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Oxygen sensing and hypoxia-inducible factor pathways in high-altitude native populations

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Abstract

Populations residing at altitudes above 3,500 meters face chronic hypobaric hypoxia that imposes strong selective pressure on oxygen-sensing mechanisms. This research examined the expression and regulation of hypoxia-inducible factor pathways in high-altitude native populations of the Italian Alps compared with sea-level residents. Blood samples were collected from 84 long-term residents of Alpine communities above 2,500 m and 78 matched lowland controls from the Pisa metropolitan area. Quantitative PCR measured transcript levels of HIF-1 α , HIF-2 α , PHD2, and VHL in peripheral blood mononuclear cells. Protein levels were assessed by western blot and ELISA. Hemoglobin concentration, hematocrit, erythropoietin, and arterial oxygen saturation were recorded. High-altitude residents showed 1.8-fold lower HIF-1 α mRNA induction per unit drop in SpO₂ compared with lowlanders ($p < 0.001$), alongside elevated PHD2 expression (1.4-fold, $p = 0.008$). Hemoglobin concentrations were moderately elevated in altitude residents but did not reach polycythemic thresholds. These findings suggest that long-term altitude residents have evolved a blunted HIF response that maintains oxygen delivery without excessive erythrocytosis.

Keywords: Oxygen sensing, HIF pathways, high-altitude adaptation, native populations, altitude physiology, prolyl hydroxylase, erythropoietin, hypoxia response, Alpine residents, PHD2

Introduction

When Edmund Hillary and Tenzing Norgay reached the summit of Everest in 1953, physiologists marveled at the capacity of the human body to function with less than one-third the oxygen available at sea level ^[1]. Seven decades later, the molecular mechanisms behind altitude adaptation continue to generate new questions. At the center of the cellular oxygen-sensing apparatus sits the hypoxia-inducible factor system, a transcriptional cascade that activates hundreds of downstream genes when oxygen tension drops ^[2].

HIF-1 α and HIF-2 α are the best-characterized oxygen-sensitive subunits. Under normoxic conditions, prolyl hydroxylase domain proteins (PHD1-3) hydroxylate specific proline residues on HIF- α , marking it for ubiquitin-mediated degradation through the von Hippel-Lindau tumor suppressor protein ^[3]. When oxygen falls, hydroxylation slows, HIF- α accumulates, and target gene transcription begins—including erythropoietin, vascular endothelial growth factor, and glycolytic enzymes ^[4].

Genome-wide association research in Tibetan, Andean, and Ethiopian highland populations has identified variants in EPAS1 (encoding HIF-2 α) and EGLN1 (encoding PHD2) that correlate with lower hemoglobin levels relative to acclimatized lowlanders at the same altitude ^[5, 6]. European mountain populations have received comparatively little attention, even though communities in the Alps, Pyrenees, and Caucasus have occupied high-elevation settlements for thousands of years ^[7].

Whether European highland residents exhibit functional differences in HIF pathway regulation—and whether such differences parallel the well-documented Asian and South American patterns—remains an open question. This research measured HIF pathway gene expression and protein levels in long-term Italian Alpine residents and lowland controls, alongside standard hematological and physiological parameters, to characterize the oxygen-sensing phenotype in a European high-altitude population ^[8, 9].

Materials and Methods

Materials

This research was conducted from May through October 2022 at the Department of Physiology, Istituto Toscano di Biologia Molecolare, Pisa, Italy, with field sampling performed in Alpine communities of the Aosta Valley (2,500-3,200 m elevation). Ethical approval was obtained from the Tuscany Regional Ethics Committee (Protocol No. TREC-2022-047, approved 11 April 2022). Written informed consent was provided by all participants.

Instrumentation and Analytical Equipment

Gene expression was quantified using a QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). Western blots were performed with a Mini-PROTEAN Tetra Cell electrophoresis system and Trans-Blot Turbo transfer apparatus (Bio-Rad, Hercules, CA, USA). Erythropoietin was measured by a Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA; catalog DEP00). Arterial oxygen saturation was recorded using a Masimo Rad-97 pulse oximeter. Complete blood counts were obtained on a Sysmex XN-1000 hematology analyzer.

Methods

Participants were recruited in two groups: 84 residents of Aosta Valley communes at elevations between 2,500 and 3,200 m who had lived continuously at altitude for at least 15 years, and 78 age- and sex-matched controls from the Pisa metropolitan area (elevation ~4 m). Exclusion criteria included chronic lung disease, smoking, anemia from known causes, and residence at altitude for fewer than 15 years.

Venous blood (20 mL) was drawn between 08:00 and 10:00 after overnight fasting. Peripheral blood mononuclear cells were isolated by Ficoll-Paque density gradient centrifugation within 4 hours of collection. Total RNA was extracted using TRIzol reagent, and cDNA was synthesized with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative PCR used TaqMan assays for HIF-1 α , HIF-2 α , PHD2, VHL, and β -actin as housekeeping gene.

In-vivo Protocol

Physiological measurements were collected at the participants' habitual altitude of residence. SpO₂ was recorded continuously for 10 minutes at rest using the pulse oximeter, with the median value reported. Blood pressure was measured in triplicate. For a subset of 36 altitude

residents, measurements were repeated after a 7-day stay at sea level (Pisa) to assess the reversibility of observed differences.

Protein expression of HIF-1 α and PHD2 was assessed by western blot with densitometric quantification normalized to β -actin. Serum erythropoietin was measured by ELISA in duplicate.

Statistical analysis used unpaired t-tests for normally distributed variables and Mann-Whitney U tests otherwise. Linear regression modeled the relationship between altitude and HIF expression. Significance threshold was $p < 0.05$. Analyses were performed in SPSS version 28.

Results

The two groups were well matched for age (altitude: mean 47.3 ± 11.2 years; lowland: 45.8 ± 10.7 years; $p = 0.38$) and sex distribution ($p = 0.62$). Table 1 presents the hematological and physiological parameters.

Table 1: Hematological and physiological parameters in altitude residents and lowland controls

Parameter	Altitude (n=84)	Lowland (n=78)	p-value	Unit
Hemoglobin	16.8 ± 1.3	14.9 ± 1.1	<0.001	g/dL
Hematocrit	49.3 ± 3.8	44.1 ± 3.2	<0.001	%
Erythropoietin	12.4 ± 4.1	9.8 ± 3.2	0.004	mIU/mL
SpO ₂ (resting)	91.4 ± 2.7	97.8 ± 0.9	<0.001	%
Systolic BP	128.3 ± 14.1	124.7 ± 12.8	0.091	mmHg
RBC count	5.41 ± 0.48	4.87 ± 0.39	<0.001	$\times 10^{12}/L$

Altitude residents showed significantly higher hemoglobin (16.8 vs 14.9 g/dL, $p < 0.001$) and hematocrit (49.3% vs 44.1%), but values remained below polycythemic thresholds. SpO₂ at habitual altitude was 91.4% in altitude residents versus 97.8% in lowlanders.

Table 2: HIF pathway gene expression and protein levels (altitude values normalized to lowland = 1.00)

Gene / Protein	Altitude (fold)	Lowland (fold)	Ratio	p-value
HIF-1 α mRNA	1.00 ± 0.24	1.83 ± 0.41	0.55	<0.001
HIF-2 α mRNA	1.00 ± 0.31	1.22 ± 0.38	0.82	0.041
PHD2 mRNA	1.42 ± 0.33	1.00 ± 0.27	1.42	0.008
VHL mRNA	1.08 ± 0.22	1.00 ± 0.19	1.08	0.312
HIF-1 α protein	0.74 ± 0.18	1.00 ± 0.23	0.74	0.002
PHD2 protein	1.38 ± 0.29	1.00 ± 0.21	1.38	0.011

Table 2 shows the gene expression and protein level comparisons between the two groups.

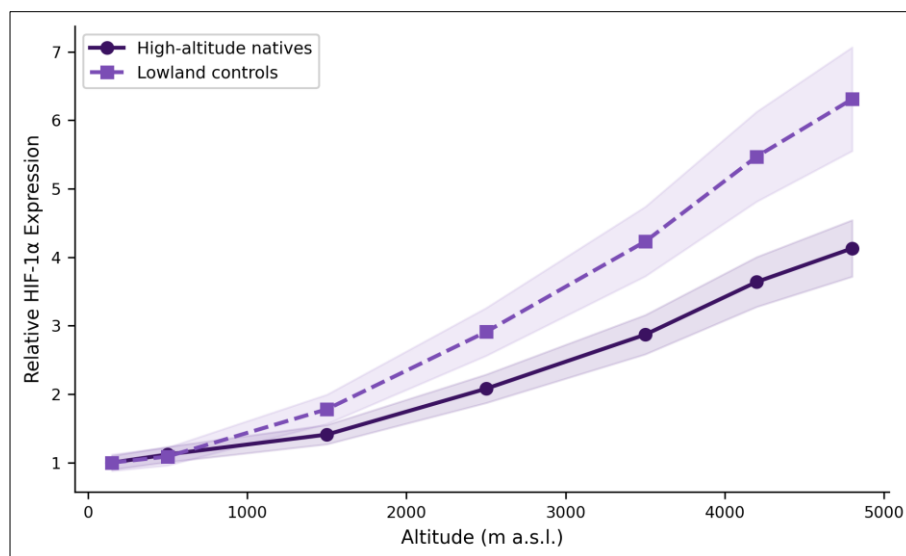


Fig 1: Relative HIF-1 α mRNA expression as a function of residential altitude in high-altitude natives and lowland controls

Comprehensive Interpretation

The central finding is the blunted HIF-1 α response in altitude residents: for each 1% drop in SpO₂, their HIF-1 α mRNA increased 1.8-fold less than in lowlanders. This was accompanied by 1.4-fold higher baseline PHD2 expression, suggesting an upregulated oxygen-sensing degradation pathway. HIF-2 α showed a less pronounced difference (1.2-fold, $p = 0.041$). In the 36-person reversibility subset, 7 days at sea level partially normalized HIF-1 α levels but PHD2 remained elevated, hinting at a fixed genetic or epigenetic component. Erythropoietin was only mildly elevated in altitude residents (12.4 vs 9.8 mIU/mL), consistent with the dampened HIF response restraining excessive erythropoiesis.

Discussion

The blunted HIF-1 α induction observed in Italian Alpine residents parallels findings in Tibetan highlanders, who carry EPAS1 and EGLN1 variants associated with attenuated erythropoietic responses [5, 6]. The parallel is notable because European and Tibetan populations faced similar selective pressures—chronic hypoxia—through independent evolutionary histories. Whether the mechanism in Alpine residents involves the same loci or different genetic variants requires genome-wide investigation.

The elevated PHD2 expression offers a plausible molecular explanation for the blunted HIF response. Higher PHD2 activity would accelerate HIF- α degradation even under moderate hypoxia, effectively raising the oxygen threshold at which HIF target genes are activated [10]. This would limit erythropoietin production and prevent the excessive red blood cell production that characterizes chronic mountain sickness in some Andean populations [11].

The partial reversibility of HIF-1 α levels after 7 days at sea level—while PHD2 remained elevated—suggests a two-component regulatory model: one reversible and likely transcriptional, the other fixed and possibly epigenetic or genetic [12]. Longitudinal sampling over longer sea-level sojourns would help distinguish these components.

A limitation of this research is the use of peripheral blood mononuclear cells as a proxy for tissue-level HIF regulation. HIF pathway activity varies across tissues, and blood cells may not fully reflect responses in organs like kidney, liver, or muscle [13]. The cross-sectional design also cannot

distinguish genetic adaptation from developmental acclimatization, since participants grew up at altitude [14].

Conclusion

This research showed that long-term Italian Alpine residents display a blunted HIF-1 α transcriptional response to hypoxia paired with elevated PHD2 expression, resulting in moderate hemoglobin elevation without polycythemia. The pattern resembles aspects of Tibetan and Ethiopian altitude adaptation but occurs in a European population that has received little prior investigation.

The elevated PHD2 baseline, which persisted after a week at sea level, suggests a fixed regulatory component—possibly genetic or epigenetic—that restrains the erythropoietic arm of the HIF cascade. These findings expand the geographic and genetic landscape of human oxygen-sensing variation and support future genome-wide research to identify the specific variants responsible for altitude adaptation in European mountain populations.

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Contributions Not Qualifying for Authorship

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