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Research Article

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Effects on Oxygen Consumption and Metabolic Gene Expression when Determining Experimental Exercise Intensity Based on Exercise Capacity Tests Conducted in Hypoxic and Normoxic Environments

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Abstract

Exercise intensity can be set relative to VO_2 max measured during hypoxic or control conditions in studies investigating exercise in hypoxic environments. It currently is not clear which is the most appropriate method.

Objective

The objective of this brief report is to determine the response to 1 hour of cycling at 60% of peak power when measured in either normoxic or hypoxic conditions.

Methods

Eleven recreationally active male participants (24 ± 4 yrs, 173 ± 20 cm, 82 ± 12 kg, 15.2 ± 7.1% fat, 4.0 ± 0.6 L x min^{-1} VO_2 max) completed two 1 hour cycling exercise trials at 60% of peak power followed by 4 hours of recovery in ambient environmental conditions (975 m) and at normobaric hypoxic conditions simulating 3000 m in a randomized counter balanced order.

Results

VO_2 max was not different between trials in relative (p=0.272) or absolute terms (p=0.105) but peak power at VO_2 max was higher in the 975 m trial (288 ± 17 watts) than the 3000 m trial (262 ± 12 watts, p=0.003) corresponding to differences at 60% of VO_2 max power. Gene expression of HIF-1α, COX, PGC-1α, HK, and PFK increased with exercise (p<0.05) but did not differ between trials. There was a trend (p=0.072) toward increased muscle glycogen use in 975m.

Conclusions

Although there were not statistical differences for muscle markers in the current study, these data should be considered when determining exercise intensity in hypoxia related research.

Keywords

Altitude; VO2 max; HIF-1α; PGC-1α; Glycolytic Enzymes; mRNA

Introduction

Exercise intensities utilized in experimental designs are usually determined relative to maximal aerobic capacity (i.e. 65% VO_2 max). This becomes a challenge when studying the effects of hypoxia and altitude versus normoxic control conditions since acute hypoxia decreases VO_2 max when compared to normoxic conditions [1-8]. Endurance trained individuals with an average sea level VO_2 max of 65.5 ml x kg^{-1} min^{-1} would be projected to have an average VO_2 max at 2500 m of 57.7 ml x kg^{-1} min^{-1}, or a 7.8% decrease [9]. When exercise intensity is set at 65% of normoxic or hypoxic VO_2 max, the absolute exercise intensities will differ in that the hypoxic intensity will be at a lower absolute intensity (42.58 ml × kg^{-1} × min^{-1} during normoxic conditions and 37.51 ml × kg^{-1} x min^{-1} during hypoxic conditions). Thus, difference in absolute intensity may be a contributing factor (other than hypoxia) to studies incorporating this method of selecting experimental exercise intensity. Larger differences will occur if testing is done at increased levels of hypoxia or higher altitudes.

The rate of oxygen consumption and thus exercise intensity has been related to increased oxidative stress [10,11] and increased transcription of metabolic gene expression [12]. We have recently reported that blood oxidative stress and skeletal muscle gene expression is blunted during recovery from exercise at a simulated altitude of 5000 m as compared to 975 m [13]. No differences in blood oxidative stress markers are noted when participants exercise in a normoxic environment (975m) relative to either absolute or relative exercise intensity and only when hypoxia was introduced (3000 m) during the exercise trial was increased oxidative stress noted [14]. Thus, in terms of oxidative stress it appears that a hypoxic challenge greater than that created by scaling exercise intensity in absolute terms or relative terms is necessary. However, it is currently unknown if the small differences in oxygen consumption created by absolute versus relative exercise intensity scaling impact the expression of metabolic genes in the skeletal muscle.

The purpose of this study is to compare differences in absolute exercise intensity and gene expression of select metabolic genes when exercise intensities are determined by maximal exercise capacity tests performed in hypoxic vs. normoxic environmental conditions. This data may aid researchers in hypoxia related research to determine the most appropriate method to determine exercise intensities and the impact of small differences in exercise intensity.

Methods

Participants

Eleven Caucasian male participants were recruited to participate in this study (see results for descriptive data). All participants were briefed on the experimental protocol and possible risks prior to giving written informed consent. All procedures were approved by the University of Montana Institutional Review Board.

Preliminary testing

Body composition was measured using hydrodensitometry. Underwater mass was measured with a digital scale (Exertech, Dreshbach, MN). Body Density was corrected for estimated residual

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Table 1: Primers and Probes used for real time RT-PCR.

Gene	Primer 1	Primer 2	Probe
B-actin	CCTTGCACATGCCGGAG	ACAGAGCCTCGCCTTTG	TCATCCATGGTGAGCTGGCGG
Cyclophillin	CCGAGGAAAACCGTGTACTATTAG	TGCTGTCTTTGGGACCTTG	CGCGTCTCCTTTGAGCTGTTTGC
RPS-18	GAGGATGAGGTGGAACGTGT	TCTTCAGTCGCTCCAGGTCT	TGGAAAATACAGCCAGGTCC
GAPDH	AGGGCCCTGACAACTCTTTT	AGGGGTCTACATGGCAACTG	CACCACACTGAATCTCCCCT
HIF-1α	GAAAGCGCAAGTCCTCAAAG	TGGGTAGGAGATGGAGATGC	TGCTAATGCCACCACTACCA
PGC-1α	AGCTGCTGAAGAGGCAAGAG	TTCCCCTAAACCAAGCACAC	GTCAGCTTTGCTTTTCCTGG
COX	GCCATGTTCTTCATCGGTTT	GGCCAGCATCTCTCACTTCT	AAGCACTATGTGTACGGCCC
HK	TAGGGCTTGAGAGCACCTGT	CCACACCCACTGTCACTTTG	TAAGGAGGTGTGCACTGTGG
PFK	CCCGTGTCTTCTTTGTCCAT	GTTGTAGGCAGCTCGGAGTC	AGAGCGTTTCGATGATGCTT

lung volume and converted to percent body fat using the Siri equation. Two graded maximal exercise tests (starting at 95 W, and increasing 35 W every 3 minutes) were completed (minimum 2 days between tests) on an electronically braked cycle ergometer (Velotron, RacerMate Inc., Seattle, WA) to determine maximal aerobic capacity (VO_2max) and the power output associated with VO_2max (Wmax) at 975 m and 3000 m. Expired gases were collected and analyzed during the test, using a calibrated metabolic cart (ParvoMedics, Inc., Salt Lake City, UT). VO_2max was assigned to the highest achieved oxygen uptake recorded during the test. Wmax was calculated by adding the Watts in the last completed stage to the fraction of time spent in the uncompleted stage multiplied by the 35 watt stage increment.

Experimental protocol

Participants completed 2 trials using a randomized, counterbalanced cross-over design over the span of 3 weeks, with a minimum of 7 days between trials. Half the participants were randomly assigned to complete the 975 m trial first while the other half completed the 3000 m trial first. Each participant completed each trial (repeated-measures). All trials were completed in a temperature, humidity, and hypoxia (Colorado Altitude Training, Louisville, CO) controlled environmental chamber (Tescor, Warminster, PA) set at 12°C and 40% relative humidity environmental conditions. These environmental conditions were selected based on a typical temperature that may be encountered at altitude. Participants kept an exercise record for 2 days before and a dietary record for 24 h before the initial trial and replicated exercise and diet for these periods before the remaining trial. Additionally, participants abstained from exercise 24 hours before each trial. Following an overnight 12 hour fast, participants arrived at the laboratory in the early morning to complete testing. Upon arrival to the laboratory participants cycled for 1 hour at 60% of workload corresponding to peak VO_2 as measured at 975 m or at 60% of workload corresponding to peak VO_2 as measured at 3000 m and then recovered in the lab for 4 hours. Participants remained in a sitting position throughout the 4 hour recovery period.

Biopsies

Muscle biopsies were taken before exercise and at the end of the 4 hour recovery period for each trial. Biopsies were taken from the vastus lateralis muscle using a 4-5 mm Bergstrom percutaneous muscle biopsy needle with the aid of suction. All subsequent biopsies during a given trial were obtained from the same leg using a separate incision 2 cm proximal to the previous biopsy. After excess blood, connective tissue, and fat were removed, a tissue aliquot was stored in RNA later and an additional aliquot was flash frozen in liquid nitrogen and stored at -80°C for later analysis.

Oxygen utilization

Expired gases were collected for 5 min, with the last 2 min averaged to represent the sample period during exercise at 0, 27.5, and 53 min of exercise. These three sampling periods were then averaged to represent the average VO_2 associated with the exercise bout.

Gene expression

An 8-20 mg piece of skeletal muscle was homogenized in 800 µl of Trizol (Invitrogen, Carlsbad CA, Cat# 15596-018) using an electric homogenizer (Tissue Tearor, Biosped Products Inc, Bartlesville OK). RNA from samples were extracted and further purified using the RNeasy mini kit (Qiagen, Valencia CA, Cat#74104) according to the manufacturer's protocol using the additional DNase digestion step (RNase-free DNase set, Qiagen, Valencia CA). RNA was quantified using a nano-spectrophotometer (nano-drop ND-1000, Wilmington DE). First-strand cDNA synthesis was achieved using Superscript-first-strand synthesis system for RT-PCR kit (Invitrogen, Carlsbad CA) according to the manufacturer's protocol. Each sample within a given subject was adjusted to contain the same amount of RNA. The resulting cDNA was then diluted 2x using RNase free water in order to have ample volume for RT-PCR and frozen for later analysis. For Real time RT-PCR, each 25 µl reaction volume contained 500 nM primers, 250 nM probe (PimeTime qPCR assay, Integrated DNA technologies), 1x FastStart TaqMan Probe master (Roche Applied Science, Indianapolis IN), and 2.5 µl of sample cDNA. Probes and primers were obtained and designed by Integrated DNA technologies (Coralville, Iowa). PCR was then run using the Bio-Rad I Cycler iQ5 Real-Time PCR Detection system (Bio-Rad, Hercules CA) using a 2-step Roche protocol. Quantification of mRNA for genes of interest was calculated on pre and 4 hr post muscle samples using the 2-ΔΔCT method and stability of the house keeping genes was determined using the 2-ΔCT method, both as previously described [15]. Four housekeeping genes were analyzed (β-actin, cyclophillin, RPS18, and GAPDH) and the most stable gene (cyclophillin) between trials ($p=0.276$) and with exercise ($p=0.366$) was used to normalize genes of interest. Our genes of interest for this investigation were: hypoxia-inducible factor 1 alpha (HIF-1α), Peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α), cytochrome c oxidase subunit 4 (COX), , hexokinase (HK), and phosphofuctokinase (PFK). See table 1 for primer and probe sequences used for real time RT-PCR.

Muscle Glycogen

Muscle glycogen was analyzed using an enzymatic spectrophotometric method. Samples were weighed upon removal from a -80°C freezer and placed in 0.5 ml, 2N HCl solution. The

Table 2: Aerobic capacity when measured at 975 m and 3000 m.

	975 m	3000 m	p-value
VO_2 max (L × min^{-1})	4.24 ± 0.28	4.00 ± 0.19	0.015
VO_2 max (ml × kg^{-1}×min^{-1})	51.4 ± 3.31	49.5 ± 8.8	0.272
VO_2 max power (watts)	288 ± 17	262 ± 12*	0.003
60% VO_2 max power (watts)	173 ± 10	157 ± 7*	0.003
VO_2 at 60% max power (L× min^{-1})	2.51 ± 0.13	2.38 ± 0.13*	0.044

*p < 0.05 from 975 m
Data are means ± SE.

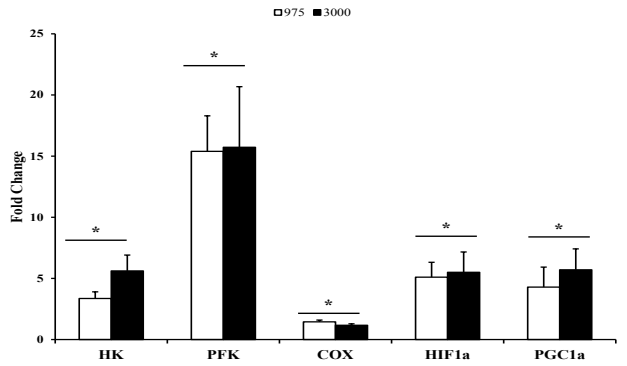

Figure 1: Post exercise gene expression for HK, PFK,COX, HIF-1α, and PGC-1α. * p< 0.05 from pre-exercise (main effect of exercise). No statistical differences were observed between trials (HK, p = 0.09; PFK, p = 0.948; COX, p = 0.191; HIF-1α, p = 0.616; and PGC-1α, p = 0.552). Data are means ± SE.

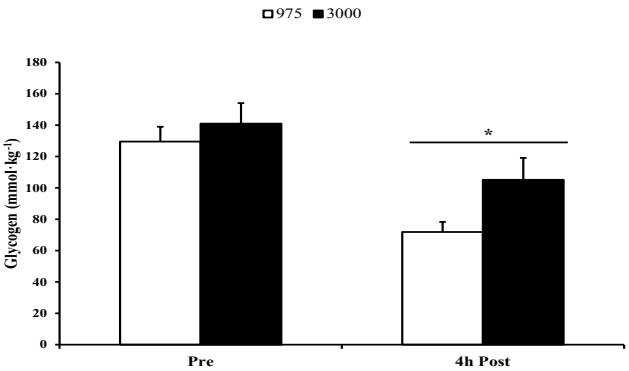

Figure 2: Muscle glycogen Pre exercise and after 4 hours of recovery. *p< 0.05 from pre-exercise (main effect of exercise). Data are means ± SE..

sample solutions were weighed, incubated for two hours at 100°C in an oven, then re-weighed and re-constituted to their original weight using distilled water. To normalize pH, 1.5 ml of 0.67 NaOH was added. An aliquot of this muscle extract (100 µl) was added to 1 ml of Infinity glucose (HK) liquid stable reagent (Thermo Fisher Scientific, Waltham, MA) and the absorbance read on a spectrophotometer at 340 nm. Glycogen concentration was calculated using the extinction co-efficient of NADH. Muscle glycogen concentrations are expressed in mmol. kg^{-1} wet weight of muscle tissue.

Statistics

Glycogen and mRNA were analyzed using a repeated measure ANOVA (trial x time). In the event of a significant F-ratio, Fishers protected least significant difference analysis was applied to determine where differences occurred. VO_2 max and power at VO_2 max were analyzed using a paired, two-tailed t-test. A probability of type I error less than 5% was considered significant ($p<0.05$). All data is reported as means ± SE.

Results

Eleven male participants (24 ± 4 yrs, 173 ± 20 cm, 82 ± 12 kg, 15.2 ± 7.1% fat) completed the study. VO_2 max was not statistically different between trials in absolute terms (p=0.105) or relative to body weight (p=0.272; Table 1). Power output at VO_2 max was higher when tested at 975 m compared to when tested at 3000 m (p=0.003) which also corresponded to a higher power output and VO_2 at 60% of power output (p=0.003 and p=0.044, respectively; Table 2).

Gene expression (mRNA) was increased as a result of exercise ($p<0.05$) but was not different between trials ($p>0.05$; Figure 1) for all our genes of interest (HK, p=0.09; PFK, p=0.948; COX, p=0.191; HIF-1α, p=0.616; and PGC-1α, p=0.552). Muscle glycogen decreased with exercise regardless of trial ($p<0.01$; main effect of exercise) and was lower in the trial that VO_2 max was measured at 975 m (p=0.04; main effect of trial). A trend in the interaction between exercise and trial was identified (p=0.072) which would indicate a trend toward increased glycogen utilization in the trial that VO_2 max was measured at 975 m (higher absolute intensity). Muscle glycogen data is presented in Figure 2.

Discussion

The purpose of this brief report was to determine the impact of standardization of absolute versus relative exercise intensities at different levels of oxygen availability on metabolic related genes and muscle glycogen. This research question revolves around determining exercise intensities for hypoxic interventions relative to a VO_2 max test conducted in normoxic or hypoxic conditions. In the current study we did not observe a statistically significant decline in VO_2 max when conducted in ambient conditions (975 m) compared to hypoxic conditions (simulated 3000 m). In the current study with 11 participants with an average difference in VO_2 max of 0.24 L x min^{-1} between tests conducted at 975 m and 3000 m the statistical power to detect this difference is only 0.374. In order detect the observed difference in VO_2 max a sample of 26 participants would be necessary (statistical power of 0.80). Despite a non-statistically significant difference in VO_2 max, we did observe a significant difference in peak power during the VO_2 max tests and the corresponding VO_2 and power at 60% of peak power that was used in this study. These results lead to an absolute exercise intensity of 173 ± 10 watts in the trial relative to VO_2 max at 975 m and 157 ± 7 watts in the trial relative to VO_2 max at 3000 m, a 9.2% difference. This difference in absolute intensity is on the order of that reported in previous literature for changes in aerobic capacity [9].

The two trials were conducted in the same environment (975 m) but at different absolute intensities. As expected, exercise did lead to an increase in the expression of our metabolic genes of interest. However, the current data indicate that the observed 16 watt difference in absolute workload was not large enough to detect differences in expression of our target genes for glycolysis (HK and PFK), oxygen sensing (HIF-1α), or aerobic metabolism (COX and PGC-1α) under normoxic conditions. This finding is an agreement with our previous

data reporting no difference in gene expression associated with oxidative stress with the same intensity difference [14]. Collectively, these data suggest that small differences in exercise intensity do not have a major impact on the transcriptional response of skeletal muscle. Further investigation is needed in order to determine if small differences in exercise intensity on a chronic basis (training) lead to differential effects on gene transcription, translation, and physical function.

Muscle glycogen use was not statistically different between the trials. However, there was a trend toward increased glycogen use in the trial that intensity was set relative to VO_2 max conducted at 975 m. Again, the power to detect the differences in glycogen use with a 16 watt difference in intensity and a 0.31 $L \bullet min^{-1}$ difference in VO_2 was likely not sufficient to statistically detect the expected differences in glycogen use.

We caution the interpretation that the initial max testing environment does not affect outcomes in hypoxic intervention studies. Differences in the degree of hypoxia surely would impact the magnitude of workload difference. The impact of subtle differences in workload in a hypoxic environment cannot be discerned from the current study design. However, this study may provide insight into what is the most appropriate procedure to determine exercise intensity when conducting hypoxia related research in order to standardize physiological response. Although many aspects of this investigation were not statistically significant, this data should be considered in study design. It is suggested that researchers select the same absolute workload between interventions as opposed to incorporating exercise intensities relative to VO_2 max as measured in each experimental environment and thus yield differences in absolute intensity. The most appropriate environment to measure VO_2 max and base trial exercise intensities may differ with study design. The most important aspect may be that there is equality in workload between trials to ensure observed differences are due to the intervention and not absolute or relative exercise intensity. Indeed, when clamping absolute workload relative workload will be different in different levels of hypoxia, but should be considered an outcome of hypoxia and not a variable to control. Furthermore, the measurement of VO_2 max in ambient environments may be the most appropriate standardization when using measures of VO_2 max to describe the aerobic fitness of participants as normative values are readily available. When determining exercise intensity during study designs dealing with hypoxia researchers should consider these aspects to maximize control of the appropriate variables.

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