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Hemoglobin concentration of young men at residential altitudes between 200 and 2000 masl mirrors Switzerland's topography: how a precise oxygen sensing mechanism might have evolved

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Short title: Serum hemoglobin predicts topography

Key points

- We analyze cross-sectional blood samples data of 71,798 Swiss men (conscripted in 2010-2012) aged 18-22 years (covering >90% of Swiss male birth cohorts).
- Hb concentrations significantly increased with residential altitude. Moderate altitude must also be considered when interpreting Hb concentrations.

The World Health Organization (WHO) recently launched an initiative to revise its recommended Hb thresholds for the diagnosis and assessment of anemia.[1,2] The physiological Hb range varies depending on age, sex, ethnicity, genetic background, possible pregnancy, smoking habits, socioeconomic and nutritional status (including iron availability), as well residential altitude (meters above sea level (masl)).[3] There is little data about whether modest increases in altitude below 2000 masl impact Hb concentration.[4,5] This knowledge helps to determine people's health status and diagnose anemia.[6] Here we analyze the association between Hb and residential altitude in healthy young Swiss men living between 200 and 2000 masl.

We use data collected between 2010-12 from young Swiss male conscripts (covering >90% of Swiss male birth cohorts due to mandatory conscription).[7] The total cohort consisted of N=110,810 males (18-21 years), and from those N=71,798 (64.8%) voluntarily consented to blood testing. Medical personnel took samples at the six conscription centers, which were shipped to a single certified laboratory. All blood samples have been analyzed using the same hemoglobinometer. See the supplementary material for further information on the participants and methods.

Blood test participants resided at an average altitude of 543.5 masl (min=205 masl, max=1,989 masl), with 91.1% living between 300-900 masl (see descriptive statistics in Supplementary Table 1). Average Hb was 156.28 g/l (95%CI 156.22-156.34, SD=8.66 g/l; min=67 g/l, max=217 g/l). Anemia (Hb<130 g/l) affected overall N=178 (0.25%) of the young men, with decreasing prevalence when residential altitude increased. There was only N=1 (0.002%) young man with excessive erythrocytosis as defined by Hb>=210 g/l. Overall, the Hb distribution shifted to the right on the x-axis with increasing altitude (Supplementary Figure 1), which is also reflected by decreasing percentages of lower Hb (<140 g/l) and increasing percentages of higher Hb (>=175 g/l) when residential altitude increased (Supplementary Table 1). Mapping unadjusted average Hb against average residential altitude in all 705 MedStat-regions (the official geographical regions of Switzerland) revealed that regions of higher residential altitude had higher mean Hb concentrations (Figure 1). In other words, the Hb values mirrored Switzerland's topography ("hemoglobinography").

Mean Hb increased by a maximum of 2.84% from 155.20 g/l for conscripts living below 300 masl to 159.61 g/l for those living over 1800 masl. Hb concentrations rose significantly and stepwise by every gain of altitude starting from 300 masl (Figure 2A). To exclude the possible impact of non-physiological parameters, we restricted the data to conscripts with normal ferritin, CRP and BMI values (Figure 2B). Hb still significantly increased with altitude gain. However, the coefficients of the regressions (Supplementary Table 3) show that the Hb increase from one altitude step of 300 meters to the next is not always the same (range 0.6-1.3 g/l). Between the altitude levels 900-1199masl and 1200-1499masl there is no further increase. If the association between Hb and altitude is considered continuously (Supplementary Figure 1B), the linear and the smoothed modelling (via fine local polynomials with a bandwidth of 100masl) differ modestly.

Ferritin significantly increased with altitude (Figure 2D), whereas odds ratios (OR) for elevated CRP did not

(Figure 2C). This is important because systemic inflammation shows profound impact on hematopoiesis, gastrointestinal iron absorption, and ferritin plasma levels.[8,9] To assess whether increased ferritin serum levels, a marker for body iron stores, directly depend on a concomitant increase of Hb concentrations, we analyzed the interrelation between altitude, Hb, ferritin, CRP and BMI. Analysis of conscripts with normal Hb concentrations (<175 g/l), normal CRP (<5.0 mg/l) and BMI (18.5 – 24.9 kg/m^2), revealed that ferritin levels still significantly rose with altitude (Figure 2E). We further plotted average ferritin levels within a matrix of altitude vs Hb quintiles (Figure 2F). Conscripts with high Hb, and who lived at higher altitudes had the highest mean ferritin levels. Within the five Hb quintile levels, mean ferritin increased with residential altitude. This suggests that additionally to the adaptation of Hb concentrations to altitude, there are mechanisms that increase iron stores depending on altitude.

The most important observation is the significant increase of Hb concentrations with every 300m increase of residential altitude. Remarkably, Mabel Purefoy FitzGerald speculated over 110 years ago that small altitude changes affect Hb serum, but the number of measurements (including her own blood!) was too low to reach statistical significance [10]. In contrast to WHO and CDC studies on Hb adjustment [11], we found a significant change in Hb concentrations below 1000 masl (Supplementary Table 4). While we observe that Hb increases from one altitude level of 300masl to the next, another recent study models an almost linear increase of 3 g/l for every altitude increase by 500m between 1 and 2000masl.[5] The differences may be due to the more narrow study population in our case (only young men in Switzerland), decreasing sample size at altitudes >=1500 masl, and the fine-grained modelling.

Since Hb concentrations are mainly regulated by the cellular oxygen sensor mechanism involving the prolyl-hydroxylase-2 (PHD-2) : hypoxia-inducible factor-2 (HIF-2) : erythropoietin (Epo) axis[12], our observation implies that the oxygen-sensor is very precise, enabling detection of even subtle changes in oxygen at low to moderate altitude. We hypothesize that the oxygen sensing mechanism did not originally evolve to increase red blood cell production by elevating Epo synthesis. The observations that (i) Epo and its receptor are expressed in mouse and human brains in an oxygen-dependent manner [13,14], (ii) Epo exerts neuroprotective effects [15,16], and (iii) cerebral Epo improves exercise performance in mice [17] might points towards Epo's function in enhancing cognitive and physical performance in conditions of oxygen deprivation as it occurs at sea level upon (menstrual or accidental) blood loss. We hypothesize that in hypoxic conditions this precise oxygen-sensor was originally thought to enhance Epo synthesis for neuroprotection and -regeneration and was later re-used to increase erythropoiesis.

This study provides the first description that blood ferritin levels rise with altitude increase (by a maximum of 16.83% for conscripts living below 300 masl to those living over 1800 masl) in an Hb-independent manner. We expected that increased erythropoiesis leading to elevated Hb concentrations requires more iron that is supplied by dietary absorption and mobilization from iron stores[18]. Consequently, we hypothesized that ferritin concentrations would directly correlate with elevated Hb. But this was not the case. We explain this phenomenon by the presence of the oxygen-sensing pathway in duodenal enterocytes, which acts independently of Hb concentrations. Accordingly, HIF-2 activity increases with low oxygenation in duodenal enterocytes to activate dietary iron absorption and iron release to the circulation. It does so by activating transcription of dimetal transporter-1, an iron transporter at the enterocyte's apical side of the enterocyte, as well as of ferroportin, an iron exporter releasing iron at the basolateral side into the circulation.[12] Thus, enterocytes sense oxygen partial pressure and supply more iron when oxygen levels drop. While Hb concentrations are not directly involved in this process, a direct crosstalk occurs between the expression of the iron hormone hepcidin in the liver and HIF-2α expressed in duodenal cells. Hepcidin-mediated ferroportin degradation in enterocytes controls HIF-2α levels in a cell-autonomous manner, thereby determining the availability of iron required for the oxygen sensor PHD2 to function.[19] Our finding suggests that additionally to the adaptation of Hb concentrations, certain mechanisms increase iron stores dependent on altitude exposure.

In summary, even very modest increases in residential altitude significantly elevate blood Hb and ferritin values in young men. Thus, altitude should be considered when defining the Hb threshold for anemia, even for populations below 1500 masl. As there appear to be different mechanisms of adaptation to high altitude in different populations [3], future analysis of Hb concentrations (and other physiological parameters) in populations living at low to moderate altitude, and studies on underlying mechanisms of adaption, are required to provide adequate thresholds.

Competing interests

None declared.

Authors' contributions

Obtained data from the Swiss Army: KS FJR.

Conceived data presentation: All authors.

Designed the data presentation: MG KS MZ RP MUM.

Prepared the SNC altitude data: MZ RP.

Analyzed the data: KS.

Wrote first draft: MG KS.

Wrote the paper: All authors.

Obtained funding: KS MG FJR.

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Figure 1: Hemoglobinography: serum Hb concentrations mirror the topography of Switzerland. Top: Map of 705 MedStat-regions which represents the official geographical regions allowing an anonymous indication of a place of residence for each person hospitalized in Switzerland. The map shows conscripts' average residential altitude (areas in greys) and average Hb level (points in colors, size proportional to sample size, N_{min}=12, $N_{n<30}$=12, N_{max}=335, N_{mean}=102). The numbers in brackets behind the altitudes in the legend key indicate the numbers of MedStat-regions in the particular category. The scatterplot on the right side shows the same Med-Stat data (altitude vs. Hb), the red solid line indicates a smoothed local polynominal trend (bandwidth = 100 masl), the yellow dashed line is a linear fit, and the size of the circle is again proportional to sample size.

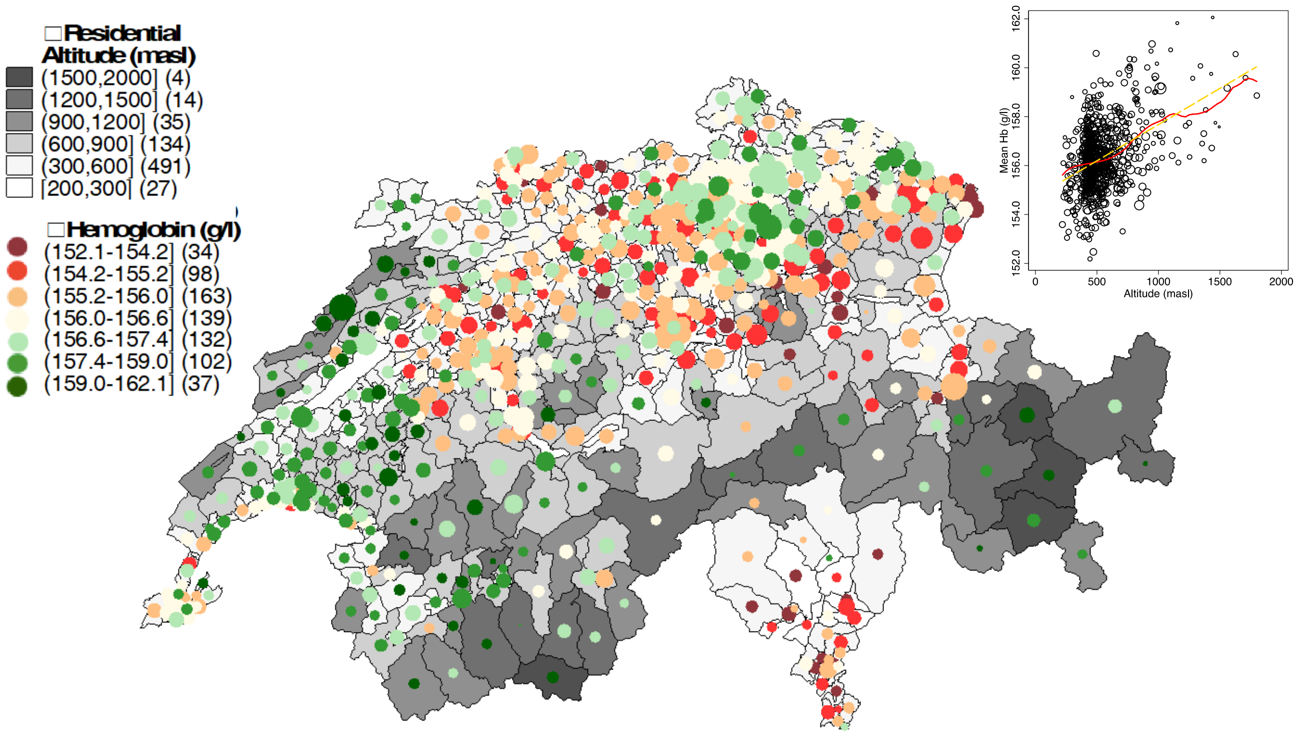

Figure 2: Coefficient plots showing the linear regression results for residential altitude as explanatory variable against (A) Hb, (B) Hb for conscripts with normal ferritin (15-200 ng/ml), CRP (<5.0 mg/l) and BMI (18.5 – 24.9 kg/m^2) only, (D) ferritin, (E) ferritin for conscripts with normal Hb (<175 g/l), CRP (<5.0 mg/l) and BMI (18.5 – 24.9 kg/m^2) only. For CRP (C), odds ratios (OR) for elevated CRP (>=5.0 mg/l) were calculated in a logistic regression. The vertical lines indicate the reference levels (constant, intercept, alpha) of the regressions, the point estimates with 95% confidence intervals indicate the coefficients (beta). The detailed results of the regression A, B, D, and E can be found in Supplementary Table 3. The robustness of these associations has been checked in a sensitivity analysis by stratification for age, occupational background, and recruitment centers (see Supplementary Figure 2). (F) Matrix plot of average ferritin levels (ng/ml, all blood test participants) by altitude (y-axis) and quintiles of Hb (x-axis, 1st quintile=the lowest 20% Hb values, 5th quintile=the highest 20% Hb values). The colors indicate 5 ng/ml-levels of increase in mean ferritin (see legend).