-and-reach. Session 3 will incorporate the 505 change-of-direction speed test, bilateral and unilateral standing broad jump, unilateral lateral jump, and bilateral and unilateral seated medicine ball throws. Sessions 4 and 5 will involve one-repetition maximum squat tests, whilst wearing non-invasive, telemetric electromyography and near infrared spectroscopy units on selected muscles. Sessions 6 and 7 will involve completing squats at different intensities (70%, 80%, and 90% of 1RM), whilst wearing non-invasive, telemetric electromyography and near infrared spectroscopy units on selected muscles. Testing will be conducted in the Biomechanics

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laboratory and gymnasium at the University of Newcastle, Ourimbah campus, and will include specific warm-up and cool-down exercises

- Wear appropriate clothing for exercise during testing.
- Exposure to those risks associated with short-term maximal exercise, which is according to previous research, generally minimal. However, during exercise, certain changes may occur including muscle soreness, muscle strain (or similar) increased heart rate and general fatigue. Every effort is made to minimise these occurrences. The usual first aid procedures will be followed where necessary.

I understand that my personal information will remain confidential to the researchers.

I have had the opportunity to have questions answered to my satisfaction.

Print Name: _____

Signature: _____

Date: _____

Contact Details

Mobile:_____

Email: _____

Team Sports Played: _____

Appendix C

Information Statement (Study 2-5)

FACULTY OF SCIENCE AND INFORMATION TECHNOLOGY

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

Dr Ben Dascombe
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Participant Information Statement for the Research Project:
Effect of altitude on selected physiological and performance measures
Document Version 3; dated 09/07/15

You are invited to participate in the research project being conducted by Mr Brendan Scott and Ms Catriona Lockhart from the School of Environmental and Life Sciences at the University of Newcastle. Brendan is completing his PhD (Exercise and Sport Science), and is supervised in this program by Dr Ben Dascombe, Dr Dean Sculley and Dr Katie Slattery. Catriona Lockhart is completing an Honours degree in Exercise and Sports Science and will also be supervised by Dr Ben Dascombe.

Why is the research being done?
The benefits of hypoxic training stimulus (including altitude training) have been well documented in endurance athletes. The proposed benefits in endurance athletes pertain to increases in aerobic capacity and performance, via haematological changes in the blood's oxygen carrying ability. However, the effects of hypoxic training on resistance exercise are less clear. Recent evidence suggests that hypoxic stimulus during resistance exercise may augment the morphological and physiological responses in skeletal muscle, though the mechanisms underpinning these altered responses are not fully yet understood. Currently, many athletes engage in hypoxic training practices during their yearly training cycle. The purpose of this research is therefore to investigate the effect of hypoxia on physiological and adaptive responses to resistance exercise.

Who can participate in the research?
We are seeking subjects with resistance training experience (at least 1 year), who are currently engaged in recreational resistance training. To be able to participate you must be aged between 18-35 years of age and will be required to be free of any medical conditions that may identify you to be at higher risk of injury or discomfort during the activities. Participants should have no pre-existing medical issues that may be worsened as a result of completing the research project. Applicants must not have completed any form of hypoxic training over the past six months.

What choice do you have?
Participation in this research is entirely your choice. Only those people who provide informed consent will be included in the project. Whether or not you decide to participate, your decision will not disadvantage you. If you do decide to participate, you may withdraw from the project at any time without giving a reason and have the option of withdrawing any data that identifies you.

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What would you be asked to do?

If you agree to participate, you will be asked to complete the following questionnaires and testing protocols:

Pre Exercise Health Screening Questionnaire

The purpose of the Pre-Exercise Health Screening Questionnaire is to ensure you do not have any current or previous health issues that may place you at a risk of injury during the testing.

Standardised Nutrition and Physical Activity

You will be required to standardise your nutrition and physical activity habits in the 48 hr prior to the exercise testing.

Experimental Trials

Participants will be asked to attend the laboratory 3 days per week for 6 weeks for assessment during a resistance training program. Training and testing sessions will involve:

1. Familiarisation with equipment and 1 repetition max (1RM) testing:
   - Subjects will be tested for 1RM in the back squat and deadlift exercises
2. Resistance exercises in normoxia (sea level)
   - 5 x 5-10 repetitions at 50-80% 1RM for back squat and deadlift exercises
3. Resistance exercises breathing air with a fraction of inspired oxygen (F_IO_2) of 16% and 13%
   - 5 x 5-10 repetitions at 50-80% 1RM for back squat and deadlift exercises

Physiological/Anthropometrical Measures

During or prior to exercise testing, physiological responses will be measured to gain an understanding of what is happing within the body. As such, the following physiological measures will be monitored during the exercise testing. All measures are non-invasive with the exception of collection of blood samples.

Anthropometrical measures: to gain data regarding subject characteristics, you will have skinfold measurements taken from several sites on the body, as well as your height, weight and age recorded.

Strength and power testing: To assess acute and adaptive responses to hypoxic or normoxic resistance exercise measures of maximal strength (1RM) and power (vertical jump and sprint tests) will be assessed before and after the training period.

Heart rate: will be collected across all exercise testing using a standard heart rate monitor placed on the chest.

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Capillary blood collection: small capillary samples will be collected before, during and following the tests from an earlobe or fingertip. Samples will be very small and will be of little discomfort to you. These samples will be analysed for changes in blood pH and lactate concentration.

Venus blood collection: Venus blood samples will be collected by a member of the research team who is qualified in venepuncture. Samples of blood will be taken from a vein in the arm prior to and following exercise. These samples will be analysed for changes in hormone concentration and other growth factors.

Electromyography (EMG): surface electrodes will be placed on the skin to measure muscle activation during exercise. These electrodes are not invasive and do not control the muscle but simply measure the degree of activation during exercise.

Force and power output: a linear position transducer will be attached to the bar during all resistance exercises, to monitor the force and power output during each repetition. This will not affect the performance of any exercise.

Perceptual responses: you will be asked to gauge your perception of exertion, perceived recovery, muscular soreness/stiffness and complete a wellness questionnaire during/following all trials.

Near-infrared spectroscopy (NIRS): you will have a NIRS device affixed to your thigh to measure muscle oxygen saturation and muscle blood flow.

Pulse oximetry: you will have a pulse oximeter attached on your index finger to measure arterial blood oxygen saturation immediately following each set of exercise.

How much time will it take?

Each data collection session will last a total of approximately 2 hours to allow for health screening and exercise testing. The testing session will be scheduled to suit your time demands. Each training session will last ~30 minutes.

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***What are the risks and benefits of participating*?**
The current study possesses little risk to participants as a result of your involvement. All exercise testing is performed in a controlled environment and will be stopped by the researcher if deemed dangerous. As a risk of physical injury may still exist, the researchers will ensure that you have completed a warm-up and are familiarised prior to completing the testing. Some muscle pain or fatigue may be present following exercise trials, though this will be similar to that felt following any strenuous activity. The non-invasive measures offer little risk to you. The collection of the capillary blood samples may potentially result in some bruising and soreness at the puncture site. All measures will be taken to ensure that the sampling is completed in a clean and safe environment and sampling will be completed as gently as possible.

As a result of participating in the study, you will receive several benefits. You will undergo a laboratory based physiological exercise assessment and this will be explained in detailed by the researchers. The test itself will be conducted in a safe and controlled environment with the supervision of an experienced exercise physiologist and fellow researchers. You will also be exposed to the latest in technological innovations in regards to both exercise testing and altitude-based practices and trends. Following the study, you will also receive a report detailing the findings of the study and arising benefits.

How will your privacy be protected?
The confidentiality of this study is assured. Under no circumstances will any names appear on publications associated with this research. The individual results will be provided to you in verbal and written form with no one else being given the results unless you request it. Hard copies of results will be stored in a locked filing cabinet along with backed up data stored securely in the filing cabinet. The researchers are the only personnel who have access to the data. Your confidentiality will be ensured by replacing your name with a numerical code. The data will be retained for at least five (5) years, at the University of Newcastle. This is in accordance with the University of Newcastle Research Data and Materials Management Policy.

How will the information collected be used?
Data will be presented in scientific journals and conferences following the conclusion of the project. All presentation and use of data will be as group and descriptive measures, not individual responses.

***What do you need to do to participate*?**
Please read this Information Statement and be sure you understand its contents before you consent to participate. If there is anything you do not understand, or you have questions, contact the researcher.

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If you would like to participate, please email the chief investigator on the details below. You will then be required to complete a pre-exercise health-screening questionnaire and consent form prior to participation.

If you are a suitable participant for this study, you will be informed of this upon completion of the pre-exercise health-screening questionnaire. If the pre-exercise health screen has identified any medical issues, you will be required to seek a medical clearance before being included in the study.

Further information

If you would like further information please contact Ben Dascombe for further information regarding the study.

Ben Dascombe
Phone: (02) 4348 4150
Mobile: 0417 712 381
Email: Ben.Dascombe@newcastle.edu.au

Thank you for considering this invitation.

Dr Ben Dascombe
Project Supervisor

Complaints about this research

This project has been approved by the University's Human Research Ethics Committee, Approval No.
H-2011-0082

Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to the Human Research Ethics Officer, Research Office, The Chancellery, The University of Newcastle, University Drive, Callaghan NSW 2308, Australia, telephone (02) 4921 6333, email Human-Ethics@newcastle.edu.au.

Appendix D

Consent Form (Study 2-5)

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THE UNIVERSITY OF NEWCASTLE AUSTRALIA

Dr Ben Dascombe
Science Offices
School of Environmental and Life Sciences
Faculty of Science and Information Technology
Brush Rd, Ourimbah, NSW 2258
02 4348 4150
Ben.Dascombe@newcastle.edu.au

Consent Form for the Research Project:
Effect of altitude on selected physiological and performance measures
Document Version 3; dated 09/07/15

I agree to participate in the above research project and give my consent freely.

I understand that the project will be conducted as described in the Information Statement, a copy of which I have retained.

I understand the purposes of this study is to investigate the effect of hypoxia on intermittent activity and resistance exercise, and the efficacy of training in hypoxia on simulated team sport performance and selected strength parameters at sea level.

I understand I can withdraw from the project at any time and do not have to give any reason for withdrawing.

I consent to (please tick):

- ☐ Undergoing pre-screening procedures that involve a health questionnaire and the collection of personal details
- ☐ Undergoing an anthropometrical assessment
- ☐ Completing 1 repetition maximum (1RM) testing of the back squat and deadlift exercises in normoxia, and power testing (vertical jumps and sprints)
- ☐ Completing a resistance training program in normoxia (F_IO_2 = 21%) or hypoxia (F_IO_2 = 16% and 13%)
- ☐ Wearing a facemask during exercise to provide normobaric hypoxic air
- ☐ Allowing a researcher to obtain capillary blood samples from the earlobe and venous blood samples from a vein in the arm
- ☐ Wearing patches to measure EMG activity
- ☐ Wearing a near-infrared spectroscopy unit taped to the *vastus lateralis* and a pulse oximeter on the index finger
- ☐ Providing ratings of perceived exertion, perceived recovery, muscular soreness/stiffness and completing a wellness questionnaire during all trials
- ☐ Abstaining from vigorous exercise 48hr prior to all experimental trials
- ☐ Eating a meal high in carbohydrate and drink 500ml of water 3hr prior to all experimental trials
- ☐ Wearing a heart rate monitor during all trials
- ☐ Abstaining from caffeine and alcohol for 24hr prior to all experimental trials
- ☐ Allowing body weight to be measured before and after exercise testing

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- ☐ Allowing the researchers to quantify force and power output during all repetitions

OR

- ☐ **I consent to ALL of the Part A procedures listed above**

I understand that participation in the research will expose participants to those risks associated with exercise.

I understand that my personal information will remain confidential to the researchers.

I have had the opportunity to have questions answered to my satisfaction.

Print Name: _____

Signature: _____

Date: _____

Please indicate whether or not you wish to receive a summary of the results

Y N

Phone: _____

Email: _____

Appendix E

Expedited Approval (Study 2-5)

HUMAN RESEARCH ETHICS COMMITTEE

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

Notification of Expedited Approval

To Chief Investigator or Project Supervisor:	**Doctor Benjamin Dascombe**
Cc Co-investigators / Research Students:	**Mr Brendan Scott** **Ms Katie Slattery** **Doctor Dean Sculley** **Miss Catriona Lockhart** **Associate Professor Suzanne Snodgrass** **Mr Lewis Ingram**
Re Protocol:	**Effect of altitude on selected physiological and performance measures**
Date:	**09-Jul-2015**
Reference No:	**H-2011-0082**

Thank you for your **Variation** submission to the Human Research Ethics Committee (HREC) seeking approval in relation to a variation to the above protocol.

Variation to add Catriona Lockhart as a student researcher.

Your submission was considered under **Expedited** review by the Ethics Administrator.

I am pleased to advise that the decision on your submission is **Approved** effective **09-Jul-2015**.

The full Committee will be asked to ratify this decision at its next scheduled meeting. A formal *Certificate of Approval* will be available upon request.

Professor Allyson Holbrook
Chair, Human Research Ethics Committee

For communications and enquiries:
Human Research Ethics Administration

Research Services
Research Integrity Unit
The Chancellery
The University of Newcastle
Callaghan NSW 2308
T +61 2 492 17894
F +61 2 492 17164
Human-Ethics@newcastle.edu.au

RIMS website - https://RIMS.newcastle.edu.au/login.asp

Linked University of Newcastle administered funding:

Funding body	Funding project title	First named investigator	Grant Ref

Appendix F

Pre-Exercise Health Screening Questionnaire

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Pre-exercise screening system 2005

Sports Medicine Australia (SMA) - Stage 1 questionnaire

Name | Age | Gender M F

Address | Phone | Date

1	Have you ever had a heart attack, coronary revascularisation surgery or a stroke ?	No	Yes
2	Has your doctor ever told you that you have heart trouble or vascular disease ?	No	Yes
3	Has your doctor ever told you that you have a heart murmur ?	No	Yes
4	Do you ever suffer from pains in your chest, especially with exercise ?	No	Yes
5	Do you ever get pains in your calves, buttocks or at the back of your legs during exercise which are not due to soreness or stiffness ?	No	Yes
6	Do you ever feel faint or have spells of severe dizziness, particularly with exercise ?	No	Yes
7	Do you experience swelling or accumulation of fluid about the ankles ?	No	Yes
8	Do you ever get the feeling that your heart is suddenly beating faster, racing or skipping beats, either at rest or during exercise ?	No	Yes
9	Do you have chronic obstructive pulmonary disease, interstitial lung disease, or cystic fibrosis?	No	Yes
10	Have you ever had an attack of shortness of breath that developed when you were not doing anything strenuous, at any time in the last 12 months ?	No	Yes
11	Have you ever had an attack of shortness of breath that developed after you stopped exercising, at any time in the last 12 months ?	No	Yes
12	Have you ever been woken at night by an attack of shortness of breath, at any time in the last 12 months ?	No	Yes
13	Do you have diabetes [IDDM or NIDDM] ? If so, do you have trouble controlling your diabetes?	No	Yes
14	Do you have any ulcerated wounds or cuts on your feet that do not seem to heal?	No	Yes
15	Do you have any liver, kidney or thyroid disorders?	No	Yes
16	Do you experience unusual fatigue or shortness of breath with usual activities?	No	Yes
17	Is there any other physical reason or medical condition, or are you taking any medication(s) which could prevent you from undertaking an exercise program, or that you are concerned about? #	No	Yes

NOTES:

Some of these conditions might include a history of blood clotting, osteoporosis, bone fractures or serious musculoskeletal disorders, or if they have recently lost a large amount of body mass without trying to. Other types of conditions might include psychiatric disorders, later-stage pregnancy or those with a history of health problems during pregnancy. Those people taking medication(s) for medical conditions listed may also need medical clearance.

Also, if any one or more of the risk factors [below] are extreme then the health and fitness professional should use professional judgement as to whether medical clearance may be required.

Signature:_____

Appendix G

Intermittent Hypoxic Resistance Training: Does it Provide Added Benefit?

As per the peer-reviewed papers **Accepted and Published** in *Frontiers in Physiology*:

Scott, B. R., Slattery, K. M., & Dascombe, B. J. (2014). Intermittent hypoxic resistance training: does it provide added benefit? *Frontiers in Physiology*, 5, 397.

This paper has not been edited from the originally accepted manuscript published in Frontiers in Physiology.

Introduction

Methods to enhance the adaptive responses to resistance training are of great interest to clinical and athletic populations alike. Altering the muscular environment by restricting oxygen availability during resistance exercise has been shown to induce favourable physiological adaptations. An acute hypoxic stimulus during exercise essentially increases reliance on anaerobic pathways, augmenting metabolic stress responses and subsequent hypertrophic processes. Hypoxic strategies during resistance exercise were originally investigated using BFR methods (Takarada et al., 2000a), whereby a cuff is applied proximally to a limb to partially limit arterial inflow while occluding venous outflow from the working muscles. Another method that has been investigated more recently is performing resistance exercise in systemic hypoxia, by means of participants breathing a hypoxic air mixture.

The addition of systemic hypoxia to resistance training has previously resulted in significantly enhanced hypertrophic and strength responses to both low-load (20% 1RM) (Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b) and moderate-load (70% 1RM) (Nishimura et al., 2010) resistance training. While research into IHRT is in its infancy, some studies have reported conflicting results, which is likely due to differing research methodologies. In a recent review, it has been suggested that many of the potential mechanisms underpinning muscle adaptations to BFR training and IHRT are linked to the muscular oxygenation status and degree of metabolic stress associated with exercise (Literature Review). The purpose of this paper is to briefly summarise

the adaptive responses that have been reported following both low- and moderate-load IHRT and to highlight key areas of concern for IHRT methodology, including the level of hypoxia used and the degree of metabolic stress imposed during exercise.

Findings from Intermittent Hypoxic Resistance Training Studies

To date, six separate investigations have examined the impact of IHRT on hypertrophic and strength responses, resulting in seven published papers comparing adaptive responses following IHRT to a normoxic control group (Friedmann et al., 2003; Ho et al., 2014b; Kon et al., 2014; Kurobe et al., 2015; Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b; Nishimura et al., 2010). Research using low-load resistance training (20% 1RM) combined with moderate hypoxia (F_IO_2 adjusted to maintain SpO_2 at 80%) and very brief inter-set rest periods (30 s) has reported greater hypertrophic and strength responses following IHRT compared to work-matched normoxic training (Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b). However, another study using similar exercise loads (30% 1RM), yet longer inter-set rest periods (60 s) and a greater hypoxic stress (F_IO_2 = 12%) has observed no additive benefits of IHRT (Friedmann et al., 2003). While conflicting, these findings may indicate that for IHRT using low-loads, both the duration of inter-set rest periods and the level of hypoxia affect the adaptive responses.

Nishimura et al. (2010) used moderate-load resistance training (70% 1RM) combined with moderate-level hypoxia (F_IO_2 = 16%) and a relatively brief inter-set rest period (60 s), demonstrating enhanced hypertrophic and strength responses following IHRT compared to the equivalent training in normoxia. However, research using similar exercise loads (70% 1RM or 10RM) and levels of hypoxia (F_IO_2 = 14.4-15%) in conjunction with longer inter-set rest periods (90-120 s) has not found additive hypertrophic or strength benefits for IHRT (Ho et al., 2014b; Kon et al., 2014). Furthermore, a study that employed moderate-load exercise (10RM) and brief inter-set rest (60 s), but a high-level of hypoxia (F_IO_2 = 12.7%) noted significantly greater hypertrophic, but not strength adaptations, following IHRT compared to normoxic training (Kurobe et al., 2015). When considering the available evidence, it appears that moderate hypoxia in conjunction with relatively brief inter-set rest periods (and subsequently increased metabolic stress) are paramount in enhancing muscular development via IHRT.

Level of Hypoxia

The hypoxic stimulus used in previous IHRT investigations has ranged from F_IO_2 = 12-16%, or investigators have adjusted the hypoxic stimulus to maintain SpO_2 at 80%. Importantly, no two investigations have employed the same hypoxic stimulus, which has led to differing physiological responses and difficulty in directly comparing the findings of each study. It has been established that the level of hypoxia or altitude an individual is exposed to has a dose-response relationship on subsequent markers of endurance performance

(Chapman et al., 2014), and it is logical that a similar relationship may exist for muscular development following IHRT.

It is possible that for enhanced muscle hypertrophy and strength development from IHRT, the level of hypoxia may follow a hormetic relationship, meaning that some beneficial acute responses to hypoxia may be attenuated if the level of hypoxia is too high. Muscle function during IHRT using high levels of hypoxia could be impacted by the presence of a “central governor”, which was popularised by Noakes, Peltonen, and Rusko (2001). This theory postulates that the degree of motor unit recruitment by the central nervous system is determined by the brain’s need to protect itself and the body, ensuring survival and maintenance of integrity during and following exercise (Millet, Aubert, Favier, Busso, & Benoit, 2009). As such, a hypoxia-mediated reduction in central drive may occur above a certain hypoxic threshold. Amann et al. (2006) have hypothesised that oxygen supply affects the regulation of motor output to ensure that muscular fatigue does not exceed a critical threshold.

It has been previously reported that exercising in systemic hypoxia can induce changes in cerebral oxygenation, which in turn may limit incremental exercise performance (Subudhi, Dimmen, & Roach, 2007). Altered cerebral oxygenation may play a regulatory role during IHRT using high-level hypoxia, effectively attenuating any increases in muscle activation that may occur using moderate-level hypoxia. This cerebral mechanism is unlikely to be present during BFR exercise, where the hypoxic environment is localised to the limb being trained

and significant elevations in muscle activation are regularly reported (Takarada et al., 2000a; Yasuda et al., 2009). This is a probable difference between these two resistance training methods. Although further research is needed to examine the dose-response relationship between the level of hypoxia during IHRT and subsequent muscle activation, current evidence suggests that beneficial muscular adaptations are only possible when moderate-level hypoxia is employed in conjunction with brief inter-set rest periods (Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b; Nishimura et al., 2010).

Metabolic Stress

The degree of metabolic stress associated with resistance training has been proposed as an important regulator of subsequent adaptive muscular responses (Schoenfeld, 2013). Intramuscular hypoxia during resistance exercise likely increases the reliance on anaerobic metabolism (Kawada, 2005), which accelerates the production of metabolites. Indeed, researchers have previously demonstrated that IHRT (F_IO_2 = 13%) using both low-loads (5 sets of 14 repetitions at 50% 1RM with 60 s inter-set rest) (Kon et al., 2012) and moderate-loads (5 sets of 10 repetitions at 70% 1RM with 60 s inter-set rest) (Kon et al., 2010) can increase metabolic stress, measured via BLa^- concentration, when compared to the equivalent exercise in normoxia. As such, increased metabolic stress may be a primary mechanism underpinning augmented muscular responses to hypoxic resistance training methods.

Schoenfeld (2013) proposed hypertrophic adaptations to resistance exercise may be mediated by metabolic stress via enhanced muscle activation, up-regulated endocrine responses, greater production of local myokines and reactive oxygen species, and cellular swelling. If hypoxia-mediated increases in metabolic stress are in fact an underlying mechanism for adaptation to IHRT, it is important to understand how best to utilise hypoxic stimuli to augment metabolic stress. If this is not considered during IHRT program design, it is likely that mechanisms downstream from the metabolic stress will not be further enhanced, and no additive benefits will be observed for IHRT. One key area factor that has received limited research attention is the duration of inter-set recovery periods. As highlighted by Bird et al. (2005), the length of inter-set rest periods not only determines the degree of ATP-PCr energy recovery, but also the extent to which BLa^- concentrations are elevated. Importantly, IHRT is vastly different to BFR methods in this regard, given that venous outflow is occluded by the BFR stimulus and metabolites therefore cannot be re-distributed away from the exercising limb. However, venous outflow is maintained during IHRT, meaning that metabolites can enter circulation and be distributed to other parts of the body. Logic therefore dictates that for augmented metabolic stress during IHRT, inter-set rest periods should be short enough to ensure that a hypoxia-mediated metabolic stress is still present within the muscles during each subsequent set.

A mechanism by which hypoxia may alter energetic metabolism during resistance exercise may by slowing the rate of PCr recovery between sets.

Resynthesis of PCr occurs primarily by oxidative processes, and is therefore sensitive to manipulations of oxygen availability (Haseler et al., 1999). It is possible that relatively brief inter-set rest periods will result in subsequent sets beginning with a lower PCr concentration when training in hypoxia, placing greater stress on anaerobic glycolysis and consequently increasing the accumulation of metabolites. This may account for the findings of no added hypertrophic or strength benefits following IHRT in previous research that has employed inter-set rest periods of 90-120 s (Ho et al., 2014b; Kon et al., 2014). Indeed, Ho et al. (2014a) have reported no differences in BLa^- concentrations between hypoxic and normoxic IHRT groups following 5 sets of 15 repetitions of squats (30% 1RM) with 90 s inter-set rest. Therefore, it is likely that inter-set rest periods of 90 s or longer are sufficient to attenuate any hypoxia-mediated rise in metabolic stress, limiting the potential anabolic effects of hypoxia.

While some research has previously demonstrated increased metabolic stress following IHRT (Kon et al., 2010; Kon et al., 2012), conflicting data have been recently reported (Ho et al., 2014a; Kurobe et al., 2015). Kurobe et al. (2015) observed no differences between hypoxic and normoxic training groups performing 3 sets of 10 repetitions of elbow extensions (10RM) with 60 s inter-set rest. These conflicting results may be partly explained by the differences in exercise volume, as the studies by Kon et al., (2010; 2012) both employed 5 sets of two exercises, whereas Kurobe et al. (2015) employed a single exercise for 3 sets. In addition, the study by Ho et al. (2014a) used very long inter-set recovery periods, especially considering the low exercise loads prescribed,

which likely attenuated any hypoxia-mediated increase in metabolic stress. It is possible that hypoxic conditions facilitate only a small increase in metabolic stress, which requires more than 3 sets with short rest intervals between to provide a significant accumulated effect. However, this explanation remains speculative and requires further research.

Conclusions

When considering the current research into IHRT, it is clear that methodological inconsistencies between studies may have caused the conflicting results. While there is currently a limited body of research examining IHRT, it appears that adaptive responses may be influenced by the level of hypoxia and the inter-set rest periods (and subsequent alterations in metabolic stress) used. We propose that for IHRT to elicit greater muscular adaptations than the equivalent training in normoxia, exercise protocols should be designed to provide a substantial metabolic stimulus, with particular care being taken to implement relatively brief rest periods between each set. However, it should also be acknowledged that a range of other factors could influence adaptive responses to IHRT. For example, neuroplasticity may be altered by long periods of intermittent hypoxic exposures, and could therefore play a role in adaptation to extended IHRT programs. Nonetheless, the purpose of this paper is to provoke thought amongst scientists regarding how resistance training program design may be manipulated in conjunction with systemic hypoxia to enhance adaptive responses. Given the available evidence, we suggest that inter-set rest periods should be very brief for low-load exercise (~20-30% 1RM; ~30 s) and brief for

moderate-load exercise (~70% 1RM; ~60 s). Furthermore, it may also be important to ensure that a moderate, rather than high, level of hypoxia is used (F_IO_2 = ~14-16%).

Appendix H

Intermittent Hypoxic Resistance Training: Is Metabolic Stress the Key Moderator?

As per the peer-reviewed papers **Accepted and Published** in *Medical Hypotheses*:

Scott, B. R., Slattery, K. M., & Dascombe, B. J. (2015). Intermittent hypoxic resistance training: is metabolic stress the key moderator? *Medical Hypotheses*, 84, 145-149.

This paper has not been edited from the originally accepted manuscript published in Frontiers in Physiology.

Abstract

Traditionally, researchers and practitioners have manipulated acute resistance exercise variables to elicit the desired responses to training. However, recent research indicates that altering the muscular environment during resistance training, namely by implementing a hypoxic stimulus, can augment muscle hypertrophy and strength. IHRT, whereby participants inspire hypoxic air during resistance training, has been previously demonstrated to increase muscle cross-sectional area and maximum strength by significantly greater amounts than the equivalent training in normoxia. However, some recent evidence has provided conflicting results, reporting that the use of systemic hypoxia during resistance training provided no added benefit. While the definitive mechanisms that may augment muscular responses to IHRT are not yet fully understood, an increased metabolic stress is thought to be important for moderating many downstream processes related to hypertrophy. It is likely that methodological differences between conflicting IHRT studies have resulted in different degrees of metabolic stress during training, particularly when considering the inter-set recovery intervals used. Given that the most fundamental physiological stresses resulting from hypoxia are disturbances to oxidative metabolism, it becomes apparent that resistance training may only benefit from additional hypoxia if the exercise is structured to elicit a strong metabolic response. We hypothesise that for IHRT to be more effective in producing muscular hypertrophy and increasing strength than the equivalent normoxic training, exercise should be performed with relatively brief inter-set recovery periods, with the aim of providing a potent metabolic stimulus to enhance anabolic responses.

Introduction

Resistance exercise is known to have a potent affect on skeletal muscle morphology and functional adaptations. Traditionally, researchers and practitioners have focused on manipulating acute resistance exercise variables to elicit the desired training response. These variables include the muscle action, loading and volume, exercise selection and order, inter-set rest periods, repetition velocity and training frequency (Bird et al., 2005). However, recent evidence suggests that methods to alter the intramuscular environment during resistance exercise can be beneficial for stimulating hypertrophy and increases in muscular strength. The use of BFR during resistance training has become increasingly popular for this purpose. This technique involves the application of a restrictive cuff, tourniquet or elastic wraps around the top of a limb, with the aim to somewhat maintain arterial inflow while occluding venous return from the exercising limb (Loenneke et al., 2012b).

This technique creates a localised hypoxic environment in the limb during exercise (Kacin & Strazar, 2011), which is proposed to impact on downstream mechanisms that promote muscular development. The novel aspect of training with BFR is that substantial improvements in muscular hypertrophy and strength are possible even when using low-loads (20-40% of concentric 1RM) for both clinical (Ohta et al., 2003) and athletic (Manimmanakorn et al., 2013a; Yamanaka et al., 2012) populations. However, while the muscles of the trunk may benefit to some degree from BFR exercise (Yasuda et al., 2010b), the trunk muscles are unable to be trained under the same conditions as the limbs.

Furthermore, due to the low-loads employed during BFR exercise, motor unit recruitment (as estimated by surface EMG) is lower than during traditional high-load exercise (Cook et al., 2013; Manini & Clark, 2009), therefore limiting the potential for neuromuscular adaptations.

Another method to manipulate the intramuscular environment during resistance exercise that is not affected by these limitations is the addition of systemic hypoxia during training. Research has demonstrated that hypertrophic and strength responses can be enhanced by breathing hypoxic air during low-load (20% 1RM) (Manimmanakorn et al., 2013a; Manimmanakorn et al., 2013b) and moderate-load (70% 1RM) (Nishimura et al., 2010) resistance training. However, some more recent evidence has provided conflicting results, reporting no additional benefit for muscular development following resistance training in systemic hypoxia (Ho et al., 2014b; Kon et al., 2014). While scientific understanding of IHRT is in its infancy, it appears that these conflicting results may be a result of differences in the research methodologies employed.

In particular, the inter-set rest periods used by researchers has varied greatly (30-120 s). Inter-set rest periods are often overlooked in the design of resistance training programs, particularly in recreational training settings. However, the rest period is a primary determinant of the overall intensity of a training session, particularly when the level of available oxygen is altered as it will directly impact on the metabolic stress induced by exercise (Kraemer & Ratamess, 2004). As the metabolic response to resistance exercise is a

proposed key moderator of subsequent adaptive responses (Schoenfeld, 2013), it stands to reason that inter-set rest periods should be carefully programmed during IHRT. In this paper, we hypothesise that due to the disturbances in energetic metabolism induced by hypoxia, the inter-set rest periods employed during IHRT are of primary importance to enhanced hypertrophic responses.

Conflicting Results of Intermittent Hypoxic Resistance Training Studies

To date, six separate investigations have assessed the efficacy of IHRT for increased muscle hypertrophy and strength, compared with the equivalent training in normoxia. These studies are summarised in Table 10.1. Two papers have used low-load resistance training (20-30% 1RM), with Manimmanakorn et al. (2013a; 2013b) employing very brief inter-set rest periods (30 s), while Friedmann et al. (2003) used longer rest intervals (60 s). Interestingly, Manimmanakorn et al. (2013a; 2013b) reported that IHRT elicited greater hypertrophic and strength responses than work-matched normoxic training, whereas Friedmann et al. (2003) observed no changes in strength or muscle CSA after either IHRT or normoxic training, though muscular endurance increased in both groups.

Table 10.1. Summary of research examining the morphological and strength responses to IHRT programs.

Study	Training conditions	Exercise (intensity)	Sets x reps (inter-set rest)	Training frequency/ duration	Hypertrophic and strength responses	Hypoxia provides added benefit?
Low-load IHRT (≤30% 1RM)						
Manimmanakorn et al. (2013a; 2013b)	• IHRT (SpO_2 at ~80%) • RT (ambient air)	Knee extension and flexion (20% 1RM)	3 x ~22-36 (30 s)	3 days/week (5 weeks)	• Greater ↑ muscular strength and endurance after IHRT • Greater ↑ in muscle CSA after IHRT	**Yes**
Friedmann et al. (2003)	• IHRT (F_IO_2 = 12%) • RT (ambient air)	Knee extension (30% 1RM)	6 x 25 (60 s)	3 days/week (4 weeks)	• ↔ Isokinetic strength and muscle CSA, • ↑ Muscular endurance (not different between groups)	**No**
Moderate-load IHRT (70% 1RM or 10RM)						
Nishimura et al. (2010)	• IHRT (F_IO_2 = 16%) • RT (F_IO_2 = 21%)	Elbow extension and flexion (70% 1RM)	4 x 10 (60 s)	2 days/week (6 weeks)	• ↑ muscle CSA only after IHRT • ↑ 1RM after just 3 weeks for IHRT	**Yes**
Kurobe et al. (2015)	• IHRT (F_IO_2 = 12.7%) • RT (F_IO_2 = 21%)	Elbow extension (10RM)	3 x 10 (60 s)	3 days/week (8 weeks)	• Greater ↑ muscle thickness after IHRT • ↑ 10RM (not different between groups)	**Yes**
Kon et al. (2014)	• IHRT (F_IO_2 = 14.4%) • RT (F_IO_2 = 21%)	Bench press and leg press (70% 1RM)	5 x 10 (90 s)	2 days/week (8 weeks)	• ↑ 1RM strength (not different between groups) • ↑ muscle CSA (not different between groups)	**No**
Ho et al. (2014b)	• IHRT (F_IO_2 = 15%) • RT (F_IO_2 = 21%)	Squat (10RM)	3 x 10 (120 s)	3 days/week (6 weeks)	• ↑ Squat 1RM (not different between groups) • ↔ Body composition in either group	**No**

reps repetitions, *IHRT* intermittent hypoxic resistance training, *SpO_2* arterial oxygen saturation, *RT* resistance training in normoxia, *F_IO_2* fraction of inspired oxygen, *1RM* 1-repetition maximum, *CSA* muscle cross sectional area, *10RM* 10-repetition maximum↑ increase, ↔ no significant change.

Similar findings have been reported for IHRT research using moderate-loads (70% 1RM or 10RM). Two investigations that employed comparable training protocols with 60 s inter-set recovery periods have demonstrated significantly enhanced hypertrophic responses for IHRT groups compared to work-matched normoxic training groups (Kurobe et al., 2015; Nishimura et al., 2010). However, other investigations using similar training loads but with extended inter-set recovery intervals (90-120 s) have demonstrated no added benefit for training under hypoxia (Ho et al., 2014b; Kon et al., 2014). Although it is too early to make definitive recommendations based on only these investigations, we hypothesise that low- and moderate-load IHRT might only provide added benefit when relatively brief inter-set rest periods are used. Indeed, when considering the fundamental physiological stress that hypoxia adds to resistance training (disturbances to oxidative metabolism), it is likely that a threshold for inter-set rest duration exists, and that the use of longer recovery periods between sets might mitigate any hypoxia-mediated effects on the muscular environment.

Effects of Inter-Set Rest Periods on Energetic Metabolism

Inter-set rest periods are most often manipulated in response to the intensity of exercise being performed. For example, maximal strength and power training (1-6 repetitions per set using heavy loads) generally utilise long rest intervals (180-480 s) to allow for sufficient neuromuscular recovery, and replenishment of ATP and PCr stores (Kraemer & Ratamess, 2004; Tan, 1999). However, training focused on hypertrophic responses (8-12 repetitions per set using

moderate loads) employs relatively short rest periods (60-120 s) in order to increase intramuscular metabolic stress (Bird et al., 2005), at the expense of contractile function in subsequent sets (Kraemer, 1997). This form of training is fuelled primarily by energy from the ATP-PCr system, with minor contributions from oxidative metabolism (Kraemer & Ratamess, 2004). Muscular endurance training (>15 repetitions per set using light loads) utilises brief rest intervals (30-45 s), with an increased reliance on aerobic metabolism.

The inter-set rest period not only determines how much of the ATP-PCr energy source is able to be recovered between sets, but also the degree to which metabolic products (e.g. lactate and H^+) are removed from the exercising musculature prior to the next set. Metabolic stress is proposed as an important moderator of hypertrophy following resistance training (Schoenfeld, 2013). As such, it appears that resistance training programs that use relatively short inter-set rest periods provide a greater stimulus for hypertrophy than those using longer rest periods. This can be observed anecdotally by comparing the moderate-load training with brief rest periods typically employed by bodybuilders, to the high-load training with long rest periods used by powerlifters. While powerlifters are generally much stronger (largely due to greater neuromuscular development), bodybuilders exhibit a greater degree of muscle hypertrophy, owing to the differences in training structure between these groups.

Hypoxia-Mediated Challenges for Energetic Metabolism

The importance of oxygen availability for PCr resynthesis was first established by Harris et al. (1976), who implemented a pneumatic cuff around the thigh (240 mmHg) for 6 minutes following a bout of isometric knee extension. As a result of the ischemic condition, PCr resynthesis was completely supressed. Furthermore, when the cuff was deflated for 25 s following 90 s of ischemic recovery and then reinflated, PCr stores recovered to levels that would be expected following 25 s of free flow recovery. Similar findings have also been presented using a systemic hypoxia model, with Haseler et al. (1999) reporting that the PCr recovery rates following plantar flexion exercise were slower when participants breathed hypoxic air (F_IO_2 = 10%) compared to normoxic air (F_IO_2 = 21%), and that PCr recovery was fastest when breathing hyeroxic air (F_IO_2 = 100%). Taken together, these findings indicate that the availability of oxygen following an exercise bout is vital for PCr resynthesis, and perhaps more importantly, that the time course of PCr resynthesis kinetics can be altered through manipulating oxygen availability.

Decreased levels of available oxygen during IHRT may also alter energetic metabolism during each set, placing more reliance on anaerobic energy production. While no data have been presented to detail whether the contributions of different energy systems is altered by performing resistance exercise in hypoxia, research examining energy production during sustained running exercise has demonstrated that short duration performance can be maintained due to a shift toward anaerobic metabolism (Weyand et al., 1999).

Participants performed maximal running efforts for durations ranging from 15-180 s in normoxia and hypoxia (F_IO_2 = 13%). Despite reductions in the oxidative energy available for sprinting under hypoxic conditions, participants were able to run just as fast for sprints of up to 60 s, and nearly as fast for sprints of up to 120 s. The authors concluded that this was possible because rates of anaerobic energy release (estimated from oxygen deficit) increased by as much as 18%, compensating for the reductions in aerobic power. Additionally, previous research has observed increased concentrations of BLa^-, which is well known as a by-product of anaerobic metabolism, following both low-load (Kon et al., 2012) and moderate-load (Kon et al., 2010) IHRT compared to the equivalent normoxic exercise. Taken together, these findings indicate that there is a shift towards anaerobic metabolism during IHRT. However, research from our laboratory has observed no significant difference in BLa^- concentrations following high-load resistance exercise with extended rest periods (180 s) in normoxia and two hypoxic conditions (F_IO_2 = 16% and 13%; unpublished findings). This may indicate that protocols designed specifically to enhance muscular strength (i.e. high-load with sufficient inter-set recovery) do not benefit substantially by the addition of systemic hypoxia.

As repeated resistance exercise sets are largely dependent on the ATP-PCr energy system, it stands to reason that if the inter-set rest periods are manipulated so that PCr stores are not completely recovered, subsequent sets will be performed under more challenging metabolic conditions. However, if inter-set recovery periods during IHRT are too long, PCr stores may return to

levels similar to normoxic resistance exercise between sets, mitigating any additive benefit from the hypoxic stimulus. This relationship has been previously illustrated in a review by Glaister (2005), who interpreted the work of Haseler et al. (1999) to demonstrate that while PCr levels may be lower at 60 s post exercise in hypoxia compared with normoxia, after 90-120 s of recovery, there does not appear to be a difference between the conditions. However, these data are specific to submaximal plantar flexion exercise, and caution should be taken when considering these findings for IHRT. Figure 10.1 represents a hypothetical model of how hypoxia-related metabolic stress may be attenuated over time following a set of resistance exercise. These hypothetical relationships are likely to be affected by both the rate of recovery from metabolic stress (i.e. PCr resynthesis and removal of metabolites), as well as the degree of metabolic stress induced by the hypoxic condition the and exercise stimulus.

Anabolic Effects of Metabolic Stress

The hypertrophic responses to resistance training are thought to be largely related to the metabolic stress induced by exercise, which is typically estimated via the accumulation of metabolites such as lactate, H^+ and Pi, and by changes in pH levels. Recently, it has been proposed that increased levels of metabolic stress can impact on several downstream mechanisms to facilitate muscular hypertrophy (Schoenfeld, 2013). For example, it is possible that metabolic stress can increase the recruitment of muscle fibres. Under normal conditions, muscle fibre recruitment follows the size principle, which dictates that smaller

motor units are recruit first, with the larger and more powerful motor units being recruited with increasing exercise loads (Henneman et al., 1965). However, given that metabolic acidosis can stimulate group III and IV afferents (Rotto & Kaufman, 1988), mechanistically speaking, a reflexive net inhibitory effect on the α-motor neuron may result (Leonard et al., 1994), facilitating increased fibre recruitment to protect against conduction failure (Yasuda et al., 2010a). Simply stated, if a greater number of muscle fibres are stimulated during a training session, then a greater portion of the muscle must respond to the exercise stress and undergo adaptation.

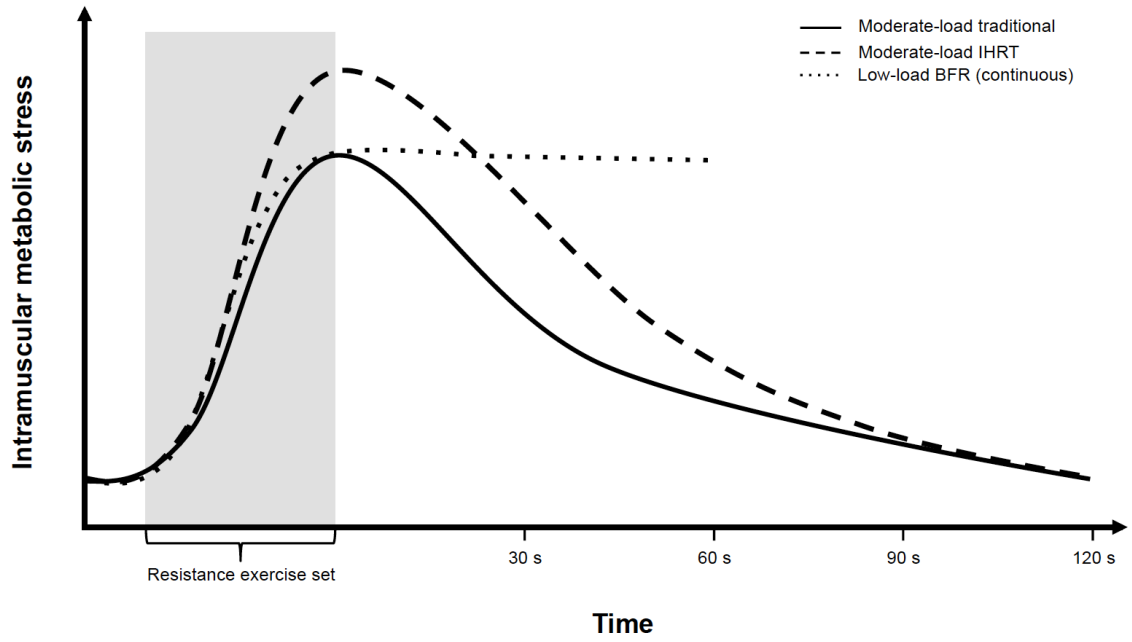

Figure 10.1. Theoretical time-course changes of intramuscular metabolic stress following a set of moderate-load traditional resistance exercise, moderate-load IHRT and low-load BFR exercise (BFR maintained following exercise). We propose that inter-set rest periods during IHRT should be structured to take advantage of hypoxia-mediated metabolic stress (30-60 s in this hypothetical example).
Note: As it is not common to employ continuous BFR for greater than 60 s between sets, this hypothetical response has not been illustrated for the same duration as other responses.

Another potential role for metabolic stress in muscular hypertrophy is increased cellular swelling. Transient cellular swelling is a well known response to the resistance training protocols commonly performed by bodybuilders to elicit hypertrophy, and has been proposed as a mediator for adaptive muscular responses (Schoenfeld & Contreras, 2014). Loenneke et al. (2012a) have recently hypothesised that the anabolic benefits of low-load resistance training with BFR may be induced by acute swelling of muscle cells. Cell swelling is maximised in exercise that relies on anaerobic metabolism, due to the osmotic changes caused by lactate accumulation (Sjogaard et al., 1985). Thus a resultant increase in the flow of water into the muscle cell is required to equilibrate the osmotic gradient (Loenneke et al., 2012a). Although it is likely that cellular swelling may be induced to a greater degree during BFR exercise than IHRT, due to the substantial venous pooling resulting from occluded venous return under BFR conditions, an accumulation of metabolites during IHRT (Kon et al., 2010; Kon et al., 2012) may increase cellular swelling alone. Given that hydration-mediated cellular swelling can increase protein synthesis and decrease protein degradation in hepatocytes (Haussinger, Lang, & Gerok, 1994) and a range of other cells (Lang et al., 1998), it is possible that similar responses occur in muscle cells. Muscle cell swelling may be detected by an intrinsic volume sensor (Loenneke et al., 2012a), that registers a threat to cellular integrity causing the cell to initiate a signalling response to reinforce its ultrastructure (Schoenfeld, 2010, 2013). Although a paucity of research has directly examined the downstream signalling events following exercise-mediated muscle cell swelling, it is suggested that mammalian target of

rapamycin and mitogen-activated protein kinase pathways may be activated (Loenneke et al., 2012a; Schoenfeld, 2010, 2013), which are known to be important for muscle hypertrophy.

Metabolic stress is also often proposed as a mechanism by which growth-orientated hormone concentrations can be increased (Schoenfeld, 2013). Theoretically, higher concentrations of hormones increase the likelihood of receptor interactions (Crewther et al., 2006), thereby enhancing the action of these hormones. In particular, increased growth hormone concentrations have been reported following low-load (Kon et al., 2012) and moderate-load (Kon et al., 2010; Kon et al., 2014) IHRT. Post-exercise elevations in growth hormone may be mediated by increased lactate and/or hydrogen ion build-up (Gordon et al., 1994). Furthermore, reduced pH levels associated with metabolic acidosis may potentiate release of growth hormone via chemoreflex stimulation mediated by intramuscular metaboreceptors and group III and IV afferents (Loenneke, Wilson, & Wilson, 2010; Viru et al., 1998). However, while GH has often been cited as having important anabolic functions for skeletal muscle, the role of acute exercise-induced endocrine responses in muscle hypertrophy has been recently questioned, and may not have anabolic effects in healthy individuals as once thought (West & Phillips, 2010). Therefore, further research is required to clarify this, and other potential mechanisms for hypertrophy resulting from resistance training.

Considerations for Training Programs

From current evidence, it appears that relatively short inter-set rest periods are required during IHRT to take advantage of hypoxia-mediated disturbances to oxidative metabolism, and subsequently enhance hypertrophic responses. Furthermore, it is likely that, as with traditional resistance training, the inter-set rest period is related to the intensity of the loads lifted. Low-load IHRT appears beneficial when using very brief rest periods (30 s), whereas moderate-load IHRT is most effective when using slightly longer (60 s) rest periods. However, if the inter-set recovery interval is extended past these durations, the hypoxia-mediated alterations in energy system contributions and subsequent metabolic stress are likely to be attenuated. This is highlighted by recent research from Ho et al. (2014b) and Kon et al. (2014), who used relatively long inter-set rest periods (120 and 90 s, respectively) and observed no added benefit for hypertrophic or strength responses following IHRT compared to the equivalent training in normoxia. We believe that while investigations using longer rest periods are necessary to broaden scientific understanding of IHRT methods, it is premature to conclude that the addition of hypoxia to resistance training does not provide added muscular benefit based on the results of research using longer inter-set rest periods.

In order to test the hypotheses presented in this paper, future research should aim to assess whether resistance training programs with varying inter-set rest intervals result in different morphological changes when performed in hypoxia. Furthermore, it is possible that some resistance training methods that have

been developed to increase the degree of metabolic stress (e.g. drop sets and assisted-repetition sets) may provide the best responses when used during IHRT. It is also likely that if appropriate inter-set rest periods are used during IHRT, the metabolic stress will continue to accumulate with each new set, effectively beginning each set at a higher level of metabolic stress. Therefore, we propose that multiple sets using the same muscle groups would exaggerate the intramuscular metabolic stress, and indeed a number of sets may be required to observe large differences in hypertrophic responses between IHRT and the equivalent training in normoxia. Further research to determine the optimal resistance training methods to use in combination with systemic hypoxia is therefore warranted.

However, it is important to highlight that exaggerated metabolic stress resulting from IHRT could adversely affect exercise performance during the each training session. If this is the case, caution should be taken not to use IHRT as a sole method of muscular development. Additional research is warranted to assess the impact of systemic hypoxia on resistance training performance, particularly when exercise is structured to facilitate a potent metabolic response. Furthermore, as the mechanisms underpinning adaptive responses to IHRT are not yet fully understood, it is possible that factors not related to metabolic stress (e.g. increased production of reactive oxygen species) may play a role in muscular development following this form of training. Although it is likely that metabolic stress plays an important role in hypertrophic responses, it would be

remiss not to recognise that skeletal muscle adaptations are vastly complex, and most probably affected by numerous physiological processes.

Conclusions

In conclusion, it is important when designing IHRT research studies that we consider the fundamental physiological stressors imposed by hypoxia (disturbances to oxidative metabolic processes). We hypothesise that the added benefits for muscular growth and strength will only be facilitated by IHRT programs that make use of short inter-set rest periods, with the aim to enhance the metabolic responses to exercise. More specifically, it appears that low-load IHRT requires rest intervals of ~30 s, whereas moderate-load IHRT should employ intervals of ~60 s. Future studies aiming to assess the potential hypertrophic and strength benefits of IHRT should implement a resistance training program that induce a marked degree of metabolic stress, as this is both a likely variable to be affected by hypoxia, and is proposed to act as a moderator for downstream process that can enhance hypertrophy and strength. In addition, future research should examine whether IHRT can evoke more substantial muscular gains than volume-equated traditional resistance training methods.