The Acute Metabolic Response of Intermittent Hypoxic Resistance Exercise

A Cross-Over RCT

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Master Degree Project 30:2019
Master program in Sport Science 2017–2019
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Abstract

Aim The aim for this present study was to investigate the acute metabolic response from intermittent resistance exercise during hypoxia, with the following research questions: (1) Are blood levels of lactate and glucose different between hypoxia and normoxia? (2) Does hypoxia induce higher lactate accumulation and pH reduction in the human skeletal muscle? (3) Is there a relationship between plasma-, blood- and muscle lactate? **Method** Eight healthy males (30 ± 2 years) performed 6 sets of unilateral leg extension on each leg (75% of 1RM) with randomized normoxic (20,9% inspired O_2) and normobaric hypoxic (12% inspired O_2) conditions. A total of 5 muscle biopsies was extracted from m. Vastus Lateralis (pre-, post exercise, 90-, 180min and 24h post exercise) during both normoxia and hypoxia trials, separated by one week for all participants. Blood samples were repeatedly taken with 20 min intervals. Heart Rate (HR) and saturation (SpO_2) were measured by a pulsoximeter during resistance exercise. **Results** No significant main effect was observed for blood lactate and glucose levels as well as the muscle lactate accumulation and pH between normoxia and hypoxia. However, pH in muscle showed a trend between the conditions post exercise where hypoxia reached lower levels in total (P=0.08). Significant correlations were observed for blood- and plasma lactate, where hypoxia showed a stronger relationship than normoxia (r=0.98 compared to r=0.87). Equal findings for the correlation of muscle- and plasma lactate showed an even greater coefficient value for hypoxia compared to normoxia (r=0.860 compared to r=0.59). **Conclusion** Summarized data indicated that no significant difference between hypoxia and normoxia was evident. Nonetheless, tendencies illustrate that hypoxia may alter the metabolic response slightly. However, further research is needed to draw a conclusion between the conditions.

Abstrakt

Syftet med denna studie är att undersöka kroppens akuta metabola svar från intermittent styrketräning under hypoxi, med följande frågeställningar: (1) Skiljer sig nivåerna av laktat och glukos i blodet mellan hypoxi och normoxi? (2) Skapar hypoxi större laktatansamling och pH reduktion i människoskelettmuskeln? (3) Finns det en relation mellan plasma-, blod- och muskellaktat? **Metod** Åtta friska män (30 ± 2 år) deltog där deltagarna utförde 6 set unilateral benextension för varje ben (75% 1RM). Intermittent styrketräning randomiserades med hypoxi som utfördes med 12% syrgas och normoxi som bibehöll normal syrgasnivå (20,9% syrgas). Under två testdagar togs 5 muskelbiopsier från m. Vastus Lateralis (före-, efter träning, 90-, 180min och 24h efter träning) på vartannat ben per testdag. Hjärtfrekvensen och SpO_2 mättes via pulsoximeter under träningen. **Resultat** Ingen signifikant huvudeffekt påvisades mellan hypoxi och normoxi för blodlaktat samt glukos, såväl som laktatackumulationen och pH värdet i muskeln. Muskel pH visade en trend där hypoxi efter styrketräning nådde lägre totalnivå än normoxi (P=0,08). Vidare observerades hypoxi att ha starka relationer mellan blod- och plasmalaktat jämfört med normoxi (r=0,98 vs. r=0,87). Större skillnad framgick för korrelationen mellan muskel- och plasmalaktat där hypoxi-försöket utgav starkare koefficient jämfört med normoxi (r=0,86 vs. r=0,59). **Konklusion** Sammanfattad data visar att hypoxi inte skapar större metabolisk respons vid intermittent styrketräning. Trots detta framkom tendenser som illustrerar att hypoxi kan påverka den metabola stressen under styrketräning. Däremot krävs vidare forskning för att kunna säkerställa effekten av hypoxi på kroppens metabola svar.

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1. Introduction

1.1 Strength training: Routines and Muscular Adaptions

Strength training is an effective stimulus to increase muscle mass and strength for both untrained and trained individuals (Morton et. al. 2016; Dankel et. al. 2019; DeFreitas et. al. 2011). The American Collage of Sports Medicine (2009) recommend individuals who want to increase muscle mass to perform 2-4 sets of loaded muscle contractions for each muscle group 2-3 times per week. The training program is recommended to be performed with 8-12 repetitions per set using a load equal to ≥70 % of the individual's one-repetition maximum (1RM). For example, employing such a training protocol for the knee extensor muscles over 21 weeks of strength training, for both trained and untrained individuals, has been documented to result in significant increases in muscle mass and strenght. Untrained individuals showed even greater increases in muscle volume (Ahtiainen et. al. 2003). Furhtermore, resistance exercise increases muscle protein synthesis rate acutely following exericse (Biolo et. al. 1995; Phillips et. al. 1999), thus creating a positive net protein balance when combined with nutrition during the post exericse period. These repeated stimuli of anabolism are likely behind the postivite aspects of higher strength training frequecies for muscle growth (Dankel et. al. 2017).

1.2 New Strength Training Models for Hypertrophy

High-intensity, as well as, low-intensity resistance exercise both have the potential to elicit strength training adaptations. Evidence suggests that low-intensity with high-volume (30% of 1RM to failure) can create a stimulation of post exercise protein synthesis rate and anabolic response equal to a high-intensity training protocol (90% 1RM) (Burd et. al. 2010). Schoenfeld et. al. (2014; 2015) propose that the muscles hypertrophic response is equivalent between high-intensity and low-intensity strength training. However, with regards to maximal muscle strength, the increases seem to be significantly greater for high-intensity strength training. The mechanism underlying the notion that low-intensity high volume resistance training can result in a similar anabolic response as conventional high-intensity training is yet to be determined but proposed to involve the greater degree of metabolic stress.

Blood flow occlusion during resistance exercise is a well-studied exercise regimen where the blood flow to the working muscle is restricted due to compression. Blood flow occlusion is only possible to achieve on superficial muscles on the limbs, and has been hypothesized to

increase the intra-muscular metabolic stress and therefore contribute to the hypertrophic response. Performing low-intensity resistance exercise e.g. 20% of 1RM combined with blood flow occlusion acutely stimulates anabolic signaling and protein synthesis (Fry et. al. 2010) as well as stimulates muscle growth over time (Loenneke et. al. 2012). This is interesting since muscle growth is not seen at such low-intensity without occlusion. Furthermore, a review stated that the metabolite accumulation is significantly elevated with occlusion, and suggested a relationship between metabolic stress and skeletal muscle hypertrophy (Loenneke, Wilson, & Wilson, 2010). Suga et. al. (2010) showed evidence for that blood flow occlusion increases the metabolite accumulation more than regular low-intensity resistance exercise due to greater muscular hypoxia.

When inducing the acute ischemic condition in the muscle, levels of anabolic hormones are elevated and intracellular swelling increases. These responses are likely the result of excessive phosphocreatine depletion, decreased pH and increased lactate production. Scott et. al. (2014) argue that these findings suggest that due to restricted blood flow, the oxygen availability decrease for the working muscles which promotes anaerobic metabolism and restricts lactate clearance.

Intermittent Hypoxic Resistance Exercise (HRE) is a further step from occlusion where the total blood oxygen availability (saturation) decrease. Here, a blood saturation of approximately 85% is considered reasonable and risk free for exercise applications (Tannheimer, Thomas, & Gerngroß, 2002). In contrast to occlusion on superficial muscles, HRE can safely reduce oxygen availability for central musculature and decreases the total hemoglobin oxygen availability for human muscle tissue (Katayama et. al. 2010; Subudhi, Dimmen, & Roach, 2007). Ramos-Campo et. al. (2018) have summarized data from studies in the field of HRE in relation to Normoxic Resistance Exercise (NRE) with regard to the effects on strength and muscle growth over a 4-8 weeks period on both trained and untrained individuals. This meta-analysis showed no difference on muscle growth after the training period, although it must be emphasized that limited number (n = 9) of studies were included, and also with variation in exercise protocols and degree of hypoxia. Furthermore, significant increases on muscle strength were observed in HRE which may not be larger than NRE. Furthermore, hypoxia with approx. 85% saturation may induce a greater metabolite accumulation acutely after a full body high-intensity circuit session (100% of 6 repetition max). Acute hypoxia has the potential to reduce performance, increase lactate levels and

reduce pH after the exercise protocol which suggest that HRE may create a greater metabolic stress than NRE (Ramos-Campo et. al. 2017). Equal findings have been shown during 5 set x 14 repetitions of low-intensity exercise (50% 1RM) with 85% saturation where the blood lactate accumulation showed a significant increase in HRE compared to NRE (Kon et. al. 2012). This accumulation of metabolites might stimulate hypertrophic adaptions, as postulated by Schoenfeld (2013).

As previous research suggests, hypoxic- and occlusion resistance exercise have an advantage on creating greater increases strength, but an increased hypertrophic response has not yet been shown in HRE protocols. Hypoxia both derived from either occlusion or simulated normobaric hypoxic conditions (hypoxia created on sea level), have shown tendencies to create additional metabolic stress with different types of resistance exercise protocols which may or may not influence the skeletal muscles adaption to training.

1.3 Hypoxia: Effects on The Human Body

Hypoxia is a state of lowered arterial oxygen content in the blood (SpO_2) and is mainly caused by a lowered atmospheric pressure at altitude which influences the SpO_2 (Tannheimer, Thomas, & Gerngroß, 2002). Therefore, when altitude increases the SpO_2 decrease in the same manner due to the change in O_2 pressure in the air. This change in oxygen availability induces a drastic strain on the human body which creates a physiological response where the heart rate and respiration increases to keep the saturation stable (Tannheimer, Thomas, & Gerngroß, 2002; Glanfield, 1988). More specifically, when the SpO_2 levels decrease to 93-70% the body respond with increased ventilatory drive, decreased limb O_2 tension and decreased arterial O_2 content (Dempsey & Morgan, 2015). A further chronic drop to ≤ 80% SpO_2 is defined as a critical oxygen deficit where cerebral and pulmonary damage can occur, even discomfort and illness has been recorded when being affected by ≤ 80% SpO_2 under 31 days total. To be able to perform, or even exercise, under a hypoxic condition, saturation should not decrease below 80% SpO_2 (Tannheimer, Thomas, & Gerngroß, 2002).

The influence of hypoxia on the human body is a growing field of interest in Sport Science. Firstly, chronic exposure of hypoxic air does alter the skeletal muscles physiology. For instance, individuals who live on high altitude or are exposed to more than 6 weeks of insufficient SpO_2, decreased their muscle mass over given period (Hoppeler & Vogt, 2001). Furthermore, when comparing high altitude- with sea level strength training of the elbow

flexors, the altitude strength training did have a lower impact on muscle strength (9.5% vs. 13.6%) and volume (11.3% vs. 17.7%) after one month training (Narici & Kayser, 1995). While being subjected to hypoxia (>5250m) for up to 75 days has been shown to decrease total mean muscle fiber area (15% decrease on m. Vastus Lateralis and m. Biceps Brachii) regardless of physical activity (Mizuno et. al. 2008). Normobaric hypoxia in a controlled resistance exercise environment has shown to not potentiate a short-term anabolic response (Gnimassou et. al. 2018)

1.4 The Metabolic response of HRE

Several studies have reported that hypoxia during resistance exercise acutely increase the blood lactate levels compared to normoxia (Ramos-Campo et. al. 2017; Kon et. al. 2010; Scott et. al. 2016). However, the blood lactate levels seems to depend on the saturation and training volume. Jen-Yu Hoo (2014) used saturation levels between ≈90-92% SpO_2 and an exercise protocol based with 30% 1RM (squats) on untrained individuals and saw no difference in blood lactate levels. Moreover when the load is increased to 50% 1RM (bench press and leg press) with ≈ 85% SpO_2, there was no difference on blood lactate levels between HRE and NRE were noted in the study by Kon et. al. (2012). In comparison, when Kon et. al. (2010) based the training protocol on 70% 1RM (bench press and leg press) with ≈ 85% SpO_2 a significant increase on mean blood lactate levels for hypoxia was evident when combined all timepoints and compared to NRE (5.7 ± 0.4 vs. 4.6 ± 0.4 mmol/l). These results indicate that the available data is lacking to draw firm conclusions on the effect of HRE on the lactate levels. Although, there is indications that higher intensities are needed to induce greater metabolic stress by HRE

In the field of normobaric HRE, although a majority of focus is directed at metabolic stress, no previous study has assessed the metabolic effects in terms of lactate, pH and glycogen within the muscle (in form of muscle biopsies) during isolated muscle contraction. Etheridge et. al. (2011) have previously examined the acute muscle protein synthesis and anabolic signaling after HRE. However, the protocol involved measurment on only 3 muscle biopsies and achieved ≈ 86% SpO_2. Additionally, the study did not asses the acute response of hypoxia on muscle lactate and pH. Furthermore, Gnimassou et. al. (2018) achieved ≈ 93% SpO_2 and likewise, only investigated protein synthesis rate acutely following HRE. Accordingly, the main purpose of the present study was to investigate how intermittent

controlled HRE influence the metabolic response of lactate and glucose in blood as well as lactate and pH in the human skeletal muscle with an improved study design.

2. Aim and Research questions

Intermittent hypoxic resistance exercise may be a promising training model for increasing muscle hypertrophy and maintaining muscle protein synthesis, effects that are suggested to be a result of increased metabolic perturbations. At present, it is however not known if hypoxic resistance exercise actually increases metabolite accumulation within the human skeletal muscle compared to exercise in the normoxic state. The primary objective of the present study is therefore to examine the metabolic response in the human skeletal muscle, and secondarily blood lactate and -glucose in connection with acute hypoxic resistance exercise.

The present study's specific research questions are as follows:

- Are blood levels of lactate and glucose different following high-intensity hypoxic resistance exercise compared to exercise in the normoxic state?
- Does acute hypoxia during resistance exercise alter the exercise induced lactate accumulation as well as pH reduction in skeletal muscle?
- How does the relation between plasma, blood and muscle lactate levels look during resistance exercise and is this relationship altered by hypoxia?

3. Methods

3.1 Participants

Eight healthy males (age: 30 ± 2 years; height: 184 ± 8 cm; body mass: 88 ± 8 kg) were recruited for participation in the study. All participants that participated in the study were free from injuries and illness. All participants were required to have had performed resistance exercise for the lower limbs one to two times per week for minimum a year as well as be able to lift 125% of their body weight in unilateral knee extension (Table 1). Furthermore, all participants were required to be weight stable, smoke and drug free as well as not consume any dietary supplements prior to the experiments.

Table 1: Preliminary trials data for group characeristics

Participants	Weigh (kg)	Length (cm)	1RM Left (kg)	1RM Right (kg)	Peak watt/kg	Mean Watt/kg
1	91	190	102.5	102.5	10.1	7.1
2	84	183	105	105	11.6	7.7
3	84	175	110	112.5	12	8.4
4	98	192	132.5	127.5	10.5	8.6
5	79	175	125	125	16.7	10,8
6	78	184	112.5	112.5	11.7	7.8
7	98	195	135	135	MD	MD
8	90	180	110	107.5	10.7	7.2
Mean	87.8 ± 7.8	184.3 ± 7.6	116.6 ± 12.5	115.9 ± 11.8	11.9 ± 2.2	8.2 ± 1.3

Characteristics and performance data from preliminary trials. Individuals absolute- and mean ± SD values are presented. MD = Missing Data; kg = kilogram; cm = centimeter; Peak and mean watt/kg = Watt produced on 30 second sprint per kg body weight.

3.2 Experimental Protocol

The participants visited the laboratory three times before the first experiment trial for familiarization. On the first occasion, data for group characteristics (age, weight, height and leg volume) were collected and the participants went through a health screening. Furthermore, data for 1RM on unilateral extension (right and left leg) and anaerobic 30s cycle ergometer sprint (mean and peak power output) were recorded for each participant during a normoxic condition. During the second visit, the participants performed the exercise protocol which consisted of 6 set on 8-10 repetitions on 75% of 1RM with 2 min rest between sets for familiarization purposes with normoxia, and on the third visit same protocol was used but with induced hypoxia.

Two days prior the experiments, the participants were instructed to refrain from all types of vigorous physical activity. On the day of the first trial, participants reported to the laboratory at 07.00 am in an overnight fasted state. Firstly, a catheter placed in the forearm vein for repeated blood sampling which took place at rest, after the cycle warmup, after resistance exercise warmup, after 2^{nd}-, 4^{th}- and 6^{th} set. Following the exercise, further blood samples were taken at 10, 20, 30, 45, 60, 90, 120 and 180 min of recovery. Furthermore, the experimental trials involved five skeletal muscle biopsies extracted from *m. Vastus Lateralis*. The first biopsy was taken in rest when the participants arrived to the laboratory, the second immediately after exercise and then 90-, 180 min and 24 h post exercise.

Post the first extracted muscle biopsy and after 10 min of very light ergometer cycle warmup, the participants either started to breath hypoxic air (FiO_2 12% to reach a SpO_2 of 80-85%) via

a face mask from an oxygen extracting apparatus (MAG-20 Altitude Generator, Higher Peak, USA) or normoxic air (FiO_2 20.9% ≈ 99-100% SpO_2) without a mask during normobaric conditions. The participants started with low-intensity (70 W) cycle ergometer warmup for 10 minutes to elevate blood circulation after the first biopsy and set baseline for heart rate and blood saturation level (SpO_2) in either the hypoxic- or normoxic before the exercise protocol which consisted of unilateral knee extension. Three warm-up sets (0-25-50 % 1RM) were performed with 2 minutes rest between sets followed by 6 sets of 8-10 repetitions starting at 75% of their 1-RM with 3 minutes rest between sets. Each set was designed to push the participants to volitional fatigue within the given repetition range. Furthermore, the mask for the hypoxic condition was rapidly taken off after the 6th set.

During the resistance exercise protocol, the saturation levels and heart rate (HR) were monitored by a pulsoximeter (RAD 57, Masimo, USA) with sensors placed on the forehead. Both HR and SpO_2 were measured after 30 and 120s following each exercise set. In the present study however, only measures at 30s after each set were accounted for. Following the biopsy at 180min post exercise, the participants was fed a standardized meal (34 g of protein, 21 g carbohydrate and 7 g fat) and had to refrain from exercise, keep a standardized diet and then report to the laboratory the following morning in a fasted state for a fifth muscle biopsy. Followed the first session, 7-10 days later, the participants reported back and performed the same protocol again with the other leg for further biopsy collection. Furthermore, starting conditions and exercising leg were randomized between the trials in a non-blind crossover fashion.

3.3 Measuring methods

All participants recorded base-data such as: 1 repetition maximum, mean and peak power output of 30s sprint on a ergometer cycle and group characteristics. Furthermore, the variables of load, set, repetitions and time under tension (TUT) will frame the performance outcome as if the participants can re-produce equal performance with and without hypoxia.

Blood samples

All blood samples was immediately analyzed for levels of lactate and glucose using an automized analyzer (Biosen C line, EKF diagnostics). Following the immediate blood sample analysis, the samples were centrifuged at 3000 g and 4°C to obtain plasma followed by storage in -80 C. Plasma samples were analyzed spectrophotometrically on a plate reader

(Infinite F200 Pro, Tecan, Switzerland) through adding 10 µl of blood plasma to a buffer containing LDH, NAD, Glycine, EDTA and Hydrazine (reaction solution). The solution were pipetted to a 96-well microplate and the amount of NADH formed in the reaction (measured by 340 nm absorbance) were directly proportional to the amount of lactate in the plasma sample. The samples were measured as duplicates to ensure proper reliability when calculating the lactate concentration.

Muscle samples

After being extracted from the m. Vastus Lateralis, the tissue was immediately blotted free from blood, frozen in liquid nitrogen and subsequently lyophilized for later storage in -80 C. These tissue samples were dissected free from connective tissue, lipid droplets and blood thus prepared for subsequent analysis. The muscle samples were thawed and pre-weighed (2 mg) in room temperature. The preparation before analysis involved to add 100 µl of 5% TCA (Trichloroacetic Acid) and mash the muscle thoroughly. The samples were later pipetted for 25 µl and added to 500 ml of reaction solution. Lactate concentration in the muscle is directly proportional to the amount of NADH that formed in the reaction (measured by 340 nm absorbance). Measurements for muscle lactate was calculated at rest, immediately after exercise and 90 min post exercise. Furthermore, muscle pH was measured on baseline and post-exercise muscle biopsies. The muscle samples was firstly prepared in a non-buffering solution containing 145 mM potassium chloride, 10 mM sodium chloride and 5 mM sodium fluoride and thereafter homogenized in a bullet blender (Bullet Blender, Next Advance, Troy, NY, USA) for 1min x 2 spins, and thereafter the muscle pH was determined using a microelectrode (Seven2Go pH Meter, Mettler Toledo, Greifensee, Switzerland).

3.4 Statistical Analysis

The statistical analysis were carried through with SPSS (IBM SPSS statistics version 25). All data was normally distributed across all variables. The normal distribution was tested trough Mauchly's Test of Sphericity to test if the variances of the differences were equal. Data for HR, SpO_2, blood-, plasma-, muscle lactate, muscle pH and blood glucose were analyzed with two-way repeated measure analysis of variance (two-way ANOVA, time x condition). If an interaction effect or a main effect of time was revealed by the ANOVA, a Fisher's LSD post hoc was employed to detect at which timepoints significant difference were present. To illustrate relationship and correlation strength between blood- and plasma lactate, muscle- and plasma lactate, and muscle lactate and muscle pH Pearson 's correlation coefficient was used.

The level of statistical significance was set at a value of <0.05. Furthermore, the data in the present study were presented with tables and graphs to illustrate the influence of HRE. All graphs were created in GraphPad Prism (version 8.1.1 (330)) and presented values in the graphs represent acquired mean ± Standard Error Mean (SEM). Furthermore, analyzed data which is presented in the results is representing mean values ± Standard Deviation (SD).

3.5 Ethical Considerations

In the present study, a total of 10 muscle biopsies were extracted from each participant. Both the blood plasma and muscle samples were frozen and stored in a registered Biobank at GIHs (Gymnastik och Idrottshögskolan) facilities in Stockholm for future research. All routines, protocols and sample data were stored and encrypted anonymously in a specific laboratory file. The participants' data was handled so no unauthorized personnel could access them. Furthermore, GIH is responsible for their personal data, and the data management follows strictly The Privacy Act (Personuppgiftlagen ,PUL). During the experiments the participant were fully insured by the Patient Insurance and Kammarkollegiet which is a project specific assurance. All participants gave signed, informed consent and the study had been granted ethical approval from the Regional Ethics Review Board in Stockholm for the trials. The participants were aware that they could at any time abort without mention a reason and their data would be deleted. No personal data will be published in the papers final form. An SpO_2 on 80% is safe for shorter durations and the participants saturation was constantly supervised. The participants could withdraw the experiment trial at any time and restore their saturation within seconds. When participation of the experiment was fully completed, the participants received financial compensation of 3000 SEK.

4. Results

4.1 Exercise Performance

Mean load during all exercise sets was not different between normoxia and hypoxia (67 kg respectively). Furthermore, no difference was observed for the TUT between the conditions. Hypoxia reached TUT of 23.4 ± 1.9s throughout the sets and during normoxia, the mean TUT was 25.4 ± 2.1s (see table 1). Repetitions produced were similar between the conditions throughout the resistance exercise (9 ± 1 reps, respectively).

Table 2: Performance Outcome

	Condition	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6
Load(kg)	Normoxia	80.6 ± 10.8	79.4 ± 12.1	76.2 ± 12.5	73.7 ± 11.6	71.2 ± 10.9	68.7 ± 11.6
	Hypoxia	80.6 ± 10.8	79.3 ± 12.1	76.2 ± 12.5	73.7 ± 11.6	71.2 ± 10.9	68.7 ± 11.6
Reps	Normoxia	9 ± 1	8.5 ± 1	9 ± 1	9 ± 0.5	9 ± 0.5	9 ± 0.5
	Hypoxia	8 ± 3	8.5 ± 1	9 ± 1	9 ± 0.5	8.5 ± 0.5	8.5 ± 0.5
TUT(s)	Normoxia	22.5 ± 4s	23.2 ± 4.1	22.5 ± 4.2	23.6 ± 3.7	22.4 ± 4.5	22.1 ± 3.4
	Hypoxia	22.5 ± 2.9	22.2 ± 4.1	22.7 ± 3.2	22 ± 3.9	21.5 ± 3.3	21.2 ± 4.4

Mean values ± SD for the performance outcome during the resistance exercise sessions with either normoxia and hypoxia. Load (weight lifted on 75% of 1RM in kilogram (kg)), Repetitions (reps) and Time Under Tension (TUT (s)) are split between Normoxia and Hypoxia.

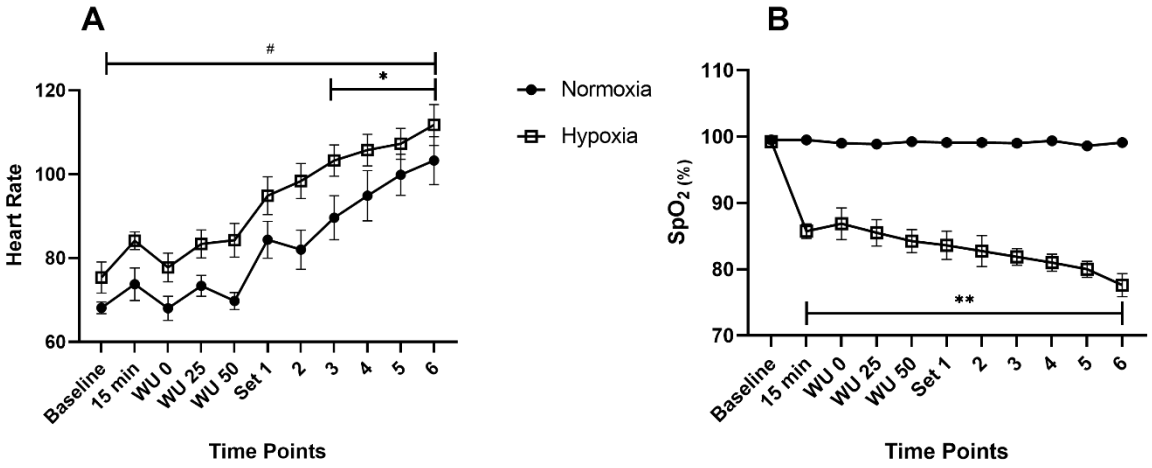
4.2 Saturation

The blood saturation is represented by mean ± SD and remained unchanged from baseline for the normoxic condition (99.1 ± 0.6% for SpO_2 at set 6 and the SpO_2 was unchanged throughout the exercise). In comparison, the hypoxic condition showed a significant interaction effect (time * condition) where the participants SpO_2 decrease from baseline within 10 min of hypoxic exposure and sustained decreased throughout exercise (85.7 ± 3.1 %, $P< 0.001$, Figure 1B). During the hypoxic trial the blood saturation decreased by a total 22.8% SpO_2 ($P< 0.001$) from baseline to the 6th set.

4.3 Heart Rate

For the HR data the statistical analysis revealed a main effect of trial and a main effect of time on the mean ± SD, but no interaction between the two conditions. The HR values for hypoxia and normoxia is shown in figure 1A. Significant increases were observed between baseline and the 6th set for both conditions ($P< 0.001$, respectively). Peak HR was observed to be higher at the 6th set for hypoxia (129) compared to normoxia (118).

Figure 1: Changes in Heart Rate (HR) and $\boldsymbol{SpO_2}$ between normoxia and hypoxia.



Mean values ± SEM is presented as changes in (A) HR and (B) Blood Saturation pre to post exercise. The figures illustrates the difference between Normoxia and Hypoxia from each timepoint during exercise (n=11 timepoints and n=8 participants). *=p< 0.05 compared to baseline, #=p< 0.05 compared to normoxia. **=p< 0.05 significant interaction effect compared to normoxia for all Timepoints.

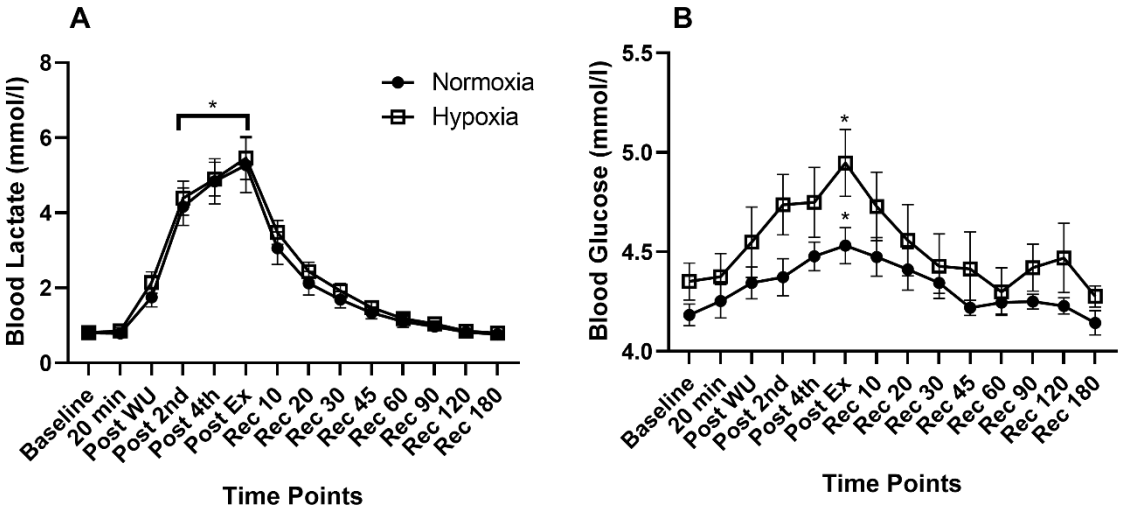
4.4 Blood Glucose

The blood glucose increased from baseline to post 6th set for both conditions (P< 0.001 for Time). However, no interaction- or main effect for conditions was evident between the conditions thought out the measured timepoints (Figure 2B). The peak mmol/l of glucose in the blood was measured to reach 4.9 ± 0.5 during hypoxia vs. 4.5 ± 0.3 during normoxia, which was significantly different compared to baseline for both conditions.

4.5 Blood Lactate

Blood levels of lactate increased as a result of exercise (Figure 2A, P< 0.05 for Time), with no differences between conditions or with an interaction effect. Peak levels of lactate were obtained after the final set of exercise (6th set), 5.3 ± 2.1 and 5.5 ± 1.6 mmol/l after normoxia and hypoxia respectively. In both trials baseline values of blood lactate were obtained again following 45 min of recovery post 6th set.

Figure 2: Change of Blood Lactate and Glucose between Normoxia and Hypoxia



Numbers are mean values ± SEM which represents changes in (A) Blood Lactate and (B) Blood Glucose during 14 different timepoints for both conditions. The figures illustrates the difference between Normoxia and Hypoxia during and after exercise, with data from n=8 participants for each timepoint. *=p< 0.05 compared to baseline, #=p< 0.05 compared to normoxia. **=p< 0.05

4.6 Plasma Lactate

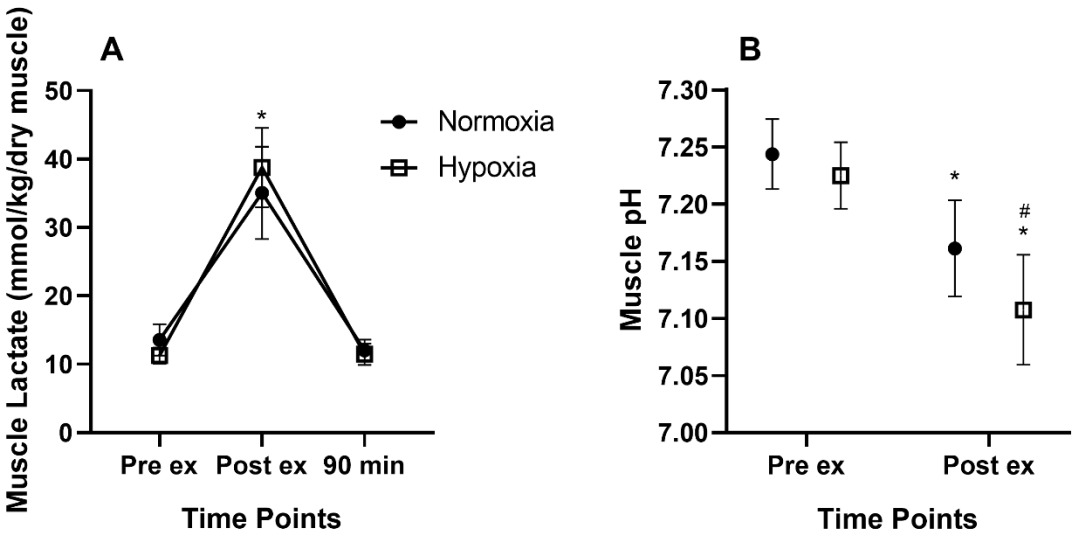
The plasma lactate increased for both conditions during the exercise bout ($P< 0.001$), with no difference between trials. From baseline to post exercise, plasma lactate levels increased 5-fold for both hypoxia (1.43 ± 0.6 to 7.2 ± 2.1 mmol/l) and normoxia (1.38 ± 0.4 to 7.2 ± 2.6 mmol/l). Furthermore, the peak values did not differ between normoxia and hypoxia (7.2 ± 2.6 vs. 7.2 ± 2.1 mmol/l) respectively for post 6th set. In both trials, baseline plasma lactate levels was again obtained after a 45 min of recovery post exercise. Furthermore, the peak values for plasma was 35.8 vs. 31% greater than the blood lactate values for normoxia and hypoxia. In contrast, the ratio of plasma lactate to blood lactate during baseline reached 58% in normoxia and 57% in hypoxia which show standardized start values for both conditions.

4.7 Muscle Lactate

Between the measured timepoints, the muscle lactate was significantly higher post exercise compared to baseline and post 90 min exercise for both conditions ($P< 0.01$). When comparing hypoxia with normoxia, no significant difference between the conditions post exercise was observed (38.8 ± 16.5 vs. 35.1 ± 19.1 mmol/kg dry muscle (figure 3B)). Furthermore, the delta muscle content of lactate (pre- and post-exercise) showed no statistically significant difference between normoxia and hypoxia (16.1 ± 6.8 vs. 26.2 ± 15.7 mmol/kg dry muscle).

To compare ratio difference between the conditions muscle lactate was recalculated from mmol/kg dry muscle to mmol/l. Thereby, when comparing muscle lactate levels against the blood plasma during normoxia, the plasma lactate was 45% of the muscle lactate at baseline (1.4 ± 0.4 vs. 3.1 ± 1.5 mmol/l). After resistance exercise, lactate concentration in the plasma increased to 90% of the muscle lactate (7.2 ± 2.6 vs. 8 ± 4,3 mmol/l). In contrast, the plasma lactate levels with hypoxia represented 56% out of the muscle lactate at baseline (1.4 ± 0.6 vs. 2.5 ± 0,8 mmol/l) which is 11% higher than with normoxia. However, the lactate ratio between muscle and plasma increased with hypoxia post exercise where plasma lactate composed of 81.9% out of the muscle lactate (7.2 ± 2.1 vs. 8.8 ± 3.75 mmol/l (8.1% less compared to normoxia)).

Figure 3: Changes in Muscle Lactate and pH values pre- to post exercise between Normoxia and Hypoxia



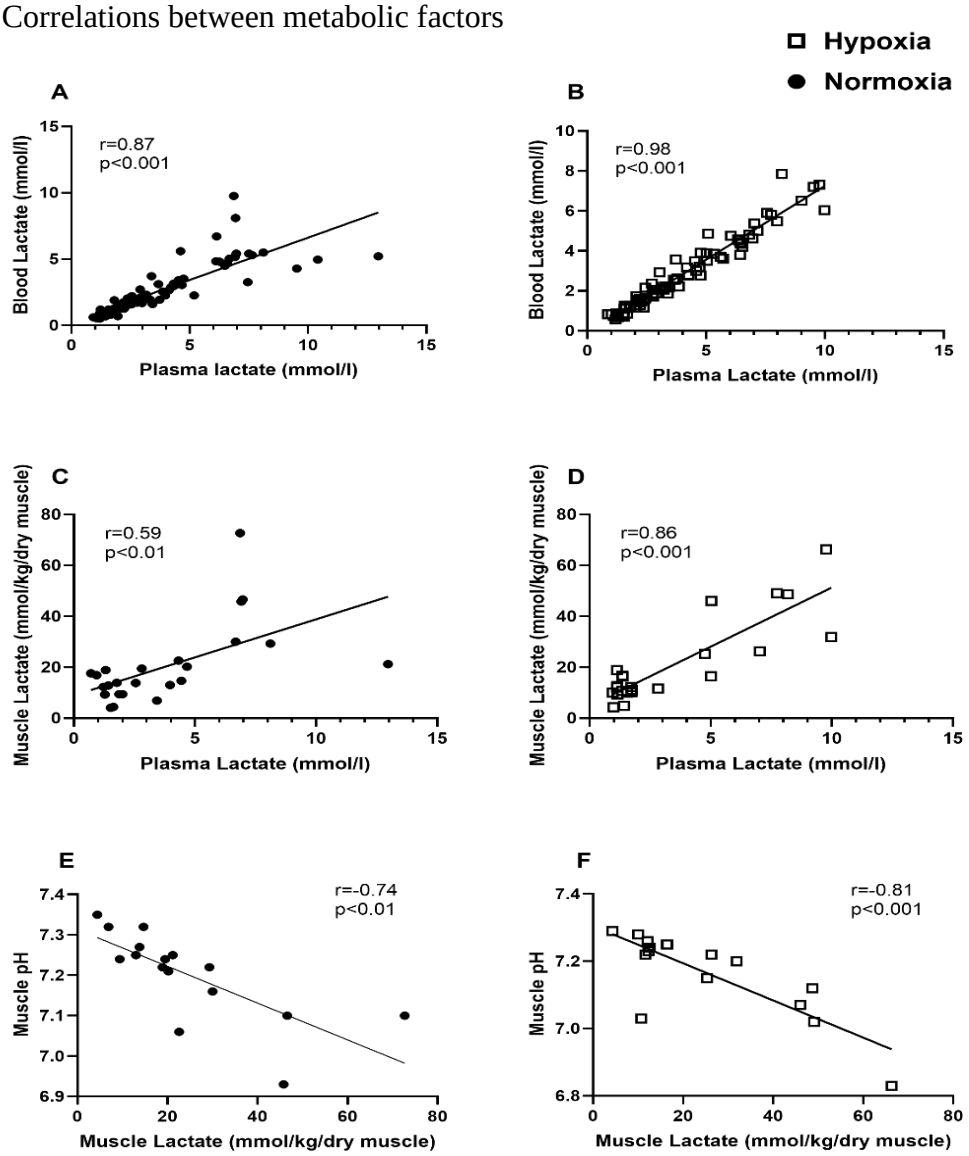
Changes in mean ± SEM (A) Muscle lactate (pre-, post- and post 90 min exercise) and (B) Muscle pH (pre- to post exercise) during resistance exercise for both conditions. The numbers are mean values for each timepoint pre- to post exercise, with data from n=8 participants for each timepoint. *=p< 0.05 compared to baseline, #=p< 0.05 compared to normoxia, **=p< 0.05 significant interaction effect compared to normoxia for all Timepoints.

4.8 Muscle pH

The muscle pH showed a significant decrease for both conditions as a result of the exercise (Normoxia: 7.24 ± 0.1 to 7.16 ± 0.1; Hypoxia: 7.23 ± 0.1 to 7.11 ± 0.1 pH, $P< 0.01$, respectively, see Figure 3B). Furthermore, there was no significant interaction between the conditions from Pre- to Post-exercise. However, the delta of the pH value from pre- to post exercise showed a tendency that hypoxia decreased more than normoxia ($P=0.080$). Thus,

further analysis trough a pairwise comparison T-test for the muscle pH post-exercise showed a significant difference between hypoxia and normoxia (7.16 ± 0.12 vs. 7.11 ± 0.14 pH, $P<0.01$). This comparison is thereby not possible to show statistically significant trough a two-way repeated ANOVA.

Figure 4: Correlations between metabolic factors



Correlation coefficient between factors, **A)** Blood Lactate (BL) vs. Plasma Lactate (PL) during Normoxia (N), **B)** BL vs. PL during Hypoxia (H), **C)** Muscle Lactate (ML) vs. PL during N, **D)** ML vs. PL during H, **E)** ML vs. Muscle pH During N, **F)** ML vs. Muscle pH during H. P< 0.01 was set to establish significant correlations between variables.

4.9 Correlations between metabolic factors

Blood- and plasma lactate showed a strong correlation trough the resistance exercise for both conditions combined (r=0.92, $P < 0.001$). Furthermore, hypoxia created a greater positive correlation between blood- and plasma lactate (figure 4B) compared to normoxia ((figure 4A)) r=0.98 compared to r=0.87). Bigger difference in correlation coefficient were observed when analyzing muscle- and plasma lactate. The relationship shift for a stronger correlation during the hypoxic condition (r=0.86, Figure 4D) compared to normoxia (r=0.59, Figure 4C). Furthermore, in combination with increased lactate, the muscle´s pH value shows a negative correlation where the pH drops consistent with muscle lactate during both hypoxia and normoxia combined (r= -0.77). Only a minor correlation difference was observed for muscle lactate and -pH between hypoxia and normoxia (r= -0.81 compared to r= -0.74 (Figure 4 E-F)).

5. Discussion

The purpose of the present study was to investigate if HRE creates a greater metabolic response in blood and skeletal muscle tissue compared to exercise in a normoxic condition. The main findings showed no significant differences between the two conditions with regard to the metabolic changes evoked by the exercise in blood or muscle. In contrast, there was a main effect for higher HR during the hypoxic trial, as the body likely compensates the reduced blood oxygen content by increasing heart rate in order to try to maintain tissue oxygen delivery. Furthermore, hypoxia did also show tendencies for a greater decrease in muscle pH, with an indication for a greater decrease from baseline to post exercise during hypoxia compared to normoxia ($P < 0.08$).

Performance

The exercise performance outcome for the participants was equal for both conditions, where set, reps and TUT did not differ. This indicates high intern validity between the participants where the performance remained unchanged. It is of great importance to match the training load between the participants to be able to establish if an metabolic effect is present, i.e. that the effect is due to hypoxia and not due to differences in training volume. Furthermore, the fact that unilateral leg extension was performed is to conceive isolated results for the quadriceps muscles which is not influenced by other musculature or physiological mechanisms.

The training protocol in the present study consisted of 6 sets x 8-10 repetitions at 75% of 1-RM with unilateral leg extension. Training intensities of ≥ 65 % of 1-RM are mainly used for muscle hypertrophy. Even higher loads are needed to maximize increases in strength (The American Collage of Sports Medicine, 2009; Kraemer & Ratamess, 2004). In the present study's experimental trials, the participants managed to produce 9 ± 1 repetitions with TUT on 23-25s, respectively. The purpose of this exercise protocol was to utilize high intensity resistance exercise to failure (fail to keep adequate Range of Motion). With a high training load, it's possible to examine hypoxias effect on the human skeletal muscle and not the muscles own metabolic processes due to workload. However, this will limit the observed metabolic effect (e.g. lactate) in both hypoxia and normoxia. Although, if potential effects emerges it will likely be due to induced hypoxia.

Katayama et. al. (2010) observed that a hypoxic condition during unilateral leg extension had a greater electro myography (EMG) amplitude compared to normoxia. Katayama concluded that a metabolic accumulation created from intermittent resistance exercise could affect the muscles ability to withstand fatigue. This is why the interset restperiod seems to play a role for lactate accumulation and between-set recovery. Scott et. al. (2016) and Kon et. al. (2010) both used 60 second rest between sets and showed increased blood lactate levels during HRE compared to NRE. However, during the present study, the rest period was 3 min between the working sets which might offset and create space for lactate clearance, and therefore may not yield significant difference in lactate concentration in blood/plasma. For practical resons, 3 min inter-set rest was necessary to have time for blood sample extraction and blood lactate analysis. However, evidence suggest that in order to maintain the performance level and total work performed of 8-12 rep on 75% 1RM, 3 min rest between sets is to prefer (Richmond & Godard, 2004).

However, both TUT and load are adjustable factors to achieve different types of muscle strain. A low repetition count for 3-5 RM has shown best effects for muscle strength. In contrast, a high repetition count of 20-28 RM resulted in increased muscular endurance (Campos et. al. 2002). Physiologically, during low load (30% 1RM fail) resistance exercise, a study found that muscle protein synthesis is significantly increased compared to exercise performed at 90% of 1RM until task failure (Burd et. al. 2010). However, high load has shown significant increases in muscle strength and volume after 21 weeks of exercise (Ahtiainen et. al. 2003). Therefore, adding HRE with loads equivalent 70-80% of 1RM, may

be a beneficial combination when high load and metabolic stress is combined to induce hypertrophy (Nishimura et. al. 2010; Kon et. al. 2014; Kon et. al. 2010).

Saturation

In the field of HRE the effects of normobaric hypoxia on blood oxygen saturation is variable, depending on training protocols, levels of inspired oxygen and acquired SpO_2 is indeed study specific. The outcome from HRE on metabolic factors seems to depend both on training load and saturation level acquired. Jen-Yu Hoo (2014) obtained SpO_2 at ≈90-92% using an exercise protocol consisting of squats at 30 % of 1-RM (with squats) on untrained individuals and saw no difference in blood lactate levels. Kon et. al. (2012) had a greater decrease in the SpO_2 to ≈ 85% with exercise performed at 50% 1RM (bench press and leg press), and showed no significant differences on blood lactate levels between hypoxia and normoxia. However, when loads increased to 60-90% of 1RM during hypoxic resistance exercise, significant increases in lactate accumulation has interestingly been shown (Ramos-Campo et. al. 2017; Kon et. al. 2010; Scott et. al. 2016). Notably, the mentioned studies used complex multi-joint exercises (benchpress, squats, legpress) which entails greater metabolic demands. Therefore, due to the nature of heavy anaerobic multi-joint exercises, more muscles are involved in the exercise bout which will likely increase the metabolic response (Scott et. al. 2016).

Previous research that have examined acute intermittent resistance exercise with induced hypoxia have not involved assessment of metabolic factors within the exercised muscle. Etheridge et. al. (2011) and Gnimassou et. al. (2018) used unilateral leg extension performed in a hypoxic state in a cross-over or two-group fashion, respectivley, and investigated muscle protein synthesis and the anabolic response. Etheridge extracted 3 muscle biopsies/participant, immediately post exercise in the non-exercised leg as baseline, and two was extracted 3.5h post exercise from both legs. Furthermore, Gnimassou had a slight different approach were two muscle biopsies (one from each leg) was immediately extracted post exercise and two more 4h post extercise. In comparison, the present study obtained 5 muscle biopsies from each participant with different timepoints for each trial (10 biopsies in total) which strengthen the data collection method with wider repeated measures. Thus, this method potentiate to observe metabolic stress during and after recistance exercise. Futermore, the SpO_2 levels are not comparable between these studies where Etheridge et. al. (2011) achieved ≈ 86% SpO_2 over a 3.5h period whereas Gnimassou et. al. (2018) achieved ≈ 93% SpO_2. Accordingly,

saturation levels achieved in the present study were lower (85.7 ± 3.1 % SpO_2). As discussed previously, a further decreased saturation can potentially result in greater metabolic demand when the degree of hypoxia is dependent on the saturation level.

In regards to the presented results, it may be possible to argue how the methods were carried through. The present study, which is based on another ongoing project with a different aim were focused on to improve the study design on HRE with repeated measure and induce lower saturation levels. However, the validity and accuracy of measurements in the present study could have been even more powerful to include an intra-muscular oxygenation measurment to evaluate the muscles local SpO_2 level. Gnimassou et. al. (2018) measured intra-muscle oxygenation (tissue saturation index = TSI) with NIRS (Near Infra-red Spectrometry) and showed no significant difference between the HRE and NRE. The TSI measurement may have been aggravated when biopsies were extracted and thus, influenced the results. Furthermore, a study was carried through with constant-load cycling where NIRS was tested for reliability and showed only a small difference on TSI but a substantial significant influence on OxyHemoglobin and DeoxyHemoglobin (Oueslati, Girard, & Ahmaidi, 2018). However, muscle oxygenation pressure has been shown to significantly decrease to very low values (<10 mmHg) during high intensity resistance exercise in a normoxic state (Richardson, Newcomer, & Noyszewski, 2001). Therefore, it is possible to hypothesis that hypoxia can create additional muscle deoxygenation during anaerobic exercise and create substantial metabolic stress. This indicates that HRE may not reach a adequate oxygen level in the muscle tissue which is enough for a different change in metabolic stress compared to NRE. This might be a weak link for this type of exercise employment. The limitations of blood SpO_2 levels inhibits the possibility for affecting the muscles oxygen depletion during exercise in a substantial manner. This is why intra-muscle oxygenation is interesting to utilize in future research, so it is possible to observe and manage the muscles local saturation level. Thus, if this instrument was implemented into the present study´s method, the intern validity would improve.

Heart Rate

Hypoxia has been shown in previous studies to increase the HR as the saturation decrease, which is a compensatory mechanism functioning to maintain oxygen delivery to the tissue (Tannheimer, Thomas, & Gerngroß, 2002). The SpO_2 level is considered safe down to 80% (Glanfield, 1988). In combination with further decrease of saturation to <80% will create

discomfort (Tannheimer, Thomas, & Gerngroß, 2002). The cardiorespiratory system is influenced differently depending on saturation level. When simulating hypxia in a hypobaric chamber, the cardiorespiratory phase synchronization (HR and respiratory cycles) showed no significant difference between on 90 ± 3% SpO_2 from baseline. However, with further decreased saturation (≈84%), showed a significant influence on the HR and ventilatory drive (Zhang et. al. 2014). Furthermore, normobaric hypoxia combined with resistance exercise have shown contradictory results on the HR. Katayama et. al. (2010) showed significant increase on the HR compared to normoxia on untrained individuals. However, experienced resistance trained participants show no significant increase for HR during HRE (Scott et. al. 2016; Gnimassou et. al. 2018). It is important to emphasize that Scott et. al. (2016) used complex, multi-joint movements and achieved HRs of >170. Furthermore, Gnimassou had a two-groups design which is not ultimate for HR comparison between hypoxia and normoxia. During the experimental trials in the present study, the HR showed to be significant between conditions but with no interaction. This indicates a possible effect of HRE on the HR, although HR values was slightly higher from baseline. It may be due to participants during hypoxia were stressed while putting on the mask from the oxygen extraction apparatus.

This implies that hypoxia may influece the HR response more than normoxia. However, depending on exercise experience, may decrease the cardiac respiratory load compared to untrained individuals. A study who compared trained with untrained individuals has used acute hypoxia during maximal aerobic exercise and showed that untrained individuals do have higher max HR than trained participants (Woorons et. al. 2005). This show tendencies of how applicible HRE is on different groups where untrained may be more affected than trained individuals. However, type of training could be a depending factor for how the HR is influenced. Furthermore, when individuals is exercising closer to maximum to max HR It is difficult to distingush differences between groups as in the study published by Scott et. al. (2016). Accordingly, if the workload is matched to submaximal HR, potential differences may more easily be interpreted. In the present study, the participants were recreational active males which were included via comfort selection method which limits the generalizability for HRE. However, a bigger sample size and more homogeneous group where muscle size and fiber type is matched, is needed to ensure interaction effect of HRE on HR and other metabolic factors. This will, in turn, increase this study´s intern validity.

Glucose

Blood glucose is not commonly reported in HRE studies. When inducing passive exposure of hypoxia, the glucose Concentration was lower in hypoxia than normoxia after a light meal (D´Hulst et. al. 2013). However, when using 30 min ergometer cycle no difference was shown in substrate metabolism between hypoxia and normoxia. The metabolism of substrates seems to depend more on exercise intensity (Wadley et. al. 2005). The influence from hypoxia on blood glucose levels is not possible to conclude from previous research and the present study. The changes of glucose levels were not significant between the conditions and no interaction was observed. In line of thought, when assessing potential effects from HRE on glucose, may potentiate that an increased adrenaline excretion from hypoxia can influence the glucose levels. However, further investigation is needed.

Blood- and Plasma Lactate

The study's results are uncertain when no significant differences were observed for the plasma- and blood lactate. It seems that hypoxia may have tendencies to create a greater lactate accumulation than normoxia. The relationships between plasma- and blood lactate showed a strong correlation coefficient for both conditions. However, hypoxias relationship resulted in stronger values than during normoxia (r=0.98 compared to r=0.87). This indicates reliable techniques for measuring blood lactate values. Although, due to the non-significant statistics, it's not possible to establish a conclusion concerning the effects of hypoxia on blood lactate through resistance exercise.

Previous research has shown increases of the blood lactate during hypoxia. As mentioned previously, these studies retrieved their result based on complex, multi-joint movements (Kon et. al. 2010; Scott et. al. 2016). For lactate production, larger muscle mass involved elicits greater metabolic stress during resistance exercise which influences lactate production (Reis et. al. 2011). Thereby, the observed lactate levels during this experiment might depend on the participants muscle Cross Sectional Area (CSA) and fibertype ratio that is active during exercise. E.g. muscles with larger amount of type II fibres is more glycolytic demaning than type I fibers, and type II fibers has a slower clearence rate for produced hydogen ions (H+), which is an byproduct from glycolysis during anaerobic work (Pette & Spamer, 1986; Bogdanis, 2012). Furthermore, with increased intensity (not only increased weight lifted) increases the excretion of H+ and causes fatigue (Sahlin, 1986). Thereby, the intensity, volume and SpO_2 level during HRE might as well be influencing factors for lactate

production as metion before. During high load exercise (80% of 1RM) on the first set uses phosphocreatine kinase as the main enegry process which has a low impact on the lactate produced. However, lactate production increases with higher ATP-demand which initiates increased glycolysis and accordingly increased lactate concentrations (MacDougall et. al. 1999).

In contrast, studies with isolated unilateral leg extension have not examined the influence on lactate during hypoxia. Although, occlusion have established positive results that muscles deprived from blood and oxygen increases metabolite accumulation and potentiate increased skeletal muscle hypertrophy (Loenneke, Wilson, & Wilson, 2010; Fry et. al. 2010; Gentil, Oliveira, & Bottaro, 2006; Suga et. al. 2010). During occlusion, the muscles metabolite clearance might be inhibited due to reduced blood flow to exercising muscle. This potential factor may explain why occlusion creates greater metabolite accumulation during exercise (Suga et. al. 2010; Fry et. al. 2010). Furthermore, metabolite accumulation has been proposed to be more important than high force for muscle growth during resistance exercise (Schoenfeld, 2013). The metabolic response seems to depend strongly on training volume, blood flow availability and TUT during exercise. Therefore, intensity play an important role to induce higher metabolite accumulation. A major limitation for occlusion is that participants not accustomed to occlusion training can experience a hypotensive effect (low blood pressure) post-exercise and pain (Araújo et. al. 2014; Wernblom, Augustsson, & Thomeé, 2006). Therefore, HRE can more safely induce muscular hypoxia on central musculature (compared to occlusion). Which can be a better alternative for long term muscle growth.

Muscle Lactate and pH

The muscle lactate and pH value showed no significant difference between hypoxia and normoxia pre- to post 90 min exercise. However, the muscle pH showed a tendency for interaction effect ($P< 0.08$) where hypoxia had slightly lower pH value. Thereby, when conducting a pairwise T-test on post-exercise pH a significant difference was observed ($P< 0.01$). However, pH and muscles lactate has been previously reported to correlate with each other (Sahlin et. al. 1976). Likewise, a correlation was observed during this experiment between pH and lactate. Nonetheless, only a minor correlation difference between hypoxia and normoxia was observed (r= -0,811 vs. r= -0,737, $P< 0.01$ respectively).

The plasma lactate ratio to muscle lactate in normoxia increased from 45% to 90% (Baseline to post exercise). In comparison, during hypoxia the plasma lactate only increased from 56% to 81.9% out of the muscle lactate which is 8.1% less than during normoxia. These values create uncertainties for potential effects from hypoxia when the baseline lactate has great differences in the lactate ratio (56% vs. 45%). However, the peak values during hypoxia is moderately higher than normoxia (38.8 ± 16.5 vs. 35.1 ± 19.1 mmol/kg dry muscle), which could be due to participant differences, measurements accuracy or that there is no effect of hypoxia. In contrast, the relationship between plasma and muscle levels of lactate was stronger during hypoxia (r=0.86) than with normoxia (r=0.59), which supports the notion that lactate clearance from muscle to blood was increased during hypoxia.

6. Conclusion

Summarized data indicates that no significant difference between hypoxia and normoxia was evident for the metabolic response during intermittent resistance exercise. However, there were tendencies for a greater intramuscular metabolic response with hypoxic resistance exercise, but the data should be regarded as inconclusive. Furthermore, if HRE truly induces a greater metabolic stress than NRE, it is most likely minor. In regard to the acquired results, the resistance exercise protocol (intensity and volume), participants exercise experience, SpO_2 level and CSA should be factors to take in consideration for further research of HRE. These factors may influence the skeletal muscles metabolic stress during exercise. Therefore, how hypoxia influence the human skeletal muscles metabolic response is unclear. Nonetheless, to falsify the null hypothesis and establish a conclusion, more data is needed for HRE.

7. Acknowledgment

This Master thesis was conducted alongside Marcus Moberg's ongoing project: "Resistance exercise under hypoxia and the acute molecular effects in human skeletal muscle". Thanks to Marcus Moberg as main scientist and supervisor in this thesis study, it was possible to write with theoretical support and use reliable measuring techniques. The data was measured and analyzed at the Åstrands Laboratory of Work Physiology in GIHs facilities (The Swedish School of Sport and Health Sciences).

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