BRIEF REPORT

Human Skeletal Muscle mRNA Response to a Single Hypoxic Exercise Bout

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Background.—The ability to physically perform at high altitude may require unique strategies to acclimatize before exposure. The effect of acute hypoxic exposure on the metabolic response of the skeletal muscle may provide insight into the value of short-term preacclimatization strategies.

Objective.—To determine the human skeletal muscle response to a single acute bout of exercise in a hypoxic environment on metabolic gene expression.

Methods.—Eleven recreationally active male participants (24 ± 4 years, 173 ± 20 cm, 82 ± 12 kg, $15.2 \pm 7.1\%$ fat, 4.0 ± 0.6 L/min maximal oxygen consumption) 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 counterbalanced order. Muscle biopsies were obtained from the vastus lateralis before exercise and 4 hours after exercise for real-time polymerase chain reaction analysis of select metabolic genes.

Results.—Gene expression of hypoxia-inducible factor 1 alpha, cytochrome c oxidase subunit 4, peroxisome proliferator-activated receptor gamma coactivator 1 alpha, hexokinase, phosphofructokinase, mitochondrial fission 1, and mitofusin-2 increased with exercise ($P < .05$) but did not differ with hypoxic exposure ($P > .05$). Optic atrophy 1 did not increase with exercise or differ between environmental conditions ($P > .05$).

Conclusions.—The improvements in mitochondrial function reported with intermittent hypoxic training may not be explained by a single acute hypoxic exposure, and thus it appears that a longer period of preacclimatization than a single exposure may be required.

Key words: HIF-1 α , PGC-1 α , glycolytic enzymes, mitochondria, mRNA, altitude exposure

Introduction

The ability to withstand high levels of altitude exposure after living at a low altitude without illness is highly desirable for recreational mountaineers, military soldiers, and others traveling to altitude. As such, interest in techniques to acclimatize before altitude exposure has gained interest. When short-term hypoxic exposure is incorporated into a training paradigm, mitochondrial density, maximum aerobic capacity, citrate synthase activity, and anaerobic performance are enhanced compared with normoxic control exercise.^{1–5} The time course and exact mechanism of metabolic adaptation to

the skeletal muscle with short-term altitude exposure is yet to be completely understood.

Two independent cellular pathways appear to play a major role in the cellular response to hypoxia. Hypoxia-inducible factor 1 alpha (HIF-1 α) is a transcription factor that stabilizes in the nucleus on exposure to hypoxic conditions and in turn induces the expression of hypoxia-induced genes, including glucose transporters and glycolytic enzymes.⁶ The activation of peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1 α) can be induced by hypoxia⁷ and stimulates mitochondrial biogenesis, increased fatty acid oxidation, and exercise performance.⁸ Although previous research has focused on the effect of repeated hypoxic exposure on these cellular pathways, limited data are available on the acute skeletal muscle metabolic response to a single bout

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of exercise in hypoxic conditions. The purpose of this project was to determine the acute impact of a lowland population exercising at 975 m (ambient) and at 3000 m (normobaric hypoxia) on select genes involved in the HIF-1 α and PGC-1 α pathways.

Methods

PARTICIPANTS

Eleven male participants (24 ± 4 years, 173 ± 20 cm, 82 ± 12 kg, $15.2 \pm 7.1\%$ fat, 4.0 ± 0.6 L/min [maximal oxygen consumption at 3000 m]) completed the study. All participants were briefed on the experimental protocol and possible risks before 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 lung volume and converted to percent body fat using the Siri equation. Graded maximal exercise tests (starting at 95 W, and increasing 35 W every 3 minutes) were completed on an electronically braked cycle ergometer (Velotron, RacerMate Inc, Seattle, WA) to determine maximal aerobic capacity (maximal oxygen consumption [$\dot{V}O_{2\max}$] and the power output associated with $\dot{V}O_{2\max}$ ($W_{\max}$) at a simulated altitude of 3000 m. Expired gases were continuously collected and averaged in 15-second intervals during the test, using a calibrated metabolic cart (ParvoMedics, Inc, Salt Lake City, UT). $\dot{V}O_{2\max}$ was assigned to the highest achieved oxygen uptake recorded during the test. $W_{\max}$ was calculated by adding the power output in the last completed stage to the fraction of time spent in the uncompleted stage multiplied by 35.

EXPERIMENTAL PROTOCOL

Participants completed 2 trials using a randomized cross-over design over the span of a maximum 3 weeks, with a minimum of 7 days between trials. All trials were completed in a temperature-, humidity-, and oxygen- (Colorado Altitude Training, Louisville, CO) controlled environmental chamber (Tescor, Warminster, PA) at 12°C and 40% relative humidity. This temperature was chosen to more closely simulate temperatures encountered at altitude. Participants kept an exercise record for 2 days before and a dietary record for 24 hours before the initial trial and replicated exercise and diet for these periods before the remaining trials. Additionally, participants abstained from exercise 24 hours before each trial.

After an overnight 12-hour fast, participants arrived at the laboratory in the early morning to complete testing. The trials consisted of cycling for 1 hour at 60% of hypoxic peak power (157 ± 7 W) at ambient altitude (975 m) and then recovering for 4 hours at 975 m, or cycling for 1 hour at 60% of hypoxic peak power (157 ± 7 W) at a simulated altitude of 3000 m and then recovering for 4 hours at 3000 m. Thus, absolute exercise intensity was held constant between trials. Recovery took place in the same environment as exercise occurred to allow time for peak gene expression and to simulate applied circumstances in which immediate return from altitude may not be possible. During the recovery period, participants changed out of their cycling clothes, towed off, and wore standardized clothing for recovery. Participants remained in a sitting position throughout the 4-hour recovery period. Participants consumed 8 mL/kg of water during the ride and 8 mL/kg of water during recovery.

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 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, tissue samples were stored in RNA Later (Qiagen, Valencia, CA) and stored at -80°C for later analysis.

PULSE OXIMETRY

Blood oxygen saturation was measured using a finger pulse oximeter (Nonin Onyx II 9550, Plymouth, MN) during exercise (average of measures taken at 4, 31.5, and 57 minutes) and at 2 and 4 hours after exercise during the passive recovery.

GENE EXPRESSION

An 8- to 20-mg piece of skeletal muscle was homogenized in 800 μL of Trizol (Invitrogen, Carlsbad, CA, catalog number 15596-018) using an electric homogenizer (Tissue Tearor, Biosped Products Inc, Bartlesville, OK). Samples were then incubated at room temperature for 5 minutes, after which 200 μL of chloroform per 1000 μL of Trizol was added and shaken vigorously by hand. After an additional incubation at room temperature for 2 to 3 minutes, the samples were centrifuged at 12,000 g for 15 minutes, and the aqueous phase was transferred to a fresh tube. Messenger RNA was

precipitated by adding 400 μ L of isopropyl alcohol and incubated overnight at -20°C . The next morning samples were further purified using the RNeasy mini kit (Qiagen, catalog number 74104) according to the manufacturer's protocol using the additional DNase digestion step (RNase-free DNase set, Qiagen). RNA was quantified using a nanospectrophotometer (ND-1000, NanoDrop, Wilmington, DE). First-strand cDNA synthesis was achieved using Superscript-first-strand synthesis system for reverse transcription polymerase chain reaction (RT-PCR) kit (Invitrogen) 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 $2\times$ using RNase free water to have ample volume for RT-PCR and frozen for later analysis. For real-time RT-PCR, each 25- μ L reaction volume contained 500 nM primers, 250 nM probe (PrimeTime qPCR assay, Integrated DNA Technologies, Coralville, IA), $1\times$ FastStart TaqMan Probe Master (Roche Applied Science, Indianapolis, IN), and 2.5 μ L of sample cDNA. Probes and primers were obtained and designed by Integrated DNA Technologies. PCR was then run using the Bio-Rad iCycler iQ5 Real-Time PCR Detection system (Bio-Rad, Hercules, CA) using a 2-step Roche protocol (1 cycle at 50°C for 10 minutes, 1 cycle at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds followed by 60°C for 1 minute). Quantification of mRNA for genes of interest was calculated on muscle samples obtained before and 3-hour after the cycling trials using the $2^{-\Delta\Delta\text{CT}}$ method, and stability of the housekeeping genes was determined using the $2^{-\Delta\text{CT}}$ method, both as previously described.⁹ Four housekeeping genes were analyzed (β -actin, cyclophilin, RPS18, and glyceraldehyde 3-phosphate dehydrogenase [GAPDH]), and the most stable gene (cyclophilin) between trials ($P = .276$) and with exercise ($P = .366$) was used to normalize genes of interest. Our genes of interest for this investigation were hypoxia-inducible factor 1 alpha (HIF-1 α), peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1 α), cytochrome c oxidase subunit 4 (COX), mitochondrial fission 1 (FIS-1), optic atrophy 1 (OPA-1), mitofusin-2 (MFN-2), hexokinase (HK), and phosphofructokinase (PFK).

STATISTICS

Blood O_2 saturation and mRNA were analyzed using a repeated-measure analysis of variance (trial $\times$ time). In the event of a significant F ratio, Fisher's protected least significant difference analysis was applied to determine where differences occurred. A probability of type I error less than 5% was considered significant ($P < .05$). All data are reported as means $\pm$ SEM.

Table. Pulse oximetry (% blood O_2 saturation)

Trial	Exercise	2 hours after	4 hours after
975 m	97.2% $\pm$ 0.3% ^b	98.4% $\pm$ 0.2%	98.3% $\pm$ 0.1%
3000 m	90.2% $\pm$ 0.6% ^{a,b}	96.3% $\pm$ 0.7% ^a	95.7% $\pm$ 0.6% ^a

^a $P < .05$ from 975 m.

^b $P < .05$ from 2 and 4 hours after. Data are means $\pm$ SEM.

Results

PULSE OXIMETRY

Blood O_2 saturation was lower in the 3000-m trial during exercise and at 2 and 4 hours after exercise than in the 975-m trial ($P < .05$). Additionally, the percent oxygen saturation was lower during exercise than at 2 and 4 hours after exercise in each trial ($P < .05$, Table).

GENE EXPRESSION

Of our 4 candidate housekeeping genes, cyclophilin was the most stable (did not change) with exercise ($P = .366$) and among the 3 trials ($P = .276$), ensuring that the relative quantification of the genes of interest to cyclophilin was minimally effected by baseline changes in cyclophilin. Therefore, all data are presented relative to cyclophilin. HK, PFK, COX, FIS-1, MFN-2, HIF-1 α , and PGC-1 α all increased as a result of exercise ($P < .05$) but were not different among trials ($P > .05$; Figure). OPA-1 did not increase with exercise ($P = .923$) and was not different among trials ($P = .068$; Figure).

Discussion

This investigation aimed to address the effect of acute exercise under hypoxic conditions on metabolic gene

Figure. Postexercise gene expression for cytochrome c oxidase subunit 4 (COX), hypoxia-inducible factor 1 alpha (HIF-1 α), peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1 α), mitochondrial fission 1 (FIS-1), mitofusin-2 (MFN-2), optic atrophy 1 (OPA-1), hexokinase (HK), and phosphofructokinase (PFK). * $P < .05$ from preexercise (main effect of exercise). No effect of trial ($P > .05$). Data are mean $\pm$ SEM.

expression. The current data demonstrate no acute effect of hypoxia (simulated altitude of 3000 m) on select metabolic gene expression compared with normoxia. Oxygen saturation in the 3000-m trial during exercise approached 90% ($90.2\% \pm 0.6\%$), which clinically indicates hypoxemia, whereas exercise oxygen saturation during the 975-m trial was higher ($97.2\% \pm 0.3\%$; $P < .05$). These data confirm a difference in oxygen saturation between our ambient 975 m and our normobaric hypoxic trials at a simulated 3000 m. We observed an acute exercise-inducing effect on COX, HIF-1 α , PGC-1 α , HK, PFK, FIS-1, and MFN-2 (no exercise effect on OPA-1), with no differences between altitudes of 975 m and 3000 m.

HIF-1 α mRNA regulates many aspects of hypoxia-induced adaptations, and its expression has been shown to increase with training (6 weeks, 5 times per week for 30 minutes at 3850 m) when hypoxia was present during training sessions.¹ This is in contrast to the current results demonstrating that HIF-1 α was not altered by an acute hypoxic exercise bout. The differences in the number of hypoxic exercise bouts over a different time course may explain differences in HIF-1 α expression between these studies. The current research is among the first human work that describes the acute mRNA response after a single hypoxic exercise bout. When the current work is taken together with previous investigations, it suggests that the duration, temporal nature, or intensity of hypoxia exposure could be a critical factor in human HIF-1 α mRNA expression and subsequent adaptations that may prove beneficial to performance at altitude. In light of the role of HIF-1 α in the induction of glycolytic genes,¹⁰ it may not be surprising that without an observed increase in HIF-1 α mRNA in the current study with hypoxic exposure there was no change in the glycolytic genes PFK or HK with hypoxia. Additionally, the current study did not demonstrate any differences in PGC-1 α or other genes related to mitochondrial development (COX, FIS-1, MFN-2, or OPA-1) with hypoxia, but the expected increase owing to exercise was observed. This finding is surprising given the previously observed increases in mitochondrial function with repeated acute bouts of hypoxia during training.¹⁻⁵ Therefore, as with HIF-1 α , the role of PGC-1 α in hypoxia-stimulated cellular adaptations may be related to dosing of hypoxic exposure.

Conclusions

The current research indicates that a single acute hypoxic exercise bout does not affect mRNA expression for select glycolytic (PFK and HK), mitochondrial biogenesis (HIF-1 α , PGC-1 α , and COX), and mitochondrial morphology (MFN-2, OPA-1, and FIS-1) genes.

Thus, the improvements in mitochondrial function with intermittent hypoxic training may not be explained by a single acute hypoxic exposure, or it may be possible that any potential mRNA changes could have required a longer acute exercise session, more-intense hypoxic exposure, longer exercise duration, or other temporal or dose-response issues. More research is needed to determine dynamics of hypoxia exposure to better understand the apparent differential mechanisms and stimuli behind a single acute hypoxic exercise bout and repeated acute hypoxic exercise to determine the most effective (in terms of time and outcome) protocols to implement in a preacclimatization situation.

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