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Serum ferritin and vitamin D evaluation in response to high altitude comparing Italians trekkers vs Nepalese porters

Running title: Serum ferritin and vit D ethnic-related response to altitude

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Type: Original investigation

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Abstract

Altitude hypoxia induces changes in iron homeostasis with serum ferritin (sFER) response being recently linked to erythropoiesis. The main aim of this study was to investigate sFER and Vitamin D (Vit D) response to hypobaric hypoxia, taking into account factors including nutrition and ethnic origin. As part of a "Kanchenjunga Exploration & Physiology" project, 6 Italian trekkers and 6 Nepalese porters took part in a 19-days long altitude trek in the Himalayas self-recording daily food consumption. Blood samples were collected and analyzed before and after the trek for sFER and Vit D. A web-based system calculated the dietary intake, generating reports that were used for later statistical analyses. sFER decreased after the trek (on average by 26% $p=0.013$, partial $\eta^2=0.479$) in both groups, whereas Vit D did not change in both groups. Nepalese tended to have lower sFER, but this difference was reduced when corrected for the dietary intake. Mean Cell Volume (MCV) and Hematocrit (HCT), in respect to baseline, remained higher 10 days after the trek (respectively, 87.37 to 88.85fL with $p=0.044$, and 43.05 to 44.63% with $p=0.065$) in Italian trekkers. The observed reduction of sFER levels was related to altitude per se as inflammation or anemia were medically excluded. sFER, therefore, may act as a primary factor in the examination of hypobaric hypoxia in field studies. The results of this study open a new door into the mechanisms of iron homeostasis in specific tissues related to hypoxia adaptations, taking into account dietary intake and ethnic origin.

Keywords

Iron status; hypobaric hypoxia; Himalayas; homeostasis; dietary intake

Introduction

High-altitude physiology has been extensively studied (West, 2016; Young & Reeves, 2002) and it is continuing to move forward, both from clinical, health and sports perspective. The hypobaric environmental condition posed by high altitude elicits through hypoxia a decrease in the partial pressure of oxygen (PpO_2) in the blood and consequently in tissues, resulting in a modification of hematological parameters (Mairbäurl, 2019; Young & Reeves, 2002). Considering the importance of erythropoiesis in hypoxic acclimatization or adaptation processes, iron homeostasis plays a crucial role (Yanamandra et al., 2019) and should be studied in hypoxic demands. Blood biomarkers from Complete Blood Count (CBC) analysis such as hemoglobin concentration (HGB), serum ferritin (sFER), red blood cell count (RBC), red cell distribution width (RDW), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) are considered as the most sensitive and accurate parameters to evaluate iron homeostasis (Tailor et al., 2017), also in response to altitude (Al-Sweedan & Alhaj, 2012; Badrick et al., 2017).

Nevertheless, these parameters are often complex to analyze as they are strongly dependent on factors such as age, gender, health and nutritional status and diseases (Morris et al., 2019). The first erythropoietic response to altitude is dependent on both hypoxic dose and iron stores (Serpell et al., 2020). Broadly speaking, the greatest effects of hypoxia on the haematological parameters occur at 2200–2500 m, with a minimum exposure of 12 h/day for a minimum period of 3 weeks (Millet et al., 2010). Research studies have specifically highlighted the role of sFER in maintaining blood cell homeostasis and turnover during living or training at altitude (Latunde-Dada et al., 2004; Mairbäurl et al., 2013; Morris et al., 2019). Ferritin is a globular protein which serves to store iron in a non-toxic form in the cells and to transport it where is required, particularly it can store up to 4500 atoms of iron. Its expression is regulated post-transcriptionally by cellular iron status: a higher intracellular iron concentration result in increased ferritin expression, whereas iron deficiency inhibits its expression. As iron storage reflects changes in iron metabolism, absorption, plasmatic turnover rate and of the RBCs' iron uptake, sFER represents a valid marker of an iron reservoir into red blood cells

(Serpell et al., 2020) and serves as an indicator of metabolic and iron-related disorders (Felipe et al., 2015).

Moreover, sFER has been strongly linked to vitamin D (Vit D), i.e. an increase in Vit D is parallel to a decrease in sFER (Munasinghe et al., 2019). Several studies have proved the relationship between iron homeostasis, erythropoiesis and Vit D, i.e. Vit D may protect against anemia by stimulating erythropoiesis (Smith & Tangpricha, 2015). Two studies investigated Vit D after 2 weeks of altitude exposure in the Alps, finding a likely reduction of Vit D levels, in correlation with irisin reduction and associated with modulation of immune processes and inflammation (Kasprzak et al., 2015; Śliwicka et al., 2017). However, the exact mechanism of Vit D in iron homeostasis and erythropoiesis in response to altitude sojourn still remains poorly understood.

The effects of acclimatization due to oxygen deprivation and physical activity induce physiological changes in the body including body mass, energy intake, resting metabolic rate and endocrine system. The severest problem is dehydration caused by increased ventilation, micturition and perspiration as a result of physical activity in altitude. For this reason, water and micronutrients intake should be ideally tracked since diverse dietary intake deficiencies may exert further pathophysiological effects, including dehydration that is already exacerbated by the hypoxic status (Stellingwerff et al., 2019). New technologies may, therefore, permit advanced monitoring to assess dietary intake accurately (Turrini et al., 2019).

Increasingly medical recommendations are needed to prevent altitude-related maladaptation (Schommer & Bärtsch, 2011). Moreover, ethnic origin, along with physical demands, may represent relevant variables, which should be considered when assessing hypoxic responses, given the ever-increasing worldwide tourism and physical training in altitude. To our knowledge, no studies have addressed ethnic differences in iron status in response to altitude sojourn yet.

The main aim of this study was to evaluate changes in selected hematological parameters, before and after an altitude trek in Caucasian lowlanders and native Nepalese porters, taking into account daily

nutritional intake. The focus was particularly placed on serum ferritin and vitamin D concentration, as they represent key factors in iron homeostasis acclimatization due to altitude hypoxia.

Methods

The research project "Kanchenjunga Exploration & Physiology" represented a subset of the project "Environmentally-modulated metabolic adaptation to hypoxia in altitude natives and sea-level dwellers: from integrative to molecular (proteomics, epigenetics, and ROS) level", approved by the Ethical Review Board of the Nepal Health Research Council (NHRC). All study procedures were performed following the ethical standards of the 1964 Helsinki declaration and its later amendments (World Medical Association, 2013). All participants provided their written informed consent.

Participants completed a combined circuit of 300 km distance in 19 days (see figure 1) with over 16,000 meters of difference in altitude and average daily walk of 6 hours involving a demanding route with ascent and descent in the Himalayas, Nepal.

Figure 1 here

The analyses were performed on two groups of participants: N = 6 Italians (It 1-6) and N = 6 Nepalese (Ne 1-6) (see table 1 for description). The expedition was under continuous supervision by an expert medical doctor, who was monitoring symptoms, SpO_2 and blood pressure data throughout the whole period. None of the participants suffered Acute Mountain Sickness.

The study protocol involved the execution of blood samples for later assessment of serum ferritin (sFER) and 25-hydroxyvitamin D (Vit D) at Kathmandu, before and after the trek. Blood samples were collected from the antecubital vein collected in tubes and immediately centrifuged (3000 rpm ×10 min). The serum was frozen and during the transport to Italy it was stored at a slightly cold temperature (−5 °C) for later analyses (transport time of two days). Italian participants were assessed twice for CBC: 1) two weeks before the start of the trip, and 2) ten days after the end of the trek (follow-up for sFER and Vit D assessment). In the latest two analyses, whole blood samples were taken from the antecubital vein and immediately analyzed for the following CBC parameters: RBC,

HGB, HCT, MCV, MCH, RDW. Counts of WBC and sub-populations of leucocytes were also assessed from these samples. All the blood analyses were carried out in the Laboratory of Clinical Pathology (Teramo Hospital). sFER levels were determined using the immunochemiluminescence assay on the ADVIA Centaur® XP Immunoassay System (Siemens Healthcare GmbH, Germany) (Munasinghe et al., 2019). Vit D levels were determined using the immunochemiluminescence assay on the LIAISON® XL analyzer with the LIAISON® 25 OH Vitamin D TOTAL Assay (DiaSorin S.p.A., Italy) (Bianchi et al., 2012; Munasinghe et al., 2019).

Daily food consumption was self-recorded by the subjects, in three non-consecutive days during the trek. No nutritional data were collected before and after the trek. After being instructed by expert field workers, participants recorded all foods, beverages and supplements ingested in 2 hard-copy diaries structured by 7 meals plan (3 principal meals and 4 snacks). For every eating occasion, they had to carefully record the time and place of consumption, detailing the exact description and quantity of food consumed. Furthermore, participants were required to detail precise recipes, ingredients, conservation and cooking methods. Quantities were specified referring to the standard household measures or standard portions of a dedicated picture atlas, developed by the same Institution below mentioned. All filled food diaries were checked by the field worker who later entered all the information into an *ad hoc* web-based software database called "Food Consumption Database (FOODCONS)" that transforms the data entered into the weight of single raw ingredients and the amounts of nutrients consumed. Reports were then provided with the average daily intake of total energy, water, as well as of macro and micro-nutrients (Protein, Fat, Saturated or Polyunsaturated fatty acid, Available carbohydrate, Sugars, Starch, Fibers, Alcohol, 6 minerals and 12 vitamins). The software additionally allows clustering the intakes by meal, food groups (12 clusters) or food sub-groups (47 clusters). The software FOODCONS and all connected instruments (hard-copy food dairy, picture atlas) were developed by Research Center for Food and Nutrition of Council for Agricultural Research and Economics (CREA) (https://www.crea.gov.it/en/web/alimenti-e-nutrizione), that tested and used in the "IV National Dietary Survey in Italy" (IV SCAI), and adapted for this study. For the

present study, we report only the average daily intake of total energy, water, Iron and Vitamin D (see table 1).

Table 1 here

Shapiro-Wilk test for the normality of distributions and Levene's test for the equality of variances were used as assumption checks (Armstrong, 2017). Repeated Measures (RM) ANOVA (Type III Sums of Squares) was performed to compare low *vs* high altitude and altitude (low *vs* high) × ethnic group (Italian vs Nepalese). General Linear Mixed Model (GLMM, with REML estimation and LRT for Random Effects, individuals as a random variable) was used to test the pre *vs* post *vs* follow-up comparison. The same analyses were repeated after correcting for Energy, Water, Iron or Vit D intake, or age. Student's and Bayesian paired *t*-test were used for pre *vs* post comparison of CBC parameters in Italian participants. Pearson method was used to test the correlation between pre-post % difference of Ferritin with pre-post % difference of Vit D. Significance (p-value), effect size (Cohen's d or partial η^2) and Bayes Factor (BF_{10}) were reported. Cauchy's *a priori* distribution was considered for the calculation of BF_{10}. For age, BMI, water, energy, Fe, and Vit D intake comparison by ethnic group, the independent sample t test (Student's t or Mann-Whitney U) was used.

Results

Overall, sFER values were in the normal range for healthy adults: > 20 µg/L (Tailor et al., 2017), before and after, for both groups. sFER values were within the reference values for the used method (15-300 µg/L). sFER decreased by 26% after the expedition (p=0.013, partial η^2=0.479), Nepalese tended to have a lower concentration (-35% at baseline, -51% after expedition; p=0.102, partial η^2=0.245), while no difference was found for the interaction ethnicity × time (see figure 2 and table 2). After correcting for Iron, Energy or Water intake, or age the above-mentioned statistical significances were reduced (when Iron intake was the covariate) or became non-significant, even if any direct effect of these covariates was not found. GLMM failed to find significant differences in

the pre vs post vs follow-up comparison (p=0.168, marginal R^2=0.042) but the random effect was significant (p<0.001, conditional R^2=0.851). The diverse dietary intakes or age as covariates did not alter GLMM results.

Overall, vit D levels were in the normal range for healthy adults (> 20 ng/mL) (Smith & Tangpricha, 2015) except in two participants in Pre values (Ne-2 and Ne-4, see Table 1 for the description of these participants). Vit D did not significantly change from pre to post-expedition (p=0.539, partial η^2=0.039) and no significant effect of ethnic origin or interaction (ethnicity × time) was found (see Figure 2 and table 2). After correcting for Energy, Water, or Vit D intake, or age no significant effect was found. GLMM failed to find significant differences in the pre *vs* post *vs* follow-up comparison (p=0.283, marginal R^2=0.061) but the random effect was significant (p<0.001, conditional R^2=0.639). The diverse dietary intakes or age as covariates did not alter GLMM results. No correlation was found between pre-post % difference of Ferritin and Vit D.

Figure 2 here

Concerning dietary intake, participants showed high energy and water intake during the altitude trek, as expected (Stellingwerff et al., 2019). Only one subject (It-6, see Table 1) had a lower Iron intake than Population Reference Intake of 11 mg/day (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2015). Iron intake of Italian trekkers was lower with respect to Nepalese porters (on average, 13.8 vs 23.9 mg/day, p<.001). Further analysis of the food groups revealed that the higher intake of Iron was primarily due to the consumption of lentils, typical of porter's diet. Instead, the higher intake of Vitamin D showed by Italian participants was primarily due to the consumption of eggs, which was commonly absent in most porters' diets (on average, 3.0 vs 0.2 μg/day, p=.004). CBC parameters, performed solely on Italian participants, were in the normal adult reference range, out of the risk of vascular disease (Kofink et al., 2018). Among the haematological parameters (see table 2), the only significant difference was found for MCV (p=0.044, Cohen's d=1.089), with an increment after the expedition. A similar trend was found in HCT (p=0.065, Cohen's d=0.964). No other parameters showed significances or strong tendencies (see table 2).

Table 2 here

Discussion

The main objective of this study was to investigate and compare changes in key factors of iron homeostasis before and after an altitude trek, in Caucasian trekkers and in native Nepalese porters (both habitually living at low altitude). The principal result of the present study is the reduction of sFER after a high-altitude trek both in Italian trekkers and Nepalese porters, independent of ethnic origin. Moreover, a significant increase was observed in the Italian participants in some CBC parameters 10 days after the end of the trek.

Our results showed that Nepalese porters tended to have lower sFER values than Italians trekkers, even without statistical significance but considering the large effect size found in our study. Previous research studies have also shown that populations living at moderate-altitude have lower sFER concentration (Yanamandra et al., 2019), as well as populations living at low-altitude (500-to-1500m a.s.l.) (Al-Sweedan & Alhaj, 2012). The only female included in the sample had the last menstruation 10 days before the blood sample collection at the end of the trek and her sFER values followed the same trend of male participants in response to the altitude trek. In our study, the Nepalese porters were not highlanders, and after correcting for dietary intakes, the statistical effect between Italian trekkers and Nepalese porters diminished. This finding lends itself for a suggestion that an advanced dietary intake assessment is crucial for the understanding and comparison of iron status in lowlanders *vs* highlanders. Therefore, besides body physiological responses to hypoxia, other variables such as physical demands and nutrition, play an essential role. Based on our findings, we, therefore, speculate that the reduction of sFER was related to altitude *per se* as a middle-term response to hypoxia regardless of ethnic origin. As reported in other studies, this reduction is related to an increased iron demand for the erythropoiesis and to a make up for adaptive changes due to hypoxia such as increased intestinal iron uptake, augmentation of serum iron-binding capacity, and enhanced mobilization of iron from cellular stores (Goetze et al., 2013; Hennigar et al., 2020).

In terms of the CBC parameters, increases were observed only in MCV (on average, from 87.37 up to 88.85fL, with increments in five out of six participants) and HCT (on average, from 43.05 up to 44.63%, with increments in five out of six participants) corpuscular parameters used as an index of red cell blood status, after 10 days after the end of the altitude sojourn, along with a partial increase of ferritin level up to pre-expedition values (regarding Italian trekkers, 19% reduction immediately after the trek and 5% reduction 10-days after were observed). These results are in line with previous studies on altitude endurance training (Hinton, 2014) and animal models (Li et al., 2019). Indeed, the sustained erythropoietic drive after early high-altitude exposure is a well-known hallmark of altitude acclimatization (Vizcardo-Galindo et al., 2020), even if the typical duration of modern altitude traveling is no longer enough to take advantage of this beneficial response during the hypobaric hypoxia exposure (West, 2012). MCV and HCT were still higher 10 days after the descent from high altitude sojourn, which is in line with the known time course of altitude de-training, i.e. 1-to-2 week (Mairbäurl, 2019). Larger samples will allow to determine ethnic-related differences in multiple traits associations with the timeline of acclimatization and de-acclimatization on CBC parameters (Peng et al., 2013).

sFER is generally considered as a marker of inflammatory disease, body iron stores, erythrocyte morphology and oxidative stress (Kell & Pretorius, 2014). Looking at the leukocyte profile, we could exclude the correlation between sFER and inflammation, and we could be sure that this reduction was due to an increase in erythropoiesis as a result of the reduction in PpO_2. Besides, sFER levels were not related to iron-poor status either (normal iron intake or non-anemic condition were checked from CBC parameters). Dietary intake, as addressed in the current work, affected the iron homeostasis adaptations. We, therefore, suggest to include accurate measures of dietary intakes in studies dedicated to iron status response to altitude. Despite the differences in iron and Vit D intake between Italian trekkers and Nepalese porters (due to the different food group consumption, i.e. eggs vs lentils), we did not observe any difference in blood concentration deficiencies, perhaps as a result of overall normal intakes of these micronutrients with respect to the population reference range.

Instead, Vit D concentrations did not change from low to high altitude, nor there was a difference in the interaction considering the ethnic origin × altitude. Vit D is involved in red cell production and can affect circulating iron status by promoting erythropoiesis through erythropoietic precursors (Alon et al., 2002) and by suppressing hepcidin expression with an increment of iron availability precursors (Masoud et al., 2018). Liu and colleagues (Liu et al., 2015) highlighted an association between vit D and poor iron status. Moreover, Vit D has been linked to iron homeostasis through stimulation of erythroid progenitor cells and down-regulation proinflammatory cytokines and hepcidin (Smith & Tangpricha, 2015). Thus, the absence of any association between Vit D and iron status in the current work was likely because participants were not affected by acute inflammation or iron-poor status, as revealed by CBC parameters. In respect to other results, where a Vit D reduction was found in alpinists in association with increased inflammation (Kasprzak et al., 2015; Śliwicka et al., 2017), we suggest that the difference may lie on the more prolonged duration (19 days of exposure in the current study, 2 weeks in the other studies), on the absence of acute inflammation in our participants, on the lower baseline values of our participants (on average, 28.4 vs 34.02 ng/mL). The low intake of vit D in Nepalese porters did not result in Vit D deficiency measured by the serum concentration, perhaps as a result of prolonged sun exposure during the trek, which protected against Vit D deficiency (Coutinho et al., 2019).

The results of the iron status (or homeostasis) approached by our study design suggest that serum ferritin may represent a key factor to examin the response of hypoxic exposure and deserves further attention. Serum ferritin closely reflects iron intra-tissue concentration (Aldred et al., 2009) and with this regard analyses of ferritin storage inside specific tissues, such as bone marrow, liver, spleen, and skeletal muscles, could help to further elucidate our findings. In addition to erythropoietic management, iron is considered as an antioxidant microelement, which plays an essential co-factor role in antioxidant enzymatic systems. Thus, it should be of particular interest to focus on muscles, considering the established effect of altitude hypoxia on skeletal muscle tissue placing a particular focus on the redox system (Doria et al., 2011; Mancinelli et al., 2016; Tam et al., 2016). For example,

extracellular vesicles could be specifically addressed, as a non-classical pathway of secretion (Truman-Rosentsvit et al., 2018). Considering the "boom" of altitude training, particularly of endurance training, the adaptations of iron status and CBC parameters (Radák, 2018), could be investigated further in the field of physical conditioning.

This study did not come without its limitations. Firstly, the CBC analysis was not performed immediately after the end of the trek, which would otherwise provide additional insight into the iron homeostasis of the study participants. However, given the nature of this work (an outdoor field study in an extreme environment), many measurements involved in this study were constrained by logistical difficulties. The small sample size, as well as age differences and heterogeneity between the ethnic groups, represented other limitations, which are again given by the nature of this study.

In conclusion, sFER appears to represent a pivotal factor to be studied in response to hypoxia across ethnic groups. We reported a disentanglement between sFER and Vit D temporal relationships in response to hypobaric hypoxia exposure, with respect to other pieces of evidence about inflammation or poor iron status. Dietary intake of water, micro and macro-nutrients is crucial for a comprehensive interpretation of adaptations to extreme environments and should be monitored in order to provide adequate advices to altitude expeditioners and medical doctors.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Authors contribution

Conceptualization: LM, DB, VV; methodology: LM, DB, TP, SF, RP, TJ, GDB, MT, VV; formal analysis: LM, DB, RP; investigation: DB, GDB, MT, VV; resources: DB, RP, GDB, MT, VV; writing – original draft: LM, DB, TP, SF, VV; writing – review & editing: LM, DB, TP, SF, RP, TJ, GDB, MT, VV; visualization: LM, DB, TJ; supervision: TP, SF, VV; project administration: VV; funding acquisition: VV

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Table 1. Descriptive characteristics of participants and dietary intake averaged from food consumption diaries. P-values of independent sample t-tests for each comparison are shown in the last row.

	Ethnicity	Gender	Age[1] (years)	BMI[2] (kg/m^2)	Energy[3] (kcal)	Water[4] (ml)	Fe[5] (mg)	Vit D[6] (μg)
It1	Italian	Female	36	25.07	2639	2617	11.4	2.5
It2	Italian	Male	63	28.91	2338	2938	12.6	3.0
It3	Italian	Male	59	21.91	2466	2357	13.9	4.4
It4	Italian	Male	25	24.31	4017	3400	22.1	4.0
It5	Italian	Male	32	24.14	3169	3354	12.9	2.6
It6	Italian	Male	48	30.54	1972	3445	9.7	1.5
	Italian group		44±15	25.81±3.25	2777±728	3019±457	13.8±4.3	3.0±1.1
Ne1	Nepalese	Male	26	26.49	2651	3394	24.2	0.0
Ne2	Nepalese	Male	18	17.51	2864	3094	25.4	0.0
Ne3	Nepalese	Male	39	22.99	2599	2862	20.8	0.0
Ne4	Nepalese	Male	40	28.83	2603	3355	20.5	1.3
Ne5	Nepalese	Male	30	29.41	3080	3719	28.0	0.0
Ne6	Nepalese	Male	29	20.94	2730	3016	24.5	0.0
	Nepalese group		30±8	24.36±4.70	2755±188	3240±310	23.9±2.9	0.2±0.5

BMI: Body Mass Index; **Fe**: Iron, **Vit D**: Vitamin D

[1]: p=.086; [2]: p=.548; [3]: p=.589; [4]: p=.349; [5]: p<.001; [6]: p=.004

Table 2. The first part shows sFER and Vit D values of Italian trekkers and Nepalese porters, before and immediately after the altitude hypoxia exposure. Plus, such parameters are provided for Italians only, 10 days after the expedition. Data are expressed as Mean ± SD (% difference in respect to the previous value). The second part shows hematological parameters of Italians 14 days before and 10 days after the expedition (Mean ± SD).

	sFER (µg/L)			**Vit D (ng/mL)**		
	Before	*After*	*10-d after*	*Before*	*After*	*10-d after*
Italian trekkers	236.0 ± 120.7	192.0 ± 115.3 (−19%)	225.2 ± 142.4 (+17%)	30.3 ± 7.1	29.4 ± 2.7 (−3%)	27.3 ± 4.4 (−7%)
Nepalese porters	153.7 ± 64.3	94.5 ± 36.4 (−39%)	N.A.	26.5 ± 10.1	29.3 ± 6.6 (+11%)	N.A.
Whole sample	194.9 ± 101.7	143.3 ± 96.1 (−26%)	N.A.	28.4 ± 8.6	29.3 ± 4.8 (+3%)	N.A.

Hematological parameters

	Italians 14-d before	*Italians 10-d after*	*p*	*Cohen's d*	BF_{10}
RBC ($\times 10^6$/µL)	4.93 ± 0.33	5.03 ± 0.26	0.224	−0.567	0.736
HGB (g/dL)	14.90 ± 0.88	15.37 ± 0.66	0.168	−0.658	0.889
HCT (%)	*43.05 ± 2.29*	*44.63 ± 1.76*	*0.065*	*−0.964*	*1.734*
MCV (fL)	*87.37 ± 1.44*	*88.85 ± 2.43*	*0.044*	*−1.089*	*2.277*
MCH (pg)	30.27 ± 1.17	30.60 ± 1.10	0.390	−0.384	0.525
RDW (%)	13.52 ± 0.91	13.40 ± 0.58	0.532	0.274	0.447
WBC ($\times 10^3$/µL)	6.04 ± 1.57	6.23 ± 1.31	0.538	−0.270	0.445
Neu ($\times 10^3$/µL)	3.45 ± 1.03	3.62 ± 0.87	0.274	−0.501	0.646
Lym ($\times 10^3$/µL)	1.89 ± 0.54	1.86 ± 0.56	0.878	0.066	0.377
Mon ($\times 10^3$/µL)	0.35 ± 0.08	0.38 ± 0.10	0.422	−0.357	0.503
Eos ($\times 10^3$/µL)	0.18 ± 0.27	0.20 ± 0.23	0.468	−0.320	0.476
Bas ($\times 10^3$/µL)	0.06 ± 0.05	0.06 ± 0.04	0.709	0.161	0.398

sFER: serum Ferritin; **Vit D**: Vitamin D; **10-d**: 10 days; **RBC**: Red Blood Cells count; **HGB**: Hemoglobin; **HCT**: Hematocrit; **MCV**: Mean Cell Volume; **MCH**: Mean Corpuscular Hemoglobin; **RDW**: Red cells Distribution Width; **WBC**: White Blood Cells count; **Neu**: Neutrophils; **Lym**: Lymphocytes; **Mon**: Monocytes; **Eos**: Eosinophils; **Bas**: Basophils.

Figure captions

Figure 1. Altimetric plan and study design of the "Kanchenjunga Exploration & Physiology" project.

Figure 2. Serum ferritin and vitamin D concentration, before (Pre) and after (Post) the expedition carried out by blood samples of Italian trekkers and Nepalese porters. Only for Italians, blood samples were collected also once back in Italy (Follow-up).