Mineral Intake and Clinical Symptoms in Adult Patients with Hypophosphatasia

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Background: Hypophosphatasia (HPP) is a rare inherited metabolic disorder characterized by deficient activity of the tissue-nonspecific alkaline phosphatase entailing impaired turnover of phosphorus metabolites. Dietary mineral intake is suspected to influence clinical symptoms of HPP, but scientific evidence is missing.

Methods: Cross-sectional matched-pairs study collecting comprehensive data on nutrient intake in 20 HPP patients and 20 unaffected, age- and gender-matched controls. Dietary information and clinical symptoms were documented in detail over 7 consecutive days using structured diaries.

Results: Baseline data and type of energy-supplying nutrients were balanced between both groups. Median nutritional intake of phosphorus and calcium were significantly lower in HPP patients versus controls, which is partially attributable to lower energy consumption in HPP patients. Differences regarding phosphorus and calcium (Ca/P) ratio and uptake of magnesium, zinc, and vitamin B6 were not statistically significant. Both high (≥ 1375 mg/d) and low intakes (< 1100 mg/d) of phosphorus were significantly associated with an increased frequency of neuropsychiatric symptoms ($P = 0.02$). Similarly, very high and very low intake of calcium was significantly associated with musculoskeletal ($P < 0.01$), gastrointestinal ($P = 0.02$), and neuropsychiatric ($P < 0.001$) symptoms. An increased Ca/P ratio was associated with increased tiredness/fatigue ($P < 0.01$), whereas a decreased Ca/P was associated with gastrointestinal issues ($P = 0.01$).

Conclusion: Phosphorus and calcium intake seem reduced in HPP patients along with reduced total energy consumption. Particularly high as well as very low absolute or unbalanced phosphorus and calcium intake are associated with an increased frequency of clinical symptoms. **(*J Clin Endocrinol Metab* 105: e2982–e2992, 2020)**

Key Words: hypophosphatasia, nutritional intake, phosphorus intake, calcium-phosphorus ratio

Hypophosphatasia (HPP) is a rare, inherited metabolic disorder caused by mutations of the ALPL gene on chromosome 1p36.12, coding for the tissue nonspecific alkaline phosphatase (1). The resulting deficiency of alkaline phosphatase (ALP) activity leads to symptoms in various organs and tissues with highly variable individual expression. Severe, potentially life-threatening forms with a presumed incidence of 1 in 300,000 typically manifest perinatally (2, 3), whereas milder forms with an estimated incidence of 1 in 6370 may be much more common (2).

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Abbreviations: AI, adequate intake; ALP, alkaline phosphatase; BMI, body mass index; EN%, energy percent; FGF, fibroblast growth factor; HPP, hypophosphatasia; PEA, phosphoethanolamine; Pi, inorganic phosphate; PLP, pyridoxal-5-phosphate; PRI, population reference intake; RI, reference intake; TmP/GFR, tubular maximum of reabsorption of Pi; Vit B6, vitamin B6

Musculoskeletal issues, including inflammatory pain perceived in bones, joints, and muscles, diminished physical performance capacity along with accelerated muscular fatigue, (stress-) fractures, and soft-tissue calcification are common findings in adult patients. Furthermore, gastrointestinal symptoms, dental abnormalities, chronic fatigue, mental complaints, and severe headaches are being reported (4-6).

Characteristic laboratory findings include reduced ALP activity in serum along with elevated levels of pyridoxal-5-phosphate (PLP), inorganic pyrophosphate, and urinary phosphoethanolamine (PEA). In addition, high serum levels of inorganic phosphate (Pi) are a common finding in adults with HPP (7-9).

Phosphorus is an essential nutrient and plays an important role in the mineralization of bones and teeth, cells' energy cycle, cell structure, signaling, and acid-base balance (10). It naturally occurs in almost every food and is widely used as a food additive for technological purposes in the food industry (11). The main sources are grain products, meat, poultry, fish, milk, and dairy products (12). Because of its abundance in the Western diet, the European value for adequate intake (AI) of 550 mg/d and the Recommended Dietary Allowance of 700 mg/d for adults both in the US and in Germany are typically exceeded in the general population and alimentary deficits are rare (11-14).

High phosphorus intake may favor hyperphosphatemia and potentially affect certain symptoms in adult HPP patients. An elevated calcium phosphate product is supposed to promote the precipitation of calcium phosphate crystals. The deposition of such crystals in soft tissues, such as tendons and bursae, can lead to inflammation, pain, and functional limitations (15-17), and calcific periarthritis is a common finding in HPP patients (18, 19). Excessive phosphorus consumption can adversely affect bone health. Animal studies show bone loss upon high phosphorus diet from an increase in parathyroid hormone (PTH) (20-24) and an increase of PTH following phosphate loading is also well-documented in humans, both as an immediate early regulatory mechanism and upon chronic phosphate loading (25-27). Gastrointestinal symptoms, such as intestinal distress, soft stools, and mild diarrhea, were detected after high phosphorus diet enriched with phosphate additives (28, 29). Additionally, Pi is a potential inhibitor of ALP activity (30), and high phosphorus intake as well as elevated serum phosphorus levels are associated with decreased bone-specific alkaline phosphatase activity (31). Zn^{2+} and Mg^{2+} are essential cofactors for appropriate enzyme activity and deficient intake of these elements is presumed to compromise enzyme activity (3).

Accordingly, dietary mineral intake, specifically nutritional phosphorus, could play an important role in HPP-related symptoms, but data on nutritional phosphorus intake in HPP patients and a potential impact of dietary phosphorus on disease-related symptoms are still lacking.

Methods

Design and setting of the study

The single-center, cross-sectional study was conducted in collaboration by the Institute of Food Science and Human Nutrition, Leibniz University Hannover, Germany, and the Orthopedic Institute, Koenig-Ludwig-Haus, University Wuerzburg, Germany. The study protocol was approved by the responsible Ethics Committee of the Medical Faculty of the University of Wuerzburg (N. 6/18). Written informed consent was obtained from all participants before any study-related procedures. The study is registered with the Germany Register for Clinical Trials (DRKS00015225).

Participants

The HPP patients were recruited at the Orthopedic Institute, University of Wuerzburg, Germany. The control group was recruited by participating HPP patients inviting clinically healthy age- and gender-matched peers among their families and friends to account for social and regional eating habits.

Selection criteria for patients were age ≥ 18 years and an established diagnosis of Hypophosphatasia, defined by reduced serum ALP activity below the age- and sex- specific reference range plus one of the following findings:

- Genetically confirmed ALPL mutation
- Elevated serum/plasma PLP (above upper limit of normal)
- Symptoms of the disease

Inclusion criteria for participants in the control group were age ≥ 18 years and absence of Hypophosphatasia. Exclusion criteria for both groups were any diagnosis of a severe chronic disease (i.e., chronic kidney disease 4/5, oncologic disease within < 5 years, coagulopathy), known gastrointestinal disorders (previous gut surgery, inflammatory bowel disease, malabsorption/chronic diarrhea/constipation) or medications that might interfere with phosphorus metabolism, specifically phosphate binders, laxatives, any antiosteoporotic medication, or enzyme replacement therapy for HPP.

Documentation of nutrition and symptoms

Subjects documented their food intake comprehensively, including drinks, snacks, and candies, in a dietary record (hardcopies) for 7 consecutive days. Consumed food had to be described as detailed as possible (variety, brand name/manufacturer, fat content). The quantities were to be calculated with a kitchen scale or, if this was not feasible, normal household sizes had to be indicated. Participants were also asked to provide the recipes of home-cooked food. The intake of medications and nutritional supplements as well as health issues and symptoms were documented every evening. Symptoms could be picked from a selection list of issues frequently described in HPP or noted as a free text. The German and English versions of the dietary record

are publicly available (32). All documents were checked for completeness, readability, and plausibility by a nutritionist and, if necessary, ambiguities were clarified. The symptoms were grouped into the following categories for statistical analysis:

- Musculoskeletal symptoms (including joint pain, joint swelling, muscle pain, muscle cramp, tired legs, bone pain, full body pain)
- Gastrointestinal symptoms (loss of appetite, diarrhea, nausea, constipation, flatulence)
- Neuropsychiatric symptoms (migraine, headache, brain fog, concentration disorders, mental complaints, paresthesia, restless legs, sleep disorders)
- Exhaustion/tiredness/fatigue

Each symptom was counted only once per day and participant. If several symptoms from 1 category were reported, these were added up. There was no weighting regarding the severity of the complaints reported.

Data analysis and statistical methods

Electronic recording of the dietary data and calculation of the nutrient intake were carried out using PRODI 6.7 expert, including database extension (Nutri Science GmbH, Freiburg, Germany). Foods that were not included in the database were researched regarding their composition and entered into the database. Similarly, the manufacturer's information on ingested dietary supplements were determined and added. Medicines were not included in the evaluation.

The totalized values for each category were related to the individual nutrient intake per day using crosstabs to delineate potential associations of diet with clinical symptoms. The nutrient intakes were ranged in intervals based on the dietary reference values for consistency. Fisher exact test and the Freeman-Halton test were used, assuming independence of the data sets, to test the dependence between the nutrient intake and the symptoms. The daily numbers of specific symptoms and number of symptoms per category were added up to give a weekly result for each participant to evaluate the absolute frequency of symptoms. Graphical evaluation was performed using Microsoft Excel (version 2019, Microsoft Corporation, Redmond, WA). The statistical analyses were performed using IBM SPSS software (version 25, IBM, Armonk, NY). Values of $P \leq 0.05$ were considered statistically significant.

The average nutrient intake per day was calculated for each subject from the 7-day food diary. No test for normal distribution was performed because of the small sample size. The data were examined by the Wilcoxon-Mann-Whitney test to compare HPP patients and controls concerning the nutrient intake. The dietary reference values for the European population were used to check if there was a sufficient supply.

The data are presented as medians (ranges) because of the small sample size. For categorical variables, the number of subjects (n) is given.

Results

Study population

Of 42 subjects enrolled, 40 (17 females + 3 males per group) returned their study documentation complete. Overall, there were no significant differences between either group regarding age, height, weight, and body mass index (BMI). Details are provided in Table 1.

A mixed diet was the predominant form of nutrition in both groups. In the HPP group, there were 3 ovo-lacto-vegetarians, 2 of whom also consumed fish. Habitual use of dietary supplements reported by 7 of 20 HPP patients and 5 of 20 controls was continued and the constituents included in the analysis. One HPP patient and 3 controls were smokers.

Intake of energy and energy-supplying nutrients

Absolute energy intake was higher in men than women in both groups. Despite similar constitution regarding height, weight, and BMI, the total energy intake was about 10.8% lower in HPP patients with a borderline significance ($P = 0.051$). In female participants, this difference was significant with a median (range) energy intake of 8.46 (6.56-14.3) MJ/d in controls and 7.43 (4.56-10.6) MJ/d in female HPP patients ($P = 0.02$). The composition of energy-supplying nutrients regarding the proportion of carbohydrates, protein, and fat was not significantly different and there was a disproportion favoring fat against carbohydrates in both groups. A total of 70% (n = 14) of participants in each group were below the lower threshold of the reference intake range (RI) of > 45 energy percent (EN%) for carbohydrates (33). Conversely, 85% (n = 17) of HPP patients and 75% (n = 15) of controls exceeded the upper threshold RI of 35 EN% for fat (34). The absolute and percentage intake of fat did not differ significantly between both groups and none of the participants had a percentage fat intake < 20 EN%.

There were no significant differences in the absolute and relative protein intake between both groups. However, the absolute protein intake in female controls was significantly higher than in female HPP patients ($P = 0.03$). All participants achieved the age-specific population reference intake (PRI) for protein, except for 5 female HPP patients (35).

Absolute fiber intake did not differ significantly between both groups and the majority of participants, 70% (n = 14) in the HPP group and 75% (n = 15) in the control group, did not attain the recommended amount for AI of at least 25 g fiber per day (33). Data about the intake of energy and energy-supplying nutrients are given in Table 2.

Intake of phosphorus, calcium-phosphorus ratio, and calcium

Median daily phosphorus intake was significantly higher in the control group compared with HPP patients ($P = 0.01$). Absolute median (range) amounts were 1374

Table 1. Characterization of the Study Population

	HPP Patients	Controls	*P* Value Group Comparison[a]
	(n = 20)	(n = 20)	(n = 40)
Age (years)			0.87
Median (range)	52.5 (26.0-61.0)	53.0 (27.0-62.0)	
Height (cm)			0.36
Median (range)	168 (156-182)	169 (158-186)	
Weight (kg)			0.79
Median (range)	66.5 (52.0-108)	68.5 (51.0-100)	
BMI (kg/m^2)			0.67
Median (range)	24.9 (19.1-34.9)	24.0 (19.2-34.5)	
Age at HPP diagnosis (years)			
Median (range)	49.0 (0.00-60.0)		
Form of nutrition			
Mixed diet (n)	17	20	
Ovo-lacto-vegetarian diet with fish (n)	2	0	
Ovo-lacto-vegetarian diet (n)	1	0	
Vegan (n)	0	0	
Nutritional supplement users during time of 7-day dietary record (n)	7	5	
Smokers (n)	1	3	

[a]Wilcoxon-Mann-Whitney test.

(946-2587) mg/d and 988 (570-1893) mg/d in the control group and HPP patients, respectively, indicating that phosphorus intake was 39% higher in control subjects. Men had higher phosphorus intake than women in both groups. All subjects attained the AI of > 550 mg phosphorus per day (13). Dietary phosphorus related to the energy intake was also lower in HPP patients than in controls, though this difference narrowly missed statistical significance (P = 0.06). Calcium intake was significantly lower in HPP patients (P = 0.04), with a median (range) calcium intake of 1089 (470-1865) mg/d in controls and 737 (263-1607) mg/d in HPP patients. There was generally no significant difference between both groups regarding calcium intake relative to energy intake, but 30% (n = 6) of the controls and 75% (n = 15) of the HPP patients did not reach the PRI of 950 mg calcium per day (36).

The intake of phosphorus was generally higher than the calcium intake in both groups and the calcium-phosphorus ratio did not differ significantly, with 0.74 and 0.75 in the HPP and control group, respectively. Only 3 HPP patients and none of the controls attained an average calcium-phosphorus ratio ≥ 1. Details are given in Table 3.

Intake of magnesium, zinc, and vitamin B6

The intake of magnesium and vitamin B6 (Vit B6) tended to be slightly lower in HPP patients, especially in women, compared with controls, but there were no significant differences between both groups. However, 40% (n = 8) of the HPP patients and 15% (n = 3) of the controls did not attain the gender-specific AI for magnesium (37). The intake of zinc was significantly higher in the subgroup of female controls compared with female HPP patients (P = 0.04), but there was generally no significant difference. Based on a phytate intake of 300 mg/d, 5 HPP patients and 1 control did not achieve the gender-specific PRI for zinc (38), whereas 55% (n = 11) of HPP patients and 35% (n = 7) of controls did not attain the PRI at a phytate intake of 600 mg/d. The nutritional Vit B6 intake was below gender-specific PRI for Vit B6 (39) in 60% (n = 12) of HPP patients and 50% (n = 10) of the controls. Details are provided in Table 3.

Symptoms

The frequency of clinical symptoms was collected by both incidence and adding up the days with a specific symptom per patient in both groups. Although only a few symptoms were reported in the control group (n = 19 in total), HPP patients documented numerous health issues (n = 519). The median (range) total of reported symptoms per week was 0.0 (0-8) in controls and 28.0 (2-58) in HPP patients (P < 0.001) (i.e., none of the HPP patients was completely free of symptoms during this study), whereas this was true for 14 controls. About 51.6% (268/519) of complaints reported by HPP patients were musculoskeletal, specifically joint pain (88/268), muscle pain (81/268), and tired legs (48/268). The overall incidence of musculoskeletal symptoms was higher in female HPP patients than men, with a median (range) of 16.0 (0-26) versus 12.0 (2-14) symptoms per week, respectively (Fig. 1). Neuropsychiatric symptoms accounted for 135 of the 519 reported issues and were slightly more prevalent in men than in women (8.0 vs 6.0 per week). The most prevalent neuropsychiatric complaints in HPP patients were concentration

Table 2. Intake of Energy and Energy Supplying Nutrients Per Day

	HPP Patients	Controls	*P* Value Group Comparison[a]
	(n = 20)	(n = 20)	(n = 40)
Energy (MJ)			0.051
Median (range)	7.63 (4.56-12.8)	8.55 (6.56-14.3)	
Carbohydrates (g)			0.12
Median (range)	179 (97.3-361)	197 (153-378)	
Carbohydrates (EN%)			0.65
Median (range)	42.1 (27.1-58.6)	40.6 (28.4-52.2)	
Carbohydrates < 45 EN%[b] (n)	14	14	
Protein (g)			0.07
Median (range)	63.7 (35.5-135)	78.6 (53.2-149)	
Protein (EN%)			0.57
Median (range)	14.7 (8.35-20.4)	15.4 (12.3-27.1)	
Protein < 62 g (m) / 52 g (f) respective < 61 g (m) / 55 g (f)[c] (n)	5	0	
Fat (g)			0.14
Median (range)	82.4 (40.3-145)	90.4 (63.3-134)	
Fat (EN%)			0.63
Median (range)	39.8 (24.8-49.9)	39.3 (27.4-48.3)	
Fat > 35 EN%[d] (n)	17	15	
Fiber (g)			0.37
Median (range)	18.4 (7.54-33.4)	20.0 (14.0-43.4)	
Fiber < 25 g[e] (n)	14	15	

[a]Wilcoxon-Mann-Whitney test.
[b]Lower bound of RI for adults (33).
[c]PRI for adults aged 18–59 years respective ≥ 60 years (35).
[d]Upper bound of RI for adults (34).
[e]AI for adults (33).

problems with 25.9% (35/135) and sleep disorders with 24.4% (33/135) of mentions. Exhaustion/fatigue was reported by 16 HPP patients and accounted for 14.1% (73/519) of symptoms mentioned. Gastrointestinal symptoms made up 8.3% (43/519) of all reported complaints, with nausea (15/43) and diarrhea (13/43) being the most common.

Issue documented among the control group were gastrointestinal (8/19), neuropsychiatric (6/19), musculoskeletal (3/19), and exhaustion/fatigue (2/19). Details are given in Table 4.

Intake of phosphorus and calcium and symptoms in HPP patients

Intake of phosphorus was significantly associated with the quantity of neuropsychiatric symptoms ($P = 0.02$), with the least symptoms experienced at phosphorus intakes in the interval from 1100 to 1375 mg/d, whereas both higher and lower intakes were associated with an up to 2.7-fold increase in symptoms (Fig. 2). There was no significant correlation between the phosphorus intake and the number of musculoskeletal, gastrointestinal, and tiredness/fatigue symptoms.

Calcium intake was significantly associated with the quantity of musculoskeletal ($P < 0.01$), gastrointestinal ($P = 0.02$), and neuropsychiatric symptoms ($P < 0.001$). The lowest average numbers of gastrointestinal and neuropsychiatric symptoms were observed at calcium intakes between 950 and 1425 mg/d (Fig. 3a/b). Both higher and lower intakes led to an increase in gastrointestinal and neuropsychiatric symptoms. The mean daily number of musculoskeletal symptoms was largely stable across different intake categories but increased at calcium intakes ≥ 1425 mg/d. There was no significant association between the calcium intake and the quantity of tiredness/fatigue symptoms.

The calcium-phosphorus ratio was significantly correlated with the number of gastrointestinal symptoms ($P = 0.01$) and tiredness/fatigue ($P < 0.01$). The lowest number of gastrointestinal symptoms occurred at calcium-phosphorus ratios of 0.75 to 1 (Fig. 4a). Both higher and lower calcium-phosphorus ratios were associated with an increase in gastrointestinal symptoms. Conversely, low calcium-phosphorus ratios were associated with less tiredness/fatigue symptoms, whereas these were most frequent at a Ca/P ratio of 1 to 1.25 (Fig. 4b). There was no significant correlation between the calcium-phosphorus ratio and the quantity of musculoskeletal and neuropsychiatric symptoms.

There was no significant dependency between energy intake and the overall quantity of symptoms of any symptom group. No significant correlations were seen

Table 3. Intake of Micronutrients Per Day

	HPP Patients	Controls	*P* Value Group Comparison[a]
	(n = 20)	(n = 20)	(n = 40)
P (mg)			0.01
Median (range)	988 (570-1893)	1374 (946-2587)	
P/kcal			0.06
Median (range)	0.59 (0.34-0.80)	0.62 (0.48-1.12)	
Ca (mg)			0.04
Median (range)	737 (263-1607)	1089 (470-1865)	
Ca < 950 mg[b] (n)	15	6	
Ca/kcal			0.16
Median (range)	0.43 (0.22-0.81)	0.50 (0.23-0.81)	
Ca/P			0.91
Median (range)	0.74 (0.43-1.35)	0.75 (0.41-0.97)	
Ca/P ≥ 1 (n)	3	0	
Mg (mg)			0.13
Median (range)	353 (189-824)	401 (259-698)	
Mg < 350 mg (m) / 300 mg (f)[c] (n)	8	3	
Zn (mg)			0.07
Median (range)	9.46 (5.73-16.3)	12.3 (6.69-42.1)	
Zn < 9.4 mg (m) / 7.5 mg (f)[d] (n)	5	1	
Zn < 11.7 mg (m) / 9.3 mg (f)[e] (n)	11	7	
Vitamin B6 (mg)			0.52
Median (range)	1.49 (0.80-3.10)	1.63 (1.01-3.11)	
Vitamin B6 < 1.7 mg (m) / 1.6 mg (f)[f] (n)	12	10	

[a]Wilcoxon-Mann-Whitney test.
[b]PRI for adults ≥ 25 years (36).
[c]AI for adults ≥ 18 years (37).
[d]PRI for adults ≥ 18 years and a phytate intake of 300 mg/d (38).
[e]PRI for adults ≥ 18 years and a phytate intake of 600 mg/d (38).
[f]PRI for adults (39).

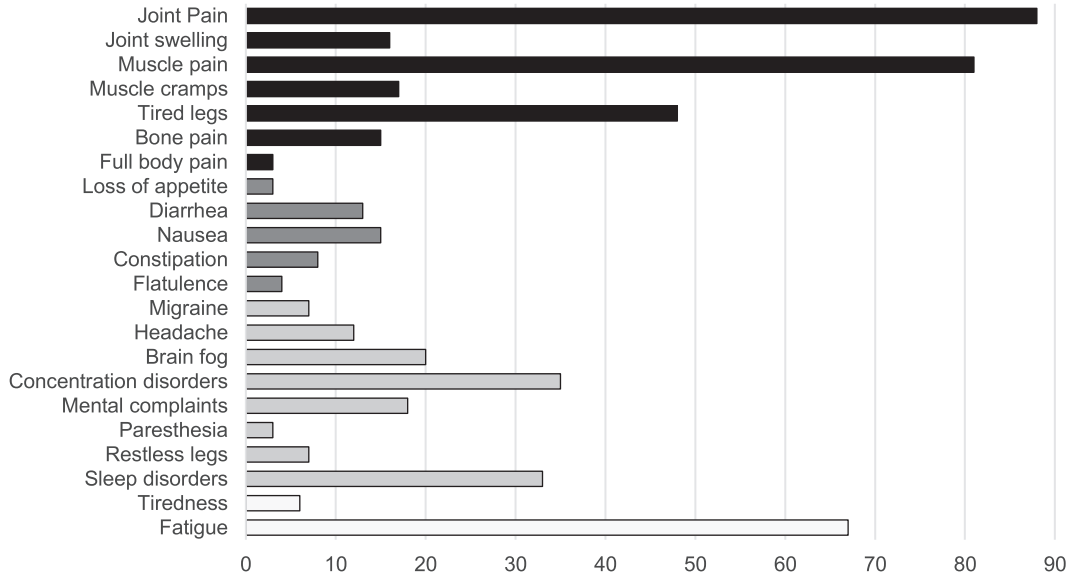

Figure 1. Absolute frequency of reported symptoms in Hypophosphatasia patients calculated out of 140 documented days. From dark to light: Musculoskeletal symptoms; Gastrointestinal symptoms; Neuropsychiatric symptoms; Exhaustion/Tiredness/Fatigue.

in healthy controls (data not shown). A summary of the statistical evaluations regarding potential dependencies of mineral and energy intake with specific clinical symptoms of HPP patients is provided in Table 5.

Discussion

This manuscript aims at providing a first overview of nutrient intake, particularly phosphorus consumption,

in HPP patients compared to non-affected, age- and gender-matched controls to discover a supposed relationship between nutrition and disease-related symptoms in HPP patients.

This pilot study has some limitations that have to be considered when interpreting the data. The overall sample size and the proportion of male subjects are relatively small. However, the number of potential participants is limited by the rareness of the disease, and a preponderance of female participants is a common observation in studies on adult HPP for reasons extensively discussed earlier (4, 7, 40). Altered dietary habits and a reporting bias cannot be excluded because results are based on patient-administered dietary records and documented symptoms.

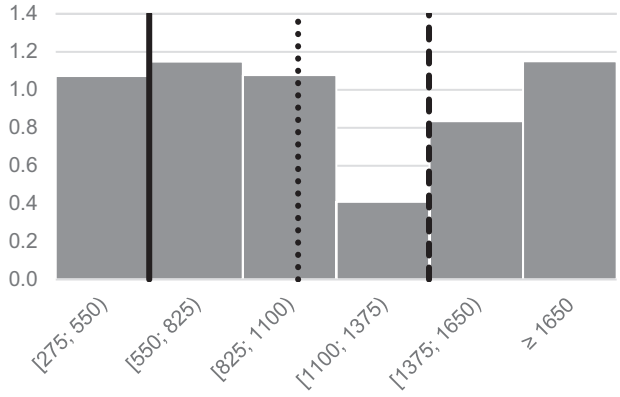

Figure 2. Phosphorus intake (mg/d) and mean incidence of neuropsychiatric symptoms reported per day in Hypophosphatasia patients. Solid line: Adequate intake for phosphorus (mg/d); Dotted line: Median phosphorus intake (mg/d) of Hypophosphatasia patients; Dashed line: Median phosphorus intake (mg/d) of controls.

The HPP patient group and the control group were generally well-balanced regarding age, gender, height, weight, BMI, nutritional habits, and even composition of energy supplying nutrients. The more it is noteworthy that total energy intake is about 10% lower in HPP patients. Specifically, female HPP patients consumed significantly less energy compared with the female controls. Although the energy intake in the control group was close to the recommended values for energy intake at a physical activity level of 1.6, which is in line with estimates for the general population (41, 42), that of female HPP patients corresponded to a physical activity level of 1.4, applicable for people with minimal activity (i.e., performing merely everyday tasks [e.g., washing, eating]) (43).

It appears reasonable to assume that the reduced activity in HPP is because of disease-related chronic health issues, which would eventually underscore the considerably high burden of the disease even in supposedly mildly affected adults. Otherwise, one could speculate that the energy metabolism in HPP may be compromised as a direct consequence of the underlying enzyme deficiency (44, 45). To understand that in more detail, activity tracking in HPP patients should be considered for further projects.

Table 4. Symptoms in HPP Patients and Controls

	HPP Patients	Controls	*P* Value Group Comparison[a]
	(n = 20)	(n = 20)	(n = 40)
Symptoms per week (n)			< 0.001
Median (range)	28.0 (2-58)	0.0 (0-8)	
Symptoms in total (n)	519	19	
Persons with at least 1 symptom per week (n)	20	6	
Symptom-free days (n)	12	125	
Persons with symptom-free days (n)	3	20	
Musculoskeletal symptoms per week (n)			< 0.001
Median (range)	14.0 (0-26)	0.0 (0-2)	
Musculoskeletal symptoms in total (n)	268	3	
Persons with at least 1 musculoskeletal symptom per week (n)	19	2	
Gastrointestinal symptoms per week (n)			< 0.01
Median (range)	1.0 (0-7)	0.0 (0-7)	
Gastrointestinal symptoms in total (n)	43	8	
Persons with at least 1 gastrointestinal symptom per week (n)	13	2	
Neuropsychiatric symptoms per week (n)			< 0.001
Median (range)	6.5 (0-20)	0.0 (0-2)	
Neuropsychiatric symptoms in total (n)	135	6	
Persons with at least 1 neuropsychiatric symptom per week (n)	17	5	
Tiredness/fatigue symptoms per week (n)			< 0.001
Median (range)	3.0 (0-7)	0.0 (0-1)	
Tiredness/fatigue symptoms in total (n)	73	2	
Persons with at least 1 tiredness/fatigue symptom per week (n)	16	2	

[a]Wilcoxon-Mann-Whitney test.

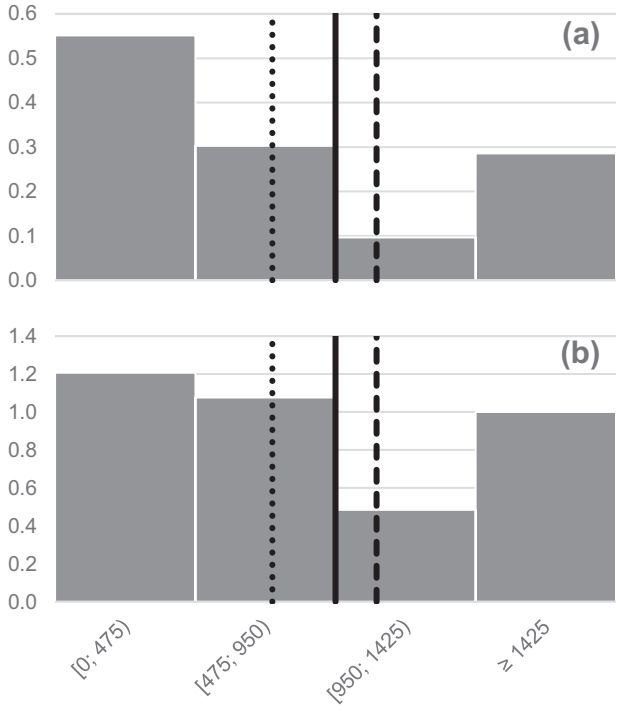

Figure 3. Calcium intake (mg/d) and mean incidence of (a) gastrointestinal and (b) neuropsychiatric symptoms reported per day in Hypophosphatasia patients. Solid line: Population Reference Intake for calcium (mg/d); Dotted line: Median calcium intake (mg/d) of Hypophosphatasia patients; Dashed line: Median calcium intake (mg/d) of controls.

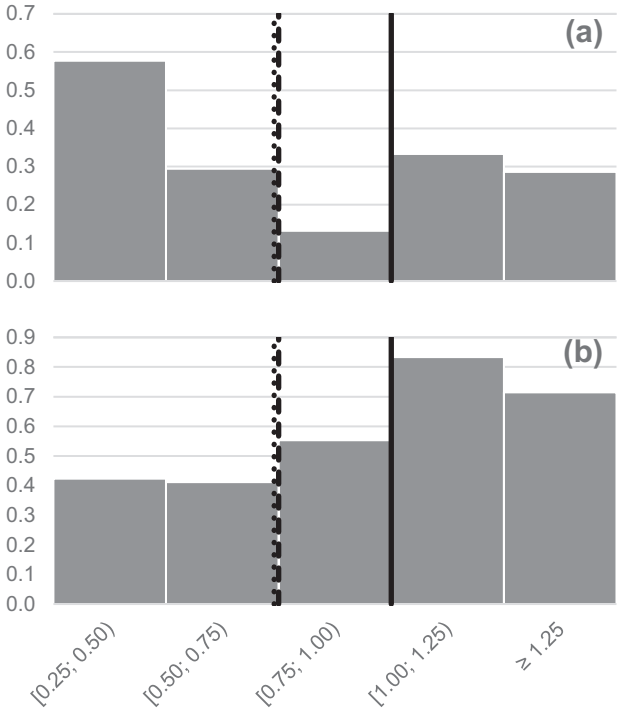

Figure 4. Calcium-phosphorus ratio and mean incidence of (a) gastrointestinal and (b) tiredness/fatigue symptoms reported per day in Hypophosphatasia patients. Solid line: Discussed lower bound of adequate calcium-phosphorus ratio; Dotted line: Median calcium-phosphorus ratio of Hypophosphatasia patients; Dashed line: Median calcium-phosphorus ratio of controls.

Magnesium and zinc are cofactors of alkaline phosphatase activity and a reduction of the enzyme's activity has been associated in zinc and magnesium deficiency (46). In our study, the intake of magnesium was largely balanced in both HPP patients and controls without significant differences. However, it is worth mentioning that the magnesium intake was below the AI in 40% of HPP patients, even though the use of dietary supplements was included in these calculations. The intake of zinc was well-balanced in HPP patients and controls on average, specifically when presuming a phytate intake in the lower range, which appears appropriate considering the low fiber intake in the European population (38).

It remains to be elucidated whether a higher magnesium and zinc intake would be favorable, but, in fact, we and others previously could not show any benefit from the supplementation of magnesium or zinc in HPP patients (8, 47).

Total Vit B6 comprises a group of numerous vitamers, found in both animal and plant foods (48). Elevated circulating PLP, along with symptoms of intracellular Vit B6 deficiency are a common finding in HPP and the question is unsolved whether a higher or lower intake of Vit B6 was favorable or detrimental. In this study, we could not find significant differences in terms of the vitamin B6 intake between HPP patients and controls. However, about one-half of all participants did not achieve the European PRI for Vit B6 (39), leaving open the question whether Vit B6 enriched diet or supplementation, adapted to the PRI or even beyond, would be beneficial.

As expected with a mixed Western diet, all subjects exceeded the AI for phosphorus of 550 mg/d (12, 13). However, the phosphorus intake was significantly higher in the controls than in the HPP patients. When accounting for the reduced energy consumption of HPP patients, the difference was narrowly no longer statistically significant ($P = 0.06$). Accordingly, the difference in energy consumption can be considered a main but not the only reason for the lower phosphorus intake in HPP patients. In addition, the calcium intake was significantly reduced and below the PRI in 75% (n = 15) of HPP patients. This appears relevant because deficient calcium intake is not only associated with bone (49) but also muscle health (50). Though not statistically significant, even the proportional calcium intake in relation to the energy consumption tended to be lower in the HPP patients. However, the intake recommendations apply to healthy individuals and the actual requirement of calcium in HPP patients is still unknown; therefore, the question is open whether higher calcium intake would contribute to musculoskeletal health in HPP (7, 40).

The finding of reduced phosphorus and calcium intake in HPP patients even after adjustment for reduced

Table 5. Dependency Between the Nutrient Intake and Symptoms Evaluated with Fisher's Exact Test

	Phosphorus		Calcium		Ca-P Ratio		Energy (MJ)	
	Value	*P*	Value	*P*	Value	*P*	Value	*P*
Musculoskeletal symptoms	29.64	0.52[a]	35.79	< 0.01[a]	31.60	0.20[a]	10.39	0.52[a]
Gastrointestinal symptoms	15.18	0.63	19.36	0.02	27.15	0.01	4.74	0.58
Neuropsychiatric symptoms	46.26	0.02[a]	41.60	< 0.001[a]	38.69	0.12[a]	13.17	0.30[a]
Tiredness/fatigue	18.36	0.32	11.26	0.44	29.04	< 0.01	3.61	0.77

[a]Monte-Carlo-Significance based on 10,000 sampling tables.

energy consumption suggests that there may be additional causes why HPP patients, consciously or unconsciously, spare phosphorus and calcium in their diet.

With that in mind, the association between phosphorus intake and the incidence of neuropsychiatric symptoms observed becomes even more relevant. Specifically, phosphorous intake levels ≥ 1375 mg/d, corresponding roughly to the average supply of the controls, were associated with an increase of these complaints in HPP patients. Strikingly, not only high but also low phosphorous intake levels (i.e., < 1100 mg/d) seemed to be unfavorable. Considering that 550 mg phosphorus per day is considered as AI for the general population (13), a reduction below this threshold does not appear advantageous for HPP patients.

Matching this U-shaped correlation for phosphorus with our findings of similarly increased neuropsychiatric and gastrointestinal issues with both elevated (≥ 1425 mg) and decreased (< 950 mg) calcium intake, it appears reasonable that an appropriately balanced supply rather than just the absolute amount of one of these minerals would be essential. A calcium-phosphorus ratio of 0.65 to 0.75 is considered appropriate for a mixed diet in adults (10). In our study, the Ca/P ratio was at the upper limit of this range in both groups and we actually observed an increase of tiredness/fatigue and gastrointestinal symptoms when the Ca/P ratio exceeded 1.0 and there was an increase in gastrointestinal symptoms when the ratio fell below 0.5. Conversely, a Ca/P ratio around 0.75 combined with an absolute daily phosphorus intake of 1100 < 1375 mg and a calcium intake above the PRI of 950 mg/d (up to 1425 mg) was associated with the lowest incidence of complaints.

In a previous evaluation of 12 HPP patients, serum intact parathyroid hormone levels in the lower along with calcium levels in the upper normal range were reported (51). Considering our finding of a diminished phosphorus intake in HPP patients, one may speculate whether, in addition to high calcium levels, diminished nutritional phosphorus intake may have contributed to that observation. From a pathophysiological perspective, both high phosphorus and low calcium are associated with an immediate increase in PTH (25, 27, 52, 53), triggering regulatory mechanisms to stabilize and increase Ca levels and reduce phosphate levels by levering renal reabsorption of Pi (54). Considering that, for reasons that are not yet completely understood and that may involve deficient renal ALP activity, the renal tubular reabsorption of phosphate tubular maximum of reabsorption of Pi (TmP/GFR) tends to be high in HPP, it appears reasonable to conclude that slight increases in PTH toward the mid-normal range or elevation of another phosphatonin (e.g., fibroblast growth factor-23 [FGF-23], sFRP4, FGF-7) recently reported to be diminished in children with the disease (55) may be advantageous in HPP. In line with that, treatment with recombinant PTH fragments has been described to lower serum phosphate, calcium-phosphate product, and TmP/GFR (56), and this same effect has been reported in a case description of HPP along with an improvement in pain and fatigue (57), whereby the latter was significantly dependent on the calcium-phosphorus ratio in this study. Accordingly, it was conceivable that a diet containing calcium and phosphorus in a ratio of, for example, > 1 could be associated with a decrease in PTH (22), entailing a further increase of the already high TmP/GFR in HPP patients. Accordingly, although a certain (calcium-balanced) extent of nutritional phosphorus may be beneficial in HPP, excessive doses might just overload the regulatory system and trigger symptoms. Conversely, we also found an increase of symptom frequency with low phosphorus intake. Reflecting that reduced phosphorus intake is typically paralleled by low calcium consumption, which was also true for the cohort under investigation here (data not shown), and considering previous reports that lowering dietary intake of both minerals leads to an increase in TmP/GFR, probably because of regulating effects of FGF-23 independent of PTH (58), this assumption appears conclusive.

This study assessed potential associations between nutritional aspects, particularly phosphorus intake and potential immediate clinical symptoms frequently reported by HPP patients. Potential effects on long-term outcome parameters, such as bone quality, prevalence

of inflammatory pain, calcific periarthritis, and renal health, were not within the scope of this study.

Based on these findings, it will be important to figure out if therapeutic interventions to optimize the nutritional intake of calcium and phosphorus can have a positive effect on the incidence of the aforementioned symptoms and whether this is merely a direct effect of mineral supply and availability or rather a consequence of the specific impact of these minerals on key regulatory hormones of mineral metabolism, specifically PTH, calcitriol, FGF-23, and other phosphatonins. And it will be important to learn if the regulatory response of these mediators might be different in the specific setting of deficient tissue nonspecific alkaline phosphatase activity (54, 55).

Conclusions

In summary, nutrient intake in HPP patients is largely balanced. However, it appears that HPP patients have a lower energy turnover than the general population, along with a reduced total intake of calcium and phosphorus. As expected, the phosphorus intake exceeded the European value for AI but was significantly lower in HPP patients than in controls. Importantly, our findings suggest that dietary phosphorus, calcium, and the calcium-phosphorus ratio may have an impact on the clinical symptoms of adults with HPP. A normalized phosphorus intake combined with a calcium intake around the PRI and a calcium-phosphorus ratio slightly < 1 seems to be favorable. Based on these findings, subsequent interventional studies should investigate whether adjusting the nutritional intake of calcium and phosphorus can improve the clinical symptoms of HPP and if and how the responsiveness of the regulatory environment to nutritional calcium and phosphorus may be different in HPP.

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