

High-altitude residence increases dietary iron absorption in young women: a prospective stable isotope study in Peru

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Key Points

- Dietary iron absorption from biofortified potatoes is higher in high-altitude residents than in matched sea-level counterparts.
- Our data suggests chronic upregulation of iron absorption at altitude occurs independently of the hepcidin-ERFE axis.

Acute high-altitude hypoxia increases erythropoietin (EPO) and erythroferrone (ERFE), which suppresses hepcidin and enhances iron absorption to support erythropoiesis. Whether these responses to acute exposure persist in long-term acclimatized individuals remains unclear. We conducted a prospective comparative study of 80 healthy Peruvian women (plasma ferritin <30 µg/L) who had lived for ≥5 years at high altitude (Huancavelica, 3670 m above sea level; n = 40) or sea level (Lima, n = 40). The groups were matched for age, body size, plasma ferritin, and altitude-adjusted hemoglobin (Hb). Participants consumed a standardized iron-biofortified potato-based meal labeled with ⁵⁷FeSO₄ twice daily for 5 consecutive days (10 iron-biofortified meals in total). We measured iron- and erythropoiesis-related biomarkers and assessed cumulative fractional iron absorption (FIA) by measuring isotopic incorporation into red blood cells 14 days later. High-altitude residents had significantly higher EPO concentrations (11.8 vs 3.2 IU/L) and unadjusted Hb concentrations (15.0 vs 11.8 g/dL; for both, *P* < .01), consistent with chronic hypoxic stimulation of erythropoiesis. There were no differences in altitude-adjusted Hb, plasma ferritin, ERFE, or hepcidin between groups. FIA was higher at altitude (15.8%) than at sea level (9.3%; *P* < .05). At altitude, FIA correlated with intestinal fatty acid-binding protein (*ρ* = 0.343; *P* < .05), a marker of enterocyte injury. Long-term residence at high altitude is associated with threefold higher EPO concentrations and ~70% greater dietary iron absorption, without changes in ERFE or hepcidin. This suggests that chronic adaptation to altitude increases iron absorption through hypoxia-mediated pathways in the gut, independent of hepcidin. This may be associated with increased iron requirements in high-altitude populations. The trial was registered at www.ClinicalTrials.gov as NCT05500014.

Introduction

Globally, an estimated 500.3 million people live at altitudes ≥1500 m above sea level, including 81.6 million living at ≥2500 m.¹ Many of these high-altitude residents are young women who are at high risk for iron deficiency (ID) and anemia.^{2,3} The adaptation of iron metabolism to acute hypoxia at high

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Proposals for access to deidentified individual participant data that underlie the reported results can be sent to the corresponding author, Diego Moretti (diego.moretti@ffhs.ch).

The full-text version of this article contains a data supplement.

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altitude, characterized by a rapid increase in erythropoiesis, is well established.⁴ In response to reduced oxygen availability, the kidney upregulates production of erythropoietin (EPO), stimulating erythropoiesis and increasing iron demand. To meet this demand, EPO induces the expression of erythroferrone (ERFE) in erythroid precursor cells.⁵ ERFE acts on the liver to suppress the iron-regulatory hormone hepcidin, and this increases iron mobilization from body stores and intestinal iron absorption.^{6,7}

However, unlike individuals undergoing acute altitude shifts, long-term residents are not exposed to transient erythropoietic stress. It remains unclear how iron metabolism is regulated in high-altitude residents chronically exposed to low PaO₂. Existing studies are hard to interpret, as iron and erythropoiesis parameters are frequently missing and comparisons to matched sea-level controls are lacking (reviewed by Muckenthaler et al⁸). However, in participants living at high altitude, the chronically expanded erythroid compartment increases plasma iron turnover and iron demand.^{9,10} As iron balance is regulated at the level of absorption,¹¹ one of the most accurate ways to determine iron status and demand is by measuring iron absorption from standardized test meals, particularly when expressed relative to plasma ferritin (PF).¹²

Whether individuals who are long-term residents at high altitude have higher dietary iron absorption is uncertain. Two previous radioisotope studies found that iron absorption in high-altitude residents was not significantly greater than in individuals living at sea level^{10,13}; however, these studies were limited by small sample sizes and provided a single isotope dose, and compared groups were not matched for iron status or body size. Therefore, our study aimed to compare iron and erythropoiesis-related biomarkers and dietary iron absorption from multiple meals between 2 groups of women—matched for age, body size, hemoglobin (Hb; adjusted for altitude) and iron status—but living either at 3670 m or at sea level. We hypothesized that even with full acclimatization during long-term residence at high altitude, plasma hepcidin would be suppressed and iron absorption would remain elevated due to increased red cell mass and iron turnover.

Participants and methods

Participants

We recruited generally healthy women aged between 18 and 40 years from 2 locations: Lima (Huascar area, at sea level) and Huancavelica (central Peruvian Andes, 3670 m). A venous blood sample was collected from participants at screening. Inclusion criteria were permanent residence in the study location for the past 5 years, (or in the Peruvian highlands: >3000 m in the case of Huancavelica), with at least 1 parent of highland origin (>3000 m); PF <30 µg/L; body weight <65 kg; body mass index (BMI) 18.5 to 26 kg/m²; no evidence of systemic inflammation (C-reactive protein [CRP] <5 mg/L); and willingness to discontinue mineral and vitamin supplements for at least 2 weeks before the study. Exclusion criteria included pregnancy or lactation, smoking (>1 cigarette per week), severe anemia (Hb <80 g/L, adjusted for altitude),¹⁴ and major blood loss or transfusion in the past 6 months.

Highland origin was assessed by recording the parental places of birth. To heuristically infer likely ethnicity, parental birthplaces were mapped to highland regions with recognized predominance of

Quechua or Aymara populations. In the high-altitude group, 97% of participants had at least 1 parent (97% both parents) originating from regions with predominantly Quechua ancestry; 1 participant (2.5%) had parental origin in a highland region with mixed Quechua and Aymara populations. In the Lima cohort, 92.2% of participants had at least 1 parent (71% both parents) originating from highland regions with predominantly Quechua ancestry; 5.3% had parental origin in regions of mixed Quechua and Aymara ancestry; and 1 participant (2.5%) had 1 parent originating from a region with predominantly Aymara ancestry.

We conducted the field work of this study between May 2022 and October 2022. ID was defined as PF of <15 µg/L or (inclusive) soluble transferrin receptor (sTfR) of >8.3 mg/L. Anemia was defined as an altitude-corrected Hb of <12.0 g/dL, in accordance with World Health Organization (WHO)–recommended thresholds in effect at the time of study enrollment (2022).¹⁴ The study was approved by the institutional ethics board of the Instituto de Investigación Nutricional, Lima (approval number 027-2022/PROY.384-IIN), and was conducted in accordance with the Declaration of Helsinki.

Study design

Participants were matched between sites for PF, Hb (adjusted for altitude),¹⁴ age, body weight, and BMI. Matching was achieved by stratifying them into predefined intervals based on the distribution of these variables within the recruitment limits. Selected participants entered the intervention phase, which involved consuming 10 standardized potato-based meals (500 g/d, as cooked, split between breakfast and lunch) over 5 days, extrinsically labeled with the iron stable isotope ⁵⁷Fe (Figure 1 study design).

Test meal composition and administration

A total of 800 labeled test meal portions were fed. The International Potato Center in Lima, Peru, has developed and registered 2 iron-biofortified potato varieties through conventional breeding: CIP-Kallpa Yawri and CIP-Puka Yawri. In this study, participants received the CIP-Kallpa Yawri variety (clone 312721.245). Potatoes were washed, boiled (~60 min), peeled, mashed, homogenized, portioned (250 g), and frozen at –18°C. Meals were thawed and reheated in a microwave oven before supervised consumption in a fasting state (8-hour overnight fast). A further 3-hour fasting period followed each meal. Each test meal was labeled with ⁵⁷Fe (Chemgas, Boulogne, France) as ⁵⁷FeSO₄, delivering a total of 3 mg of stable iron over 10 meals. For labeling the potato test meals, isotopic iron was added to 200 mL of drinking water consumed alongside the meal.

Anthropometry, meal composition, and biochemical measurements

Iron, phytic acid (PA), polyphenol, and vitamin C content analyses were carried out on freeze-dried potatoes. Subsamples were ground in a stainless-steel mill only used for potato samples to avoid contamination. Iron concentration was measured by using inductively coupled plasma mass spectrometry (Thermo Fisher Scientific iCAP-Q; Thermo Fisher Scientific).¹⁵ PA concentrations were measured using a modification of the Makower method,¹⁶ as described previously.¹⁷ Polyphenol concentrations were determined using coupled ultraperformance liquid chromatography with mass spectrometry.¹⁸

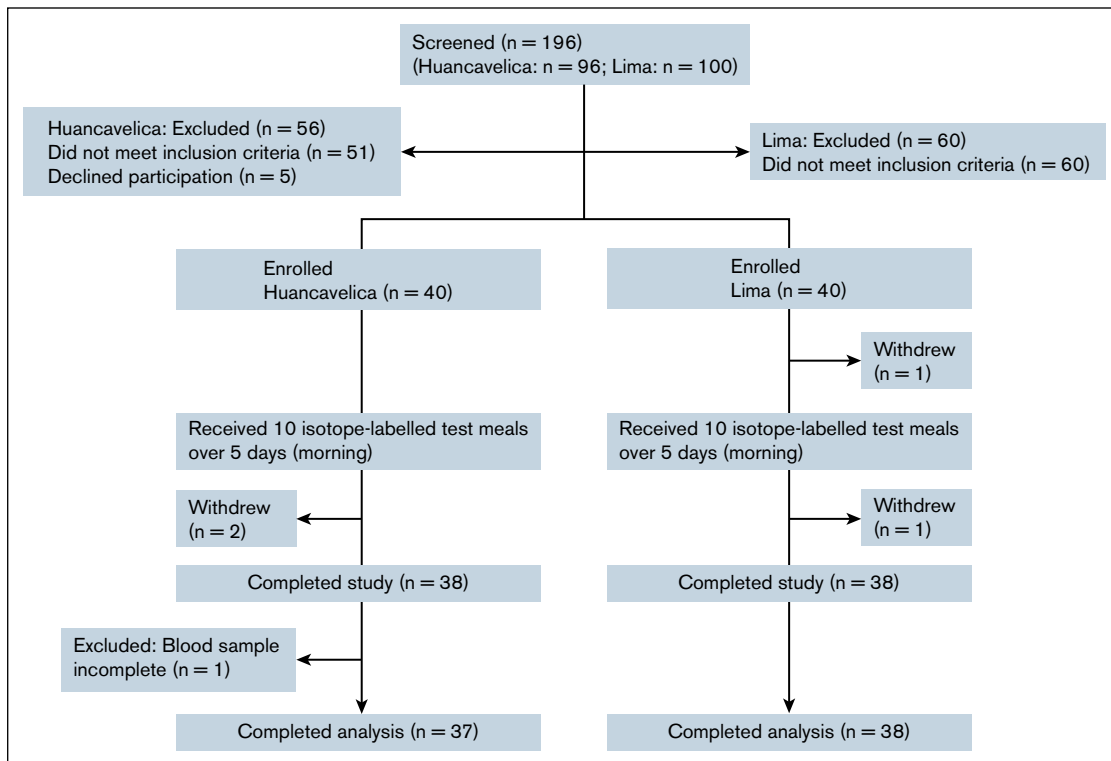


Figure 1. Study design and participant flowchart.

Ascorbic acid was analyzed in the fresh samples by UV/visible spectroscopy (UV 160A; Shimadzu Corp).¹⁹ Because of prolonged frozen storage, native vitamin C declined from 13.4 to 0.44 mg per 100 g. To restore lost vitamin C content, vitamin C was added to the test meals to achieve 10.4 mg per 100 g.

Height and weight were measured using standardized techniques. Physical activity was measured with the IPAQ (International Physical Activity Questionnaire, long version). In both locations, venous blood samples were collected at screening and stored at less than -18°C . PF at screening was measured in both locations using a Cobas E 411 analyzer (Roche Diagnostics, Lima, Peru) using control material (Liquicheck Immunology Control, Bio-Rad, Hercules, CA). Hb was measured using a Sysmex analyzer (XN1000) in Lima and a 5-differential flow cytometer (Nihon Kohden) at altitude. At screening, CRP was measured by turbidimetry (Vitros 4600; Ortho Clinical Diagnostics) at altitude and with a Cobas 501 system (Roche Diagnostics, Lima, Peru) in Lima.

After participant inclusion, study samples were all measured with identical methods, using a multiplex enzyme-linked immunosorbent assay²⁰ to measure PF, sTfR, CRP, and α_1 -acid glycoprotein and retinol-binding protein. We measured plasma iron and total iron-binding capacity by using colorimetry and calculated transferrin saturation (TSat) using the formula (plasma iron/total iron-binding capacity) $\times$ 100. We used enzyme-linked immunosorbent assays to measure hepcidin (DRG Instruments GmbH, Marburg, Germany), ERFE (Intrinsic LifeSciences, La Jolla, CA), EPO (Biovendor, Brno, Czech Republic), and intestinal fatty acid-binding protein (IFABP; Hycult Biotech, Uden, The Netherlands).

Assessment of iron absorption

Fourteen days after the last test meal administration, a second blood sample was collected from the participants. We analyzed each isotopically enriched whole blood sample for its iron isotopic composition in duplicate under chemical blank monitoring, according to previously published methods.²¹ Fractional iron absorption (FIA) was calculated as the ratio of ^{57}Fe incorporated into red blood cells (RBCs) to the estimated circulating iron pool.²² Blood volume was estimated using height, weight, and Hb concentration,²³ assuming 80% incorporation of absorbed iron into RBCs. Previous studies investigating iron turnover at different altitudes assessed systemic incorporation of absorbed iron directly into RBCs. In habitual residents of Lima, RBC incorporation rates of 82% and 86% have been reported, whereas in residents of Morococha (4540 m), rates ranged from 88% to 92%.^{9,10} Because our altitude difference was less pronounced than in these early studies, and the reported variation in RBC incorporation was only $\sim 6\%$, we applied a uniform incorporation rate of 80% and conducted a sensitivity analysis using an incorporation rate of 86% for Huancavelica residents.

Statistical analysis

Assuming a standard deviation of 0.18 for log-transformed FIA based on previous studies in young women at the ETH Zurich, 80% power, a type 1 error of 5%, and a physiologically relevant difference of 35% in absorption between sites, we calculated a required sample size of 36 per group. Allowing for 10% attrition, we recruited 40 participants per site.

Data normality was assessed visually using Q-Q plots and formally with the Shapiro-Wilk test. FIA between locations was compared using an independent sample *t* test on log₁₀-transformed data. Absorption values were further adjusted for minor differences to a reference PF concentration, defined as the geometric mean of the study population (PF of 11 µg/L).²⁴ Associations between FIA and markers of iron status, erythropoiesis, and inflammation were examined using regression models. Variables significantly correlated with FIA were entered into initial models, and minimal models were selected based on the highest standardized *R*². Models were fitted both for the combined data set (all participants) and separately for participants at high altitude (Huancavelica) and low altitude (Lima). As log transformation consistently improved model fit, both correlation and regression analyses were performed and reported using log-transformed data. Statistical significance was set at α value of 0.05. The statistical analysis was conducted with IBM SPSS Statistics (version 29.0.2.0).

Results

A total of 196 women were screened, and 40 participants per location were enrolled (Figure 1). In Huancavelica, 38 women completed the intervention. For 1 high-altitude participant the final blood sample was not analyzable for iron absorption, leaving data for analysis from 37 participants. In Lima, 38 participants completed the intervention and provided data for analysis. All participants were consuming native diets typical of the local setting and had moderate to high levels of background physical activity, without differences between study sites.

The test meals contained 1.6 mg Fe in each serving of 250 g of mashed potatoes. This was composed of 1.3 g of native iron and 0.3 mg of labeled iron ⁵⁷FeSO₄, resulting in a isotopic label-to-natural iron ratio of 0.23 and an iron-to-PA molar ratio of 13.4 (mol/mol): test meal composition is shown in supplemental Table 1.

Table 1. Age, anthropometric variables, and measured and adjusted Hb of the participating women, by study site

	High-altitude residents (Huancavelica, 3670 m)	Sea-level residents (Lima)
n	37	38
Age, y	24.2 (4.7)	24.4 (4.9)
Weight, kg	52.1 (5.3)	51.8 (5.6)
Height, m	1.52 (0.04)	1.53 (0.04)
BMI, kg/m ²	22.7 (2.1)	22.4 (2.2)
Waist circumference, cm	74.3 (5.04)	75.8 (5.25)
Measured Hb, g/dL	15.0 (14.4-15.6)*	11.8 (11.1-12.7)
Adjusted Hb, g/dL, WHO correction (2011) ¹⁴	12.2 (11.6-12.8)	11.8 (11.1-12.7)
Adjusted Hb, g/dL, WHO correction (2024) ²⁵	12.5 (11.9-13.1)†	11.8 (11.1-12.7)

Data are presented as means (standard deviation in parentheses) or as medians (interquartile range in parentheses).
*Significantly different between groups by independent *t* test (*P* < .01).
†Borderline significant difference between groups by independent *t* test (*P* = .050).

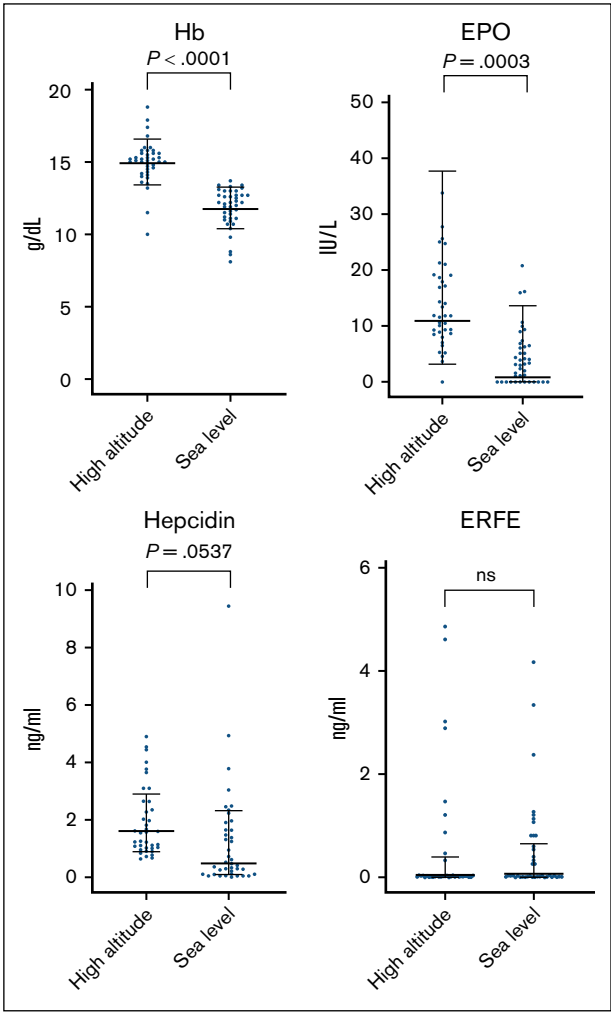


Figure 2. Hb, EPO, hepcidin, and ERFE by study site. Whiskers represent geometric mean with geometric standard deviation. Data were compared using unpaired *t* tests. For EPO, 1 data point was outside the graph range (127.7 IU/L), ns, not significant.

Baseline characteristics did not differ significantly between the 2 groups in terms of weight, age, BMI, or waist circumference (Table 1). As expected, uncorrected Hb concentrations were significantly higher in Huancavelica (15.0 ± 1.5 g/dL) than in Lima (11.8 ± 1.3 g/dL; *P* < .01; Figure 2). Altitude-corrected Hb did not significantly differ between groups when the 2011 WHO correction factors¹⁴ were used, but it was borderline significantly different between groups when the revised 2024 WHO correction factors²⁵ were used (Table 1). In Lima, the prevalence of anemia, ID, and iron deficiency anemia was 47%, 73%, and 42%, respectively; in Huancavelica, the corresponding prevalences were 27%, 51%, and 19%, indicating higher prevalence of low iron status in Lima.

There were no significant between-group differences in the concentrations of PF, plasma iron, TSat, and sTfR (Table 2). EPO concentrations were markedly elevated at high altitude (11.8 vs 3.2 mIU/mL; *P* < .01; Figure 2). Retinol-binding protein differed significantly between groups (*P* < .01), but overall vitamin A status

Table 2. Iron status markers, erythropoiesis, and inflammation variables in the participating women, by study site

	High-altitude residents (Huancavelica, 3670 m)	Sea-level residents (Lima)
PF,* $\mu\text{g/L}$	11.8 (6.9-19.6)	9.1 (5.7-16.3)
PF of <15 $\mu\text{g/L}$, n (%)	19 (51) [†]	27 (71)
sTfR, mg/L	5.4 (4.4-6.7)	4.7 (4.0-6.6)
TSat, %	15 (9-23)	13 (6-27)
Plasma iron, $\mu\text{mol/L}$	10.6 (7.3-16.8)	9.5 (5.5-18.1)
EPO, mIU/mL	11.8 (8.6-19.1) [‡]	3.2 (0-6.5)
ERFE,* ng/mL	0.05 (0.01-0.11)	0.07 (0.1-0.76)
Hepcidin, ng/mL	1.6 (1-2.6) [§]	0.5 (0.1-2.8)
Retinol-binding protein, $\mu\text{mol/L}$	1.4 (1.2-1.6) [‡]	1.1 (0.9-1.5)
CRP, mg/L	0.16 (0.07-0.5)	0.16 (0.08-0.5)
Elevated CRP, >5 mg/L, n (%)	4 (10.8)	3 (7.9)
AGP, g/L	0.57 (0.52-0.71)	0.55 (0.49-0.67)
Elevated AGP, >1 g/L, n (%)	2 (5.4)	2 (5.3)
IFABP, pg/mL	1130 (846-1414)	1179 (814-1792)

Data are presented as medians (with interquartile ranges in parentheses) unless otherwise specified.

AGP, α 1-acid glycoprotein.

*Geometric mean (interquartile range).

[†]Trend for a difference between groups, χ^2 test ($P = .080$).

[‡]Significantly different between groups, independent sample t test ($P < .01$).

[§]Trend for a difference between groups, independent sample t test ($P = .053$).

was adequate, with only 1 participant per group at risk of deficiency. ERFE and hepcidin concentrations were not significantly different between groups, but there was a trend toward higher hepcidin concentrations at high altitude (1.9 ± 1.2 vs 1.2 ± 1.7 ng/mL; $P = .053$; Figure 2). Inflammation, as measured by CRP and α 1-acid glycoprotein concentrations, did not differ significantly.

The cumulative geometric mean FIA from the 10 test meals was significantly higher in Huancavelica (15.8%) than in Lima (9.3%; $P < .01$; Figure 3). When analyzed without adjustment for differences in PF, absorption remained significantly higher at altitude (15.2% vs 11.3%; $P < .05$).

Spearman correlation analysis of the combined data set showed positive associations of absorption with EPO ($\rho = 0.288$; $P < .05$), sTfR ($\rho = 0.453$; $P < .01$), and negative associations with TS% ($\rho = -0.437$; $P < .01$), PF ($\rho = -0.428$; $P < .01$), and hepcidin ($\rho = -0.424$; $P < .01$). In the site-specific correlation analysis (Table 3), at sea level, fractional absorption correlated with PF ($\rho = -0.578$; $P < .01$), hepcidin ($\rho = -0.636$; $P < .01$), sTfR ($\rho = 0.513$; $P < .05$), TSat ($\rho = 0.632$; $P < .01$), and EPO ($\rho = 0.363$; $P < .01$), suggesting ID affecting erythropoiesis in Lima. In contrast, at high altitude, fractional absorption correlated significantly with PF ($\rho = -0.377$; $P < .01$), hepcidin ($\rho = -0.435$; $P < .01$), and IFABP ($\rho = 0.343$; $P < .05$), a marker of intestinal barrier function and enterocyte integrity.

In regression models predicting FIA in all participants, PF ($\beta = -0.271$; $P < .05$) and EPO ($\beta = 0.317$; $P < .01$) were the strongest predictors of FIA, whereas hepcidin was not significant in the minimal model ($\beta = -0.210$; $P = .098$; Table 4). Site-specific

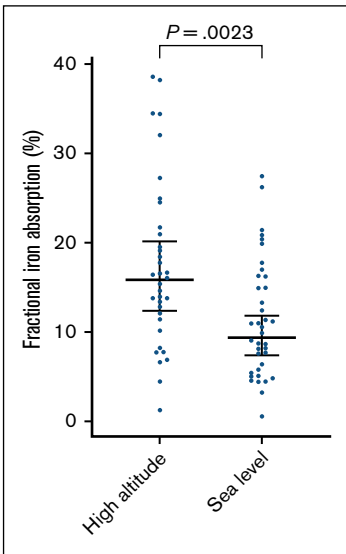


Figure 3. Dietary iron absorption (percent) from 10 potato-based test meals in young women living at high altitude in Huancavelica (3670 m; $n = 37$) and near sea level in Lima ($n = 38$). Iron absorption was determined by measuring the cumulative incorporation of stable iron isotopes into RBCs 14 days after the last test meal. Data were compared between groups using an unpaired t test on $\log_{10}$ -transformed values. To account for small differences in PF, absorption values were normalized to a PF of 11 $\mu\text{g/L}$. Two high-altitude values exceeded the graph range (51.9% and 63.3%).

analyses showed that in Huancavelica, PF was the primary predictor ($\beta = -0.561$; $P < .001$), whereas in Lima, hepcidin ($\beta = -0.479$; $P < .01$) and TSat ($\beta = -0.354$; $P < .05$) were the strongest predictors of FIA, again indicating more pronounced ID in Lima (Table 4).

Discussion

The main findings of this study are that compared to sea-level residents, residents at high altitude, matched for age, body size, and iron status, exhibit threefold higher EPO concentrations and ~70% greater fractional dietary iron absorption, despite no significant differences in ERFE or hepcidin levels.

Our findings of increased iron absorption differ from 2 previous iron absorption studies done in high-altitude residents. In a Peruvian study,¹⁰ fasted healthy young adult males residing at sea level ($n = 8$; mean Hb, 15.1 g/dL) and at 4541 m ($n = 6$; mean Hb, 19.9 g/dL) were given 100 mg of ^{50}Fe as ferrous sulfate. Blood samples taken 10 to 14 days later measured RBC radioactivity to assess iron absorption. Despite 23% higher plasma iron turnover (0.53 vs 0.43 mg Fe per day per kg) and 30% higher red cell iron turnover (0.49 vs 0.37 mg Fe per day per kg) at altitude, mean FIA was similar between groups: 4.0% at high altitude and 3.7% at sea level. However, only a single, pharmacologic iron dose was used, and most key determinants of iron absorption, such as fasting status, iron status, body size, and inflammation were not assessed, limiting group comparisons. Pizarro et al¹³ investigated iron absorption in healthy, nonpregnant women residing at high altitude and at sea level, using a double-isotope extrinsic tag radioiron method. The study enrolled women aged 28 to 45 years; 11 had lived for at least 2 years in Huancayo, Peru (3450 m), and

Table 3. Correlations between iron absorption, inflammation, nutritional, and erythropoiesis markers, by study site

	Iron absorption, %	sTfR, mg/L	AGP, g/L	CRP, mg/L	IFABP, pg/mL	TSat, %	Hepcidin, ng/mL	EPO, IU/L	PF, μ g/L	ERFE, ng/mL
Huancavelica (n = 37)										
Iron absorption, %	1	0.310	0.140	0.108	0.343*	−0.234	−0.435**	0.075	−0.377*	−0.125
sTfR, mg/L		1	0.376*	0.138	0.118	−0.488**	−0.343*	0.233	−0.588**	0.262
AGP, g/L			1	0.405*	0.357*	−0.191	−0.006	0.088	−0.086	0.156
CRP, mg/L				1	0.323*	−0.216	−0.182	0.259	−0.177	0.151
IFABP, pg/mL					1	−0.081	−0.206	0.105	−0.040	−0.229
TSat, %						1	0.362*	−0.269	0.585**	−0.209
Hepcidin, ng/mL							1	−0.188	0.515**	−0.226
EPO, IU/L								1	−0.343*	0.096
PF, μ g/L									1	−0.249
ERFE, ng/mL										1
Lima (n = 38)										
Iron absorption, %	1	0.513**	0.222	0.047	0.058	−0.632**	−0.636**	0.363*	−0.578**	0.115
sTfR, mg/L		1	0.336*	0.292	−0.017	−0.646**	−0.610**	0.430**	−0.756**	0.435**
AGP, g/L			1	0.430**	−0.306	−0.417**	−0.009	0.329*	−0.175	0.039
CRP, mg/L				1	−0.343*	−0.242	−0.040	0.057	−0.132	0.098
IFABP, pg/mL					1	−0.017	−0.159	−0.250	−0.147	−0.075
TSat, %						1	0.543**	−0.536**	0.681**	−0.318*
Hepcidin, ng/mL							1	−0.360*	0.768**	−0.404**
EPO, IU/L								1	−0.404**	.300
PF, μ g/L									1	−0.450**
ERFE, ng/mL										1

*Spearman correlations were significant (2-sided, $P < .05$)**Spearman correlations were significant (2-sided, $P < .01$).

Table 4. Minimal regression model predicting FIA in all participants and by site

	Constant (SE)	B (SE)	Standardized β	P value
All participants*	1.300 (0.118)			<.001
PF, $\mu\text{g/L}$		−0.226 (0.106)	−0.271	.037
EPO, IU/L		0.089 (0.029)	0.317	.003
Hepcidin, ng/mL		−0.099 (0.059)	−0.210	.098
Huancavelica†	1.656 (0.122)			<.001
PF, $\mu\text{g/L}$		−0.431 (0.109)	−0.561	<.001
Lima‡	1.347 (0.150)			<.001
Hepcidin, ng/mL		−0.203 (0.060)	−0.479	.002
TSat, %		−0.313 (0.126)	−0.354	.018

All variables were log transformed ($\log_{10}$) before analysis.

B, nonstandardized coefficient; SE, standard error.

*Overall regression: $R^2 = 0.315$; $P < .001$. Excluded variables: sTfR, retinol-binding protein, TSat, α_1 -acid glycoprotein, and CRP.

†Overall regression: $R^2 = 0.314$; $P < .001$. Excluded variables: sTfR, retinol-binding protein, TSat, α_1 -acid glycoprotein, CRP, hepcidin, and EPO.

‡Overall regression: $R^2 = 0.536$; $P < .001$. Excluded variables: sTfR, retinol-binding protein, α_1 -acid glycoprotein, CRP, hepcidin, EPO, and PF.

13 in Santiago, Chile (630 m). Mean Hb concentrations were 15.2 g/dL at high altitude and 13.9 g/dL at sea level, and mean PF concentrations were 21 $\mu\text{g/L}$ and 22 $\mu\text{g/L}$. Anemia was identified in 3 of 11 women at high altitude and in 1 of 13 women at sea level. Iron-deficient erythropoiesis, defined as TSat <15%, was more prevalent in the high-altitude group (6/11) compared to the sea-level group (1/13). After an overnight fast, participants consumed a single test meal of wheat porridge and milk labeled with 3 mg of iron as $^{55}\text{FeCl}_3$ and a reference dose of ^{59}Fe -labeled ferrous ascorbate. Geometric mean iron absorption from the test meal, adjusted to a standard reference dose absorption of 40%, was 9.0% in the high-altitude group and 6.9% in the sea-level group. This ~25% difference did not reach statistical significance. However, the small sample size may have introduced a type 2 error, and adjusting for reference dose absorption of ferrous ascorbate between the 2 groups may have reduced the apparent difference in dietary absorption.

In contrast to early studies,^{10,13} in our study we provided the tracer to each participant in 10 separate local meals, minimizing the amount of extrinsic tag in each meal and replicating dietary iron absorption more closely than prior single-dose protocols. Our larger sample size minimized type 2 error. Our study groups matched for age, body weight, BMI, PF and altitude-adjusted Hb, and balanced in size. However, blood volume was estimated from standard equations rather than directly measured.²² We considered a uniform 80% RBC incorporation rate for absorbed iron, and conducted a sensitivity analysis applying incorporation rates of 86% and 80% which yielded fractional absorption values of 14.7% in Huancavelica and 9.3% in Lima, respectively, which remained statistically different. A recent study conducted in iron-deficient women residing in Huancavelica similarly reported high FIA from iron-biofortified potatoes (geometric mean, 28.4%)—levels rarely observed with plant-derived foods—which is consistent with the hypothesis that high-altitude residence is associated with enhanced dietary iron absorption.²⁶ Another study in women residents of Huancavelica reported iron absorption of 12% to 17% from both biofortified and nonbiofortified potato meals,²⁷ values

consistent with those observed in our study. The relatively low PA and high ascorbic acid content of potatoes, compared with cereals, likely contributed to the high iron absorption from the iron-biofortified potato test meals.

Mean EPO concentrations in high-altitude residents were threefold higher than those in sea-level residents. This finding suggests that chronic exposure to low oxygen tension at high altitude continues to activate the hypoxia-inducible factor (HIF) signaling pathway in the kidney, resulting in sustained renal EPO production. Thus, even after acclimation to high altitude, a chronic EPO stimulus is needed to maintain increased erythropoiesis. It is likely that this same chronic hypoxic signal also activates the HIF-2 α signaling pathway in duodenal enterocytes. Stabilization of HIF-2 α in enterocytes upregulates expression of the reductase duodenal cytochrome B, the divalent metal transporter 1, and ferroportin, facilitating iron absorption.²⁸ Duodenal biopsies from acutely acclimatizing mountaineers revealed up to a 10-fold increase in duodenal divalent metal transporter 1 and ferroportin expression.⁶ Importantly, activation of the duodenal HIF-2 α signaling can occur even when hepcidin is not suppressed.²⁸ In contrast to studies of acute hypoxia at altitude generally showing hepcidin suppression,^{29,30} thought to be mediated by elevations in ERFE,³¹ in our high-altitude residents there was no evidence of hepcidin suppression and ERFE concentrations did not differ between groups (Figure 2). Plasma hepcidin concentrations tended to be slightly higher in high-altitude women (mean, 1.9 vs 1.2 ng/mL; $P = .053$), which may reflect the slightly higher iron status in Huancavelica. It appears that increased iron absorption in our high-altitude residents was not a result of ERFE-driven hepatic hepcidin suppression. This suggests chronic adaptation to altitude increases iron absorption through hypoxia-mediated pathways in duodenal enterocytes independent of the systemic ERFE-hepcidin axis.

To our knowledge, this is the first study to compare ERFE concentrations between high-altitude and sea-level resident populations. Despite the substantial elevation in EPO among high-altitude women, ERFE concentrations did not significantly differ between groups. This finding contrasts with previous studies of acute hypoxic exposure, which have reported increases in both EPO and ERFE in short term altitude stays.^{5,31,32} Our findings support are consistent with the view that ERFE may not play a regulatory role in chronic hypoxia, but may rather serve as an acute erythropoietic stress signal, responding to sudden increases in EPO and erythropoiesis.

Pleiotropic effects of EPO have been described in a variety of tissues, including the gut epithelium,³³ in which EPO can function as a immunomodulatory cytokine, reducing NF- κ B-inducible immune mediators in chemically induced colitis.³⁴ Because hypoxia in the gut epithelium may be exacerbated at altitude, and gastrointestinal lesions have been reported in short term high altitude sojourners,^{4,35,36} EPO may confer a physiological protection to the gut lining. The potential for a direct effect of EPO on intestinal function, including iron absorptive capacity, should be further investigated.

Why women residing at high altitude need to absorb more dietary iron is uncertain. High-altitude residents have higher amounts of circulating iron in erythrocytes and have higher red cell and plasma iron turnover.¹⁰ In a recent study of predominantly iron-replete Swiss male army recruits, PF levels increased with increasing

altitude,³⁷ suggesting that, under conditions of iron sufficiency, mechanisms may exist to expand iron stores at higher elevations. This finding is consistent with enlarged erythroid and plasma iron compartments at altitude. However, iron compartments are in a steady state, and iron is efficiently recycled within the body, so actual losses are dictated by processes such as epithelial shedding and blood loss, not the rate of iron movement or recycling. It is possible that menstrual iron losses and/or basal losses are increased at high altitude, which would stimulate increased iron absorption. Although there is no strong evidence that women residing at high altitude experience greater menstrual blood loss compared to those at sea level,³⁸ given equivalent menstrual blood volume, a woman at high altitude with a mean Hb concentration of 15 g/dL would lose more iron per menstrual cycle than a sea-level counterpart with a mean Hb of 11.8 g/dL (mean Hb values reported in this study). Assuming an average menstrual blood loss of 35 mL per cycle,³⁹ the high-altitude woman would lose ~3.7 mg more iron per period. This translates to an average additional iron loss of ~0.13 mg/d, potentially contributing to increased physiological iron requirements in women living at high altitude.

Increased basal iron losses from the gastrointestinal tract in high-altitude residents could also stimulate greater iron absorption. Acute hypoxic stress on the gut at high altitude increases gastrointestinal blood loss.³⁵ Fruehauf et al⁴⁰ reported that 61% of otherwise healthy mountaineers developed peptic mucosal lesions and hemorrhagic gastritis/duodenitis after 4 days at high altitude. Although these disorders are less prevalent in high-altitude residents,³⁵ it is possible that chronic hypoxic stress in such populations still induces low-level ischemic injury to enterocytes, which might increase basal iron losses through increased enterocyte shedding and/or microbleeding. This hypothesis is supported by the significant positive association between FIA and IFABP levels in the high-altitude participants ($p = 0.343$; $P < .05$; Table 3). IFABP is a cytosolic protein expressed in mature enterocytes and is a biomarker of enterocyte damage; circulating IFABP is elevated by ischemic injury to the small intestinal epithelium, but further research to confirm this finding is required.⁴¹

Together, these data indicate that high-altitude residence induces sustained adaptations in iron metabolism. Compared to women at sea level, high-altitude resident women respond to chronic hypoxia by producing high levels of EPO and have sharply higher dietary iron absorption despite lack of hepcidin suppression. The increase in iron absorption may be through hypoxia-mediated pathways in the gut independent of hepcidin. Why dietary iron absorption is increased in women at high altitude is uncertain, but it is likely a response to increased basal losses. If so, this implies an increase

in physiological iron requirements in women residing at high altitude. We studied young women in an area of predominantly Quechua descent; our findings may not be generalizable to iron-replete populations and other age groups and high-altitude ethnicities, notably in Tibetan populations, who may have evolved different adaptive mechanisms.⁴² Further research is needed to precisely define dietary requirements in long-term adapted populations at high altitude and across diverse ethnic groups. If confirmed, these findings would have implications for dietary recommendations, anemia control strategies, and iron fortification policies for high-altitude populations.

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Authorship

Contribution: M.B.Z., D.M., R.L., G.B., and N.U.S. conceptualized the study; M.B.Z., R.L., G.B., and D.M. were involved in funding acquisition; D.M., R.L., and G.B. contributed to fieldwork supervision; D.M., R.L., G.B., P.S., N.B., and C.Z. performed data and statistical analysis; D.M. and M.B.Z. wrote the first draft; and all authors were involved in manuscript editing.

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