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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 ± 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-1α, PGC-1α, 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-1α) 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-1α) can be induced by hypoxia[7] 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-1α and PGC-1α pathways.

## Methods

### PARTICIPANTS

Eleven male participants (24 ± 4 years, 173 ± 20 cm, 82 ± 12 kg, 15.2 ± 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 [$\dot{V}O_{2max}$]) and the power output associated with $\dot{V}O_{2max}$ ($W_{max}$) 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). $\dot{V}O_{2max}$ 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 crossover 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, toweled 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