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SUPPORTING INFORMATION

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Increase of plasma erythroferrone levels during high-altitude exposure: A sub-analysis of the TOP OF HOMe study

To the Editor:

In recent years, erythroferrone (ERFE) has been described as the key endocrine regulator that connects erythropoiesis with iron metabolism.[1] In animal models, erythropoietin (EPO), which is the main driver of erythropoiesis, will induce ERFE production, which subsequently suppresses hepcidin synthesis, probably by inhibiting the hepatic BMP/SMAD pathway.[1] Low hepcidin itself will allow increased iron release from enterocytes and iron-recycling macrophages and thus augment iron availability for erythropoiesis. The same pathways are of clinical importance for the pathophysiology of iron disorders in humans, particularly in patients suffering from ineffective erythropoiesis.[1]

However, the specific roles of EPO and ERFE in physiological responses in healthy individuals who are exposed to high-altitude are less well understood.[2,3]

In a field experiment, eight healthy volunteers living in Homburg, Germany (233 meters above sea level [MASL]) spent 4 days at the "Schneefernerhaus" (Garmisch-Partenkirchen, Germany), an environmental research station at 2656 MASL. Our study was initially designed to study phosphorus regulation at high-altitude. We therefore randomized the eight volunteers into two groups, who were subsequently put either on a normal phosphorus diet (1300–1400 mg phosphorus/day) or on a low phosphorus diet (700–800 mg phosphorus/day). Detailed information about the project and the baseline characteristics are summarized in an earlier publication of our group, which focused upon phosphorus regulation at high-altitude.[4] The mean atmospheric air pressure at 233 and 2656 MASL is 1012 hPa (212.01 hPa oxygen partial pressure) and 740 hPa (155.03 hPa oxygen partial pressure).

Before, during and after high-altitude exposure, blood and urine samples were collected twice (Time Point [TP] 1–2), seven times (TP 3–9) and three-times (TP 10–12), respectively, for measurements of plasma ERFE, hepcidin and EPO; at day 2, maximal aerobic exercise was performed (∼10 km high-altitude hiking with a total ascent of ∼700 m), directly before TP 6.

We discarded the hepcidin, ERFE and EPO measurements after TP 9 from further analysis, as earlier work from our group suggested that a short-term increase in plasma EPO will increase plasma ERFE for approximately 7 days[5]; thus, we did not expect plasma ERFE to return to baseline values within 48 h (i.e., at TP 10–12) after high-altitude exposure.

The study was approved by the local ethics committee and conducted in concordance with the Declaration of Helsinki. All participants provided written informed consent.

Note, ERFE was measured from plasma samples by an enzyme-linked sandwich immunosorbent assay described previously.[5] Hepcidin was measured by an enzyme-linked immunosorbent assay (DRG hepcidin 25; DRG International Inc., USA). All other metabolites were measured according to the standardized methods of the Saarland University central laboratory.

Statistical analyses were performed by SPSS 20. Continuous data are presented as median (interquartile range [IQR]). Comparison as time progressed was performed by linear mixed model analysis, with prespecified time points as the dependent variable and high-altitude exposure (TP 3–9) versus no high-altitude exposure (TP 1–2) as the independent variable. Two-sided p values <.05 were considered significant.

As shown before, EPO increased compared to baseline (TP 1: 7.12 [6.06; 11.36] mIU/mL) soon after arriving at 2656 MASL, reaching its highest levels at TP 5 (20.80 [17.48; 21.59] mIU/mL). After descending to 233 MASL, EPO returned to baseline. Plasma hepcidin initially increased from 8.36 [8.03; 22.03] ng/mL to 22.30 [11.03; 44.06] ng/mL at TP 4 and subsequently decreased. Compared to levels measured at 233 MASL (TP 1: 2.56 [1.00; 5.88] ng/mL), plasma ERFE successively increased at high altitude, reaching its peak at TP 8 (10.30 [5.87; 17.47] ng/mL) (Figure 1, Table S2), and remaining elevated after a return to low altitude until TP 12. Strenuous physical exercise had no immediate effect upon either EPO, hepcidin or ERFE. In subgroup analyses, the changes in ERFE were particularly pronounced in individuals randomized to low phosphorus intake (Tables S1A and S1B).

In the linear mixed model analysis, plasma EPO was significantly associated with high-altitude exposure (Estimate 7.78 CI [5.59; 9.97]; $p < .001$). Plasma hepcidin, however, showed no association with high-altitude exposure (Estimate 0.52 CI [−5.55; 6.58]; $p = .858$). While

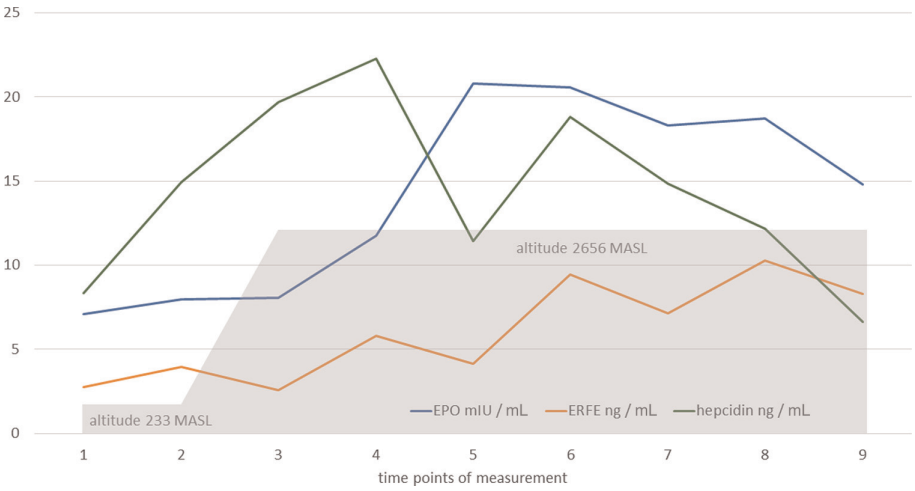

FIGURE 1 Plasma levels of erythroferrone (ERFE), hepcidin and erythropoietin (EPO) at different time points: time points one and two at 233 MASL, time points 3–9 at 2656 MASL. Results are presented as median

plasma ERFE tended to be higher at high-altitude, level of significance was marginally missed (Estimate 2.06 CI [−0.03; 4.16]; $p = .053$).

In line with previous data by Robach et al.,[2] we observed that high-altitude exposure will increase plasma ERFE and lower plasma hepcidin. We deliberately chose a more moderate altitude (Robach et al.: 3800 MASL; TOP of HOMe: 2656 MASL) and longer exposure (Robach et al.: 15 h; TOP of HOMe: 4 days). Our findings expand the results from Robach et al. to more common physiological conditions, as many people are exposed to altitudes of ∼2700 MASL either professionally or during their leisure time; moreover, an altitude of ∼2700 MASL corresponds to the air pressure in a commercial airliner. Apparently, such an altitude causes an increase in EPO that is sufficient to stimulate ERFE secretion.

To our knowledge, the only other study that analyzed the impact of high-altitude exposure on plasma ERFE was reported by Garvican-Lewis et al., who simulated high-altitude exposure during 21 subsequent nights ("live high, train low").[3] In individuals who did not receive additional pharmacological treatment, simulated high-altitude exposure did not augment plasma ERFE. However, the participants were exposed to simulated high altitude only for a part of the day, which may explain the findings.[3]

While ERFE may be expressed in skeletal muscles, strenuous mountain hiking did not affect ERFE in our study. These observations are in agreement with a study of 29 healthy athletes, in whom a marathon run did not consistently increase ERFE levels.[6]

As a limitation of this analysis, we cannot provide repetitive measurements of parameters of iron metabolism – such as ferritin or transferrin saturation – or of erythropoiesis – such as hemoglobin, reticulocyte hemoglobin content and reticulocyte counts. Moreover, the study group was relatively small, which limits the validity of subgroups analyses.

In conclusion, our study expands the observations by Robach et al., as we confirm that high- altitude exposure will raise plasma ERFE not only at very high altitude >3800 m, but also at moderate altitude, to which healthy men and women are frequently exposed.

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CONFLICT OF INTEREST

T.G. is a scientific co-founder of Intrinsic LifeSciences and Silarus Pharma, consultant for ADARx, Akebia, Pharmacosmos, Ionis, Gossamer Bio, Global Blood Therapeutics, American Regent, Disc Medicine, and Rockwell Scientific and inventor on patents related to erythroferrone. I.E.E., A.S., S.W. and G.H.H. have no conflicts of interest.

PATIENT CONSENT STATEMENT

All participants provided written informed consent.

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SUPPORTING INFORMATION

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DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are not publicly available due to organizational reasons, but they are available from the corresponding author on reasonable request.

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