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Does a rise in plasma erythropoietin after high-altitude exposure affect FGF23 in healthy volunteers on a normal or low-phosphorus diet?

Insa E. Emrich [a,*], Juliane Dederer [b], Angelika Kircher [a], Verena Klemis [c], Claudia S. Lennartz [a], Kathrin Untersteller [a], Stefan Wagenpfeil [d], Danilo Fliser [a], Myles Wolf [e], Gunnar H. Heine [a,f]

[a] Saarland University Medical Center, Internal Medicine IV – Nephrology and Hypertension, Homburg, Germany
[b] Saarland University Medical Center, Internal Medicine III – Cardiology, Angiology and Intensive Care Medicine, Homburg, Germany
[c] Saarland University, Department of Transplant and Infection Immunology, Homburg, Germany
[d] Saarland University Medical Center, Institute for Medical Biometry, Epidemiology and Medical Informatics, Homburg, Germany
[e] Division of Nephrology, Department of Medicine, Duke University School of Medicine and Duke Clinical Research Institute, Durham, NC, USA
[f] Agaplesion Markus Krankenhaus, Frankfurt (Main), Germany

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Abstract *Background and aims:* Data of experimental rodent models suggest that hypoxia with subsequent increase in erythropoietin stimulates the expression of the phosphaturic hormone fibroblast growth factor 23 (FGF23).
Methods and results: To translate the findings of animal studies into human physiology, herein we exposed eight healthy volunteers to high altitude (2656 m above sea level) for four days. The volunteers were randomized on a low-phosphorous diet (n = 4) or a normal phosphorus diet (n = 4). Although high-altitude exposure caused a significant increase in plasma erythropoietin (EPO) (before high-altitude exposure: low phosphorus: median EPO 6.6 mIU/ml [interquartile range (IQR) 6.0; 8.2], normal phosphorus: median EPO 9.0 mIU/ml [IQR 7.9; 11.5]; at day 2: low phosphorus: median EPO 21.3 mIU/ml [IQR 19.5; 23.8], normal phosphorus: median EPO 19.4 mIU/ml [IQR 18.0; 20.8]), there was no consistent increase in plasma c-terminal FGF23 or plasma intact FGF23. We observed only a single, intermittent peak in c-terminal FGF23 levels after 5 h of maximal aerobic exercise.
Conclusion: These data do not support a substantial effect of moderate hypoxia alone on the expression of FGF23, but they suggest that combined exercise and high-altitude exposure may temporarily induce FGF23 expression.

Introduction

High plasma levels of fibroblast growth factor 23 (FGF23) are associated with incident cardiovascular events in patients with chronic kidney disease (CKD) [1–3] and in the general population [4]. Therefore, lowering plasma FGF23 has been claimed to improve cardiovascular prognosis in patients with CKD, although a much more detailed understanding of FGF23 regulation is needed before such strategies can be tested in clinical studies [5,6].

* Corresponding author. Fax: +49 6841 16 15225.
E-mail address: insa.emrich@uks.eu (I.E. Emrich).

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Table 1a Plasma levels of CKD-MBD metabolites at different time points (TP1 to TP12) of all participants (n = 4) *on a normal phosphorus diet.*

	TP1 Day 1 6 am	TP2 Day 1 12 am	TP3 Day 1 4 pm	TP4 Day 1 8 pm	TP5 Day 2 7 am
Altitude (meters above sea level)	233	758	2656	2656	2656
EPO [mIU/ml]	9.0 [7.9; 11.5]	8.6 [8.2; 10.7]	8.8 [8.4; 12.4]	11.8 [10.2; 12.9]	19.4 [18.0; 20.8]
C-terminal FGF23 [RU/ml]	72.3 [65.0; 75.8]	59.5 [51.6; 63.9]	53.4 [43.6; 62.9]	78.4 [66.5; 83.0]	60.4 [54.7; 62.5]
Intact FGF23 [pg/ml]	50.1 [46.2; 74.9]	37.9 [37.7; 47.6]	40.4 [38.0; 46.2]	54.0 [41.6; 64.9]	43.3 [41.7; 46.1]
Intact PTH [pg/ml]	26.0 [22.5; 30.0]	29.5 [25.3; 35.8]	32.0 [26.0; 37.8]	23.5 [21.8; 29.5]	25.5 [20.8; 33.0]
1,25-Dihydroxyvitamin D [ng/l]	59.0 [52.5; 63.0]	–	–	–	58.5 [46.0; 75.3]
25-Hydroxyvitamin D [ng/ml]	34.1 [31.0; 34.8]	31.9 [30.5; 34.0]	32.3 [30.4; 33.5]	33.2 [31.0; 36.1]	35.0 [33.8; 35.3]
Phosphorus [mg/dl]	3.9 [3.7; 4.3]	3.9 [3.7; 4.0]	3.3 [2.9; 3.7]	3.4 [3.1; 3.7]	3.9 [3.7; 4.1]
Urine phosphorus/urine creatinine	0.58 [0.39; 0.68]	0.39 [0.21; 0.39]	0.61 [0.46; 0.73]	0.33 [0.26; 0.34]	0.50 [0.46; 0.53]
Calcium [mmol/l]	2.40 ± 0	2.40 ± 0	2.45 ± 0.06	2.48 ± 0.10	2.45 ± 0.10
Urine calcium/urine creatinine	0.03 ± 0.01	0.04 ± 0.02	0.01 ± 0	0.03 ± 0.01	0.02 ± 0.01
pH (capillary blood gases)	7.426 ± 0.022	7.444 ± 0.021	7.429 ± 0.016	7.455 ± 0.037	7.471 ± 0.020
pCO_2 [mmHg] (capillary blood gases)	33.8 ± 2.2	33.5 ± 1.0	32.7 ± 1.0	31.7 ± 1.9	28.9 ± 1.7
pO_2 [mmHg] (capillary blood gases)	74.7 ± 8.5	62.0 ± 2.6	52.3 ± 4.7	51.3 ± 3.1	49.0 ± 2.6

Data are presented as mean ± standard deviation or median and interquartile range as appropriate. EPO = erythropoietin; PTH = parathormone, FGF23 = fibroblast growth factor 23.

Traditional CKD-MBD ("chronic kidney disease – mineral and bone disorder") components that affect plasma FGF23 include phosphorus, calcium, parathormone, and vitamin D [7]. Lately, alternative regulators beyond traditional components of CKD-MBD have been identified, such as iron deficiency [8] and inflammation [9].

Iron deficiency and inflammation both activate hypoxia-inducible factor 1 (HIF1α) signaling, which may subsequently stimulate FGF23 expression [9]. Treating osteoblast-like cell lines with IL-1β results in an increased FGF23 production accompanied by increased cellular expression of HIF1α mRNA and nuclear HIF1α abundance [9]. Similarly, in rodent models, stimulation of HIF1α under hypoxic conditions increases circulating FGF23 levels [10].

Erythropoietin (EPO) stimulation may be a central intermediate that links hypoxia and subsequent FGF23 expression [11]. In murine experiments, EPO secretion preceded an increase in plasma FGF23 in experimentally induced hemorrhagic shock and sepsis, while EPO receptor blockade partly inhibited this FGF23 increase [12]. Moreover, recombinant human EPO directly induced an increase in plasma c-terminal FGF23 (which reflects intact FGF23 and inactive fragments [cFGF23]) and intact FGF23 (which mirrors only intact FGF23 [iFGF23]) [13].

However, it remains uncertain how far data from such rodent studies can be transferred to humans. An increase in plasma iFGF23 and cFGF23 was reported in four anemic patients who received a single dose of 20.000–40.000 IE recombinant EPO [14]. In contrast, plasma cFGF23 levels increased after a single infusion of 60.000 IE recombinant EPO in 32 healthy participants without a concomitant increase in plasma iFGF23 [13]. Of note, such (supra)pharmacological dosages of EPO yield plasma EPO levels that are far beyond normal physiological ranges. Whether a physiological increase in plasma EPO also provokes an increase in plasma FGF23 levels is unclear. Deciphering such pathways in human physiology is of clinical importance: if hypoxia and subsequent EPO stimulation were relevant stimulators of FGF23 in humans, FGF23 increase would be expected to occur under physiological conditions such as high-altitude exposure (to which many healthy individuals are exposed, e.g., during mountaineering or downhill skiing) and long-haul air travel (as commercial air flight exposes travelers to oxygen partial pressures, which equals a stay at 2656 MASL).

Against the background of the claimed effects of FGF23 on vascular [4] and myocardial cells [15], such temporary or persistent plasma FGF23 elevations might have direct cardiovascular sequelae. Additionally, these EPO-induced increases in FGF23 should cause hypophosphatemia, which might lead to severe complications such as neurological disorder [16], and – in case of long-term exposure to high altitude with a persistent FGF23 increase – osteomalacia or bone fractures [17,18]. Both the potential cardiovascular- and the phosphorus-lowering effects of FGF23 are required to be increased in physiologically active FGF23 rather than only an increase in inactive FGF23 fragments, which occurs in iron deficiency, where expression and degradation increase in parallel, so that plasma phosphorus is not affected [8].

Against this background, we aimed to analyze whether high-altitude exposure with hypoxia-induced EPO stimulation would increase both plasma cFGF23 and iFGF23 levels and thereby induce transient hypophosphatemia. To analyze the potential modifiers of such a transient hypophosphatemia, individuals were randomized to either normal or low-phosphorus diets and a single episode of physiological exercise was included, both of which have been claimed to affect FGF23 regulation. By analyzing the effects of diet and exercise, we aimed to allow a better transfer of our study findings to real-world scenario. Finally, to understand the potential consequences of the potential rise in FGF23, we measured a

TP6 Day 2 7 pm	TP7 Day 3 8 am	TP8 Day 3 7 pm	TP9 Day 4 6 am	TP10 Day 4 10 am	TP11 Day 4 6 pm	TP12 Day 5 9 am
2656	2656	2656	2656	758	233	233
17.6 [15.7; 22.6]	17.0 [14.5; 21.2]	19.8 [18.7; 20.2]	16.3 [14.8; 17.8]	16.6 [13.5; 19.9]	13.4 [9.3; 19.1]	8.1 [7.6; 8.9]
92.3 [78.6; 108.0]	60.4 [56.7; 63.7]	66.0 [58.5; 85.9]	67.4 [64.8; 74.0]	64.3 [60.3; 66.4]	67.3 [60.0; 68.1]	65.0 [58.4; 73.0]
39.4 [38.5; 40.9]	45.5 [43.0; 46.8]	43.3 [41.1; 43.7]	50.6 [49.0; 53.6]	46.0 [44.6; 49.1]	44.0 [42.8; 48.0]	55.0 [53.5; 62.8]
22.0 [19.3; 28.3]	24.5 [22.5; 33.8]	25.5 [22.5; 35.3]	29.0 [26.3; 34.3]	23.5 [23.0; 32.0]	25.5 [22.8; 33.8]	28.0 [23.3; 35.0]
–	–	–	59.5 [54.0; 61.0]	–	–	55.5 [48.8; 63.0]
32.8 [31.0; 33.8]	34.4 [32.8; 34.6]	34.5 [33.4; 34.9]	33.0 [31.6; 33.4]	32.6 [30.9; 33.8]	31.6 [29.5; 33.7]	32.6 [30.8; 33.4]
3.2 [2.8; 3.5]	3.7 [3.4; 4.0]	4.0 [3.6; 4.3]	4.2 [4.0; 4.2]	3.4 [3.4; 3.6]	4.2 [4.1; 4.2]	3.7 [3.5; 4.0]
0.07 [0.05; 0.10]	0.37 [0.31; 0.42]	0.67 [0.60; 0.76]	0.49 [0.49; 0.53]	0.29 [0.23; 0.30]	0.65 [0.56; 0.69]	0.41 [0.35; 0.52]
2.48 ± 0.10	2.35 ± 0.06	2.43 ± 0.05	2.35 ± 0.06	2.35 ± 0.06	2.40 ± 0.08	2.35 ± 0.06
0.05 ± 0.04	0.02 ± 0.01	0.04 ± 0.02	0.02 ± 0.01	0.06 ± 0.02	0.03 ± 0.01	0.02 ± 0.01
7.461 ± 0.005	7.464 ± 0.019	7.457 ± 0.003	7.458 ± 0.018	7.467 ± 0.010	7.454 ± 0.016	7.440 ± 0.013
29.4 ± 1.6	29.5 ± 0.9	29.6 ± 1.2	29.3 ± 2.2	29.1 ± 3.7	30.5 ± 2.5	31.8 ± 2.6
55.7 ± 2.5	56.7 ± 1.5	54.3 ± 4.5	52.7 ± 5.1	71.3 ± 10.4	73.7 ± 4.2	77.3 ± 10.2

broad spectrum of other CKD-MBD components during the study period.

Methods

To evaluate whether FGF23 levels can be influenced by high-altitude exposure, a field experiment was performed. Eight healthy volunteers living in Homburg, Germany (233 m above sea level [MASL]) spent four days at the "Schneefernerhaus", an environmental research station located at a height of 2656 MASL in the German Alps, localized directly below the summit of the Zugspitze. These healthy volunteers were randomized to either a normal phosphorus diet (n = 4; 1300−1400 mg phosphorus/day) or a low-phosphorus diet (n = 4, 700−800 mg phosphorus/day). To standardize phosphorus intake across the entire observation period, participants started the standardized normal phosphorus diet or the low-phosphorus diet seven days before the field experiment, and phosphorus intake was maintained stable during both times, i.e., the high-altitude stay and the first 24 h after descending to 233 MASL.

The mean atmospheric air pressure at 2656 MASL is 740 hPa (155.03 hPa oxygen partial pressure). As we assumed an atmospheric air pressure of 1012 hPa (212.01 hPa oxygen partial pressure) at 233 MASL, this is a 27% reduction in oxygen partial pressure at the "Schneefernerhaus" compared to that in our outpatient clinic in Homburg, Germany.

Before, during, and after high-altitude exposure, blood and urine samples were collected at two, seven, and three time points (TPs), respectively (see Table S2 for further details), for measurements of plasma cFGF23 and iFGF23. To understand potential physiological implications of the increase in FGF23, we additionally measured 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, intact parathormone, and EPO, as well as plasma and urine phosphorus and calcium, all of which could be affected by changing plasma levels of FGF23. Additionally, we performed a blood gas analysis to gain information of pH, pCO2, and pO2. At day 2, maximal aerobic exercise was performed as a five-hour high-altitude hike, directly before TP 6.

Blood samples were immediately centrifuged, and plasma and urine samples were frozen. After return from the research station, all vials were stored at −80 °C until measurement at Saarland University Medical Center in Homburg, Germany.

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

Detailed inclusion and exclusion criteria are summarized in the supplementary information.

Both iFGF23 and cFGF23 were measured from plasma samples by second-generation ELISA (Immutopics, San Clemente, USA). All other metabolites were measured according to the standardized methods of the Saarland University central laboratory.

Statistical analyses were performed with SPSS 20. Continuous data are presented as mean ± standard deviation or as median and interquartile range [IQR] in case of skewed distribution and were compared using *t-test*. Comparison as time progressed was performed using a linear mixed model, with prespecified TPs as the dependent variable and high altitude as the independent variable. Time intervals in the collection of blood samples were considered as co-variables. Two-sided p values < 0.05 were considered significant.

Table 1b Plasma levels of CKD-MBD metabolites at different time points (TP1 to TP12) of all participants (n = 4) *on a low-phosphorus diet*.

	TP1 Day 1 6 am	TP2 Day 1 12 am	TP3 Day 1 4 pm	TP4 Day 1 8 pm	TP5 Day 2 7 am
Altitude (meters above sea level)	233	758	2656	2656	2656
EPO [mIU/ml]	6.6 [6.0; 8.2]	6.4 [5.9; 8.2]	6.7 [6.0; 9.2]	12.0 [10.0; 14.1]	21.3 [19.5; 23.8]
C-terminal FGF23 [RU/ml]	59.6 [52.0; 67.7]	55.4 [48.3; 59.9]	61.1 [56.1; 66.4]	83.5 [59.0; 123.1]	67.1 [58.3; 84.8]
Intact FGF23 [pg/ml]	108.4 [57.7; 159.2]	101.5 [55.6; 144.9]	100.9 [62.3; 141.3]	99.4 [56.7; 143.7]	103.3 [66.0; 144.5]
Intact PTH [pg/ml]	32.5 [30.5; 39.0]	33.0 [29.5; 35.3]	31.5 [27.5; 36.5]	34.5 [30.5; 39.5]	36.0 [34.0; 41.5]
1,25-Dihydroxyvitamin D [ng/l]	54.0 [50.8; 57.8]	–	–	–	60.5 [52.0; 64.8]
25-Hydroxyvitamin D [ng/ml]	29.3 [26.8; 31.4]	30.8 [26.9; 33.8]	32.4 [29.5; 33.1]	29.0 [26.7; 32.6]	30.2 [26.2; 34.1]
Phosphorus [mg/dl]	4.2 [4.0; 4.5]	3.8 [3.7; 4.0]	3.5 [3.4; 3.7]	3.6 [3.6; 3.6]	4.2 [4.1; 4.3]
Urine phosphorus/urine creatinine	0.54 [0.44; 0.64]	0.35 [0.28; 0.37]	0.20 [0.14; 0.30]	0.11 [0.09; 0.18]	0.40 [0.29; 0.46]
Calcium [mmol/l]	2.35 ± 0.06	2.43 ± 0.05	2.43 ± 0.13	2.40 ± 0.08	2.40 ± 0.08
Urine calcium/urine creatinine	0.01 ± 0	0.02 ± 0.01	0.01 ± 0	0.02 ± 0.01	0.01 ± 0.01
pH (capillary blood gases)	7.429 ± 0.017	7.404 ± 0.039	7.427 ± 0.012	7.458 ± 0.018	7.475 ± 0.045
pCO_2 [mmHg] (capillary blood gases)	36.0 ± 0.9	40.1 ± 6.9	37.1 ± 1.8	34.4 ± 3.1	32.6 ± 3.3
pO_2 [mmHg] (capillary blood gases)	72.5 ± 23.3	59.5 ± 14.8	47.0 ± 5.7	49.0 ± 9.9	47.0 ± 5.7

Data are presented as mean ± standard deviation or median and interquartile range as appropriate. EPO = erythropoietin; PTH = parathormone, FGF23 = fibroblast growth factor 23.

Results

Six women and 2 men participated, none of whom was an active smoker. Participants who were randomized to a normal phosphorus diet had higher levels of cFGF23 (72 RU/ml [65; 76] vs. 60 RU/ml [52; 68]) and lower levels of iFGF23 (50 pg/ml [46; 75] vs. 108 pg/ml [58; 166]) at the baseline blood drawing scheduled seven days after initiation of the dietary interventions. Their plasma phosphorus levels were slightly lower (3.9 mg/dl [3.7; 4.3] vs. 4.2 mg/dl [4.0; 4.5]), and their urine phosphorus/urine creatinine levels were higher (0.58 [0.39; 0.68] vs. 0.54 [0.44; 0.64]) than those in the low-phosphorus diet group. Further baseline characteristics are presented in detail in Table S1. There were no statistically significant differences between the two intervention groups.

During the stay at 2656 MASL, none of the participants developed acute high-altitude sickness, and no participant left the research station prematurely.

As expected, the EPO level increased compared to that of the baseline soon after arriving at 2656 MASL in both groups. After descending back to 233 MASL, the plasma EPO level returned to baseline (Tables 1a and b). In line, levels of pO2 and pCO2 of the blood gas analysis decreased at high altitude, and pH levels increased steadily corresponding to respiratory alkalosis reaching its highest values at TP 5 (7.471 ± 0.020) in the normal phosphorus diet group and at TP 7 (7.480 ± 0.027) in the low-phosphorus diet group. In contrast, we observed no consistent increase in either cFGF23 or iFGF23. Levels of cFGF23 (but not those of iFGF23) differed during the study period, with a single peak at TP 6 (92.3 RU/ml [78.6; 108.0] in the normal phosphorus diet arm vs. 187.6 RU/ml [170.6; 242.3] in the low-phosphorus diet arm) after five hours of maximal aerobic exercise (Tables 1a and b). Interestingly, this strenuous physical exercise additionally provoked a drop in urinary phosphorus excretion and a rise in urinary calcium excretion in both groups. Plasma phosphorus dropped at TP 6, whereas the plasma calcium level slightly increased at TP 6, but no consistent changes were induced by high-altitude exposure in both groups (Tables 1a and b). In the low-phosphorus diet group, but not in the normal phosphorus diet group, high-altitude exposure tended to result in an increase in 1,25-dihydroxyvitamin D plasma levels and intact parathormone levels (Tables 1a and b).

In the linear mixed model analysis, plasma EPO, serum calcium, and urinary calcium/creatinine excretion were significantly associated with high-altitude exposure in the normal phosphorus diet group, whereas plasma EPO, cFGF23, iFGF23, urinary phosphorus excretion, and urinary calcium/creatinine excretion were significantly associated with high-altitude exposure in the low-phosphorus diet group (Tables 2a and b). Plasma 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, and intact parathormone did not change significantly during high-altitude exposure in these models.

Discussion

Recent preclinical data suggest EPO to be a central regulator of FGF23 expression [11,12]. If these experimental findings were of clinical relevance, then conditions associated with hypoxia-induced EPO expression such as high-altitude exposure would be expected to increase plasma FGF23. As FGF23 has been associated with the induction of left ventricular hypertrophy [15] and subsequent cardiovascular events [4], such an increase in plasma FGF23 might become a cardiovascular concern. Moreover, EPO-induced FGF23 expression may induce changes in mineral and bone metabolism, particularly hypophosphatemia.

In our field experiment conducted at a high-altitude research station, we, however, did not observe a consistent increase in cFGF23 and iFGF23 despite a significant rise in plasma EPO.

Only a single peak of cFGF23 could be detected at TP 6, directly after five hours of hiking, whereas levels of iFGF23

TP6 Day 2 7 pm	TP7 Day 3 8 am	TP8 Day 3 7 pm	TP9 Day 4 6 am	TP10 Day 4 10 am	TP11 Day 4 6 pm	TP12 Day 5 9 am
2656	2656	2656	2656	758	233	233
23.6 [18.9; 26.8]	19.9 [15.4; 22.6]	15.8 [13.0; 18.4]	13.7 [13.0; 16.2]	13.6 [12.3; 17.0]	11.1 [8.7; 14.4]	5.3 [4.3; 6.9]
187.6 [170.6; 242.3]	75.4 [61.1; 96.9]	72.9 [54.0; 111.6]	86.0 [67.3; 105.9]	90.7 [70.8; 114.3]	72.3 [61.6; 78.3]	74.8 [65.4; 78.6]
97.1 [53.7; 144.7]	109.7 [67.0; 149.2]	96.9 [57.5; 132.5]	108.8 [59.5; 155.6]	97.9 [50.9; 143.1]	98.8 [51.6; 147.8]	103.9 [58.7; 153.2]
28.5 [26.8; 31.3]	36.5 [31.8; 39.0]	44.5 [37.3; 49.0]	41.5 [38.5; 44.5]	35.0 [33.3; 41.5]	43.0 [37.5; 47.5]	36.0 [34.0; 37.3]
–	–	–	62.5 [48.8; 71.3]	–	–	64.0 [55.8; 69.8]
29.5 [27.8; 30.1]	28.1 [25.5; 31.7]	28.8 [26.4; 31.5]	27.6 [25.2; 29.3]	28.6 [24.7; 33.0]	27.6 [25.3; 31.7]	29.8 [26.3; 32.9]
3.6 [3.5; 3.7]	3.8 [3.7; 3.9]	3.9 [3.6; 4.2]	4.1 [4.0; 4.2]	3.6 [3.3; 3.9]	4.0 [3.9; 4.2]	4.0 [3.9; 4.1]
0.08 [0.05; 0.11]	0.22 [0.21; 0.27]	0.40 [0.36; 0.50]	0.42 [0.41; 0.43]	0.16 [0.13; 0.18]	0.36 [0.31; 0.44]	0.38 [0.28; 0.44]
2.45 ± 0.08	2.38 ± 0.10	2.48 ± 0.13	2.38 ± 0.10	2.38 ± 0.10	2.35 ± 0.06	2.30 ± 0.08
0.04 ± 0.02	0.01 ± 0.01	0.02 ± 0	0.01 ± 0.01	0.03 ± 0.03	0.02 ± 0.01	0.01 ± 0.01
7.450 ± 0.016	7.480 ± 0.027	7.424 ± 0.051	7.456 ± 0.013	7.440 ± 0.014	7.442 ± 0.012	7.430 ± 0.009
32.7 ± 3.8	29.4 ± 1.5	34.5 ± 6.2	29.8 ± 0.7	31.7 ± 0.5	31.3 ± 0.4	33.3 ± 0.2
49.5 ± 7.8	53.5 ± 9.2	51.5 ± 7.8	57.7 ± 10.6	55.5 ± 4.9	65.0 ± 11.3	71.0 ± 8.5

remained unchanged. Apparently, increased FGF23 production was coupled with increased FGF23 cleavage, so that only inactive fragments may have accumulated, which are measured by cFGF23 assays but not with iFGF23 assays [8]. This FGF23 peak was not followed by increased urinary phosphorus excretion or a drop in plasma 1,25-dihydroxyvitamin D, both of which are physiological FGF23 effects. This is in line with the results of rodent data: rats that were housed in a hypobaric atmosphere for two weeks had significantly elevated plasma cFGF23 but normal plasma iFGF23 and normal plasma phosphorus levels [10].

Thus, high-altitude exposure does not affect plasma cFGF23 in healthy volunteers at rest. We recently reported that submaximal exercise and high-intensity exercise do not increase FGF23 at sea levels [19], and murine data provide no consistent evidence for exercise-induced FGF23 expression [20,21]. Apparently, the combination of high-altitude exposure and exercise might be a stimulus to induce FGF23 expression (and its subsequent cleavage). Interestingly, *Lombardi* et al. reported elevated plasma iFGF23 in cyclists who participated in the Giro d'Italia, which notably also includes several days of high-altitude exposure [22]. Evidently, these two exercise models differed substantially, and cyclists participating in the Giro d'Italia consumed 3874.97 mg/day phosphorus, which is more than double than our participants did. Moreover, some information of the TPs of venipuncture has been presented by Lombardi et al. [22] Therefore, additionally, standardized exercise protocols are necessary to prove or reject the hypothesis that exercise at high altitude will induce FGF23 expression.

Table 2a Effect of high-altitude exposure on changes in the levels of CKD-MBD metabolites with time in all participants (n = 4) on a normal phosphorus diet.

	Estimate	CI	p
EPO [mIU/ml]	8.682	6.690; 10.939	<**0.001**
C-terminal FGF23 [RU/ml]	0.472	−6.385; 10.150	0.642
Intact FGF23 [pg/ml]	−1.827	−11.391; 0.717	0.081
Intact PTH [pg/ml]	0.228	−5.793; 7.228	0.822
1,25-Dihydroxyvitamin D [ng/l]	0.111	−13.152; 14.532	0.914
25-Hydroxyvitamin D [ng/ml]	1.015	−0.922; 2.720	0.319
Phosphorus [mg/dl]	−1.921	−0.574; 0.022	0.068
Urine phosphorus/urine creatinine	1.356	−0.036; 0.173	0.190
Calcium [mmol/l]	2.118	0.001; 0.058	**0.049**
Urine calcium/urine creatinine	0.011	−0.017; −0.003	**0.007**
pH	1.178	−0.007; 0.025	0.262
CO_2	−1.445	−3.664; 0.710	0.170
O_2	−9.473	−15.144; −9.445	<**0.001**

Bold signifies $p < 0.05$.

Table 2b Effect of high-altitude exposure on changes in the levels of CKD-MBD metabolites with time in all participants (n = 4) on a low-phosphorus diet.

	Estimate	CI	p
EPO [mIU/ml]	3.841	2.147; 7.169	**0.001**
C-terminal FGF23 [RU/ml]	2.278	1.236; 27.307	**0.033**
Intact FGF23 [pg/ml]	0.997	−33.081; 33.219	**0.004**
Intact PTH [pg/ml]	1.036	−2.146; 6.330	0.314
1,25-Dihydroxyvitamin D [ng/l]	−0.101	−16.580; 15.130	0.921
25-Hydroxyvitamin D [ng/ml]	−0.340	−3.012; 2.153	0.736
Phosphorus [mg/dl]	−1.721	−0.378; 0.049	0.116
Urine phosphorus/urine creatinine	2.320	0.005; 0.158	**0.037**
Calcium [mmol/l]	1.710	−0.007; 0.084	0.097
Urine calcium/urine creatinine	−2.803	−0.009; −0.001	**0.031**
pH	2.989	0.008; 0.042	**0.007**
CO_2	−0.884	−1.934; 0.784	0.387
O_2	−4.145	−16.079; −4.914	**0.002**

Bold signifies $p < 0.05$.

During high-altitude exposure, we additional found a transient drop in plasma phosphorus and urinary phosphorus excretion, which might potentially be explained by respiratory alkalosis and a subsequent acute transcellular phosphorus shift into myocytes [23].

It was postulated that high-altitude exposure may also affect conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D and subsequently PTH secretion [24]. We did not see any consistent changes in these hormones in our study; in line, plasma calcium remained within physiological ranges throughout our study. In a recently published work, Luehker O et al. described the difficult interpretation of changes in acid–base homeostasis when exercise and hypoxia are coincident [25]. Conforming to our data, plasma calcium levels increased during exercise and hypoxia as compared to plasma calcium levels at rest [25]. Increased levels of plasma calcium were also observed by two other previously published trials performed at high altitude. We may only speculate that this increase in plasma calcium is due to a decline in bone turnover [26].

Against our expectation, randomization of individuals into a low- or normal phosphorus diet group did not substantially affect the study findings. Interestingly, randomization into a low-phosphorus diet arm did not decrease plasma phosphorus during the study period; potential limitations of our approach are discussed below. Earlier studies in this field, which had a similar study size, yielded partly inconsistent results [27,28]. Admittedly, we cannot rule out that reducing dietary phosphorus in a more aggressive manner or for a longer time period might have affected FGF23. Moreover, we did not quantify phosphorus intake before our intervention or design a run-in period with the same phosphorus supplementation in both groups.

Consistent with our results, Mehta et al. reported that patients suffering from sleep-disordered breathing manifested no differences in iFGF23 and cFGF23 relative to controls, although the authors noted that the severity of hypoxia may also have been insufficiently severe to demonstrate an effect [29]. Nevertheless, we performed a study in sustained hypoxia, whereas sleep-disordered breathing is known to be intermittent hypoxia [24]. We cannot exclude that more prolonged or more severe hypoxia – either induced at altitudes higher than 4000 MASL for longer durations or by pathophysiological states such as lung disease – might affect plasma FGF23. Furthermore, as mentioned above, pharmacological interventions elevate plasma EPO to levels substantially higher than those after high-altitude exposure [30].

Several limitations of our trial should be discussed. First, the number of participants was limited, and this resulted in inclusion of a low number of participants in the two randomization groups. Research at high altitude is challenging, and study sizes are restricted both by the availability of healthy volunteers willing to stay several days at a remote research station and by the limited capacity of this high-altitude station to accommodate volunteers. Despite this limited study size, we aimed to assess the effect of three different modifiers on plasma FGF23: high-altitude exposure, phosphorus intake, and exercise. We therefore aimed to provide a more comprehensive analysis, which considers major physiological confounders. It may be claimed, however, that this approach was overzealous to some degree.

Next, unfortunately, we did not measure cFGF23 and iFGF23 under a standard diet before randomization to normal and low-phosphorus diets. Instead, baseline blood samples were collected after seven days of standardized diet, as we focused our analysis on the comparison of CKD-MBD components at sea level and at high altitude in individuals with low- or normal phosphorus intake, rather than on a direct comparison of standardized low- and/or normal phosphorus diet with a standard diet. Thus, we cannot exclude that the two dietary groups differed in plasma cFGF23, iFGF23, and phosphorus before dietary intervention.

Finally, despite a broad array of laboratory measurements, we did not assess plasma magnesium, calcitonin, or bicarbonate nor FGF23 mRNA or HIF1α mRNA, all of which might have provided complementary information.

In summary, we provide data on the effect of high-altitude exposure with a subsequent increase in plasma EPO upon iFGF23, cFGF23, and other CKD-MBD components in healthy individuals randomized to low- or normal phosphorus intake. We found that high-altitude exposure alone does not affect FGF23 expression or FGF23 degradation, suggesting an accumulation of inactive FGF23 fragments. Subsequently, this peak was not followed by urinary phosphorus losses.

Conflicts of interest

None.

Acknowledgments

The results presented in this paper have not been published previously in whole or part.

Our research work meets the WHO criteria of clinical trials, and it was published on 14 August 2017 in the German Clinical Trials Register (registration number: DRKS 000 12 771).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2019.09.002.

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