ORIGINAL ARTICLE

Bone turnover and mineral metabolism in adult patients with hypophosphatasia treated with asfotase alfa

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Abstract

Summary There is limited understanding of how asfotase alfa affects mineral metabolism and bone turnover in adults with pediatric-onset hypophosphatasia. This study showed that adults with hypophosphatasia treated with asfotase alfa experienced significant changes in biochemical markers of bone and mineral metabolism, possibly reflecting enhanced bone remodeling of previously osteomalacic bone.

Introduction Hypophosphatasia (HPP), due to a tissue nonspecific alkaline phosphatase (TNSALP) deficiency, can cause impaired bone mineralization and turnover. Although HPP may be treated with asfotase alfa, an enzyme replacement therapy, limited data are available on how treatment with asfotase alfa affects mineral metabolism and bone turnover in adults with HPP.

Methods ALP substrates, bone turnover and mineral metabolism markers, and bone mineral density (BMD) data from EmPATHY, a single-center, observational study of adults ($\geq$ 18 years) with pediatric-onset HPP treated with asfotase alfa (NCT03418389), were collected during routine clinical care and analyzed from baseline through 24 months of treatment.

Results Data from 21 patients showed significantly increased ALP activity and reduced urine phosphoethanolamine (PEA)/creatinine (Cr) ratios after baseline through 24 months of asfotase alfa treatment. There were significant transient increases in parathyroid hormone 1-84 (PTH), osteocalcin, and procollagen type 1 N-propeptide (P1NP) levels at 3 and 6 months and in tartrate-resistant acid phosphatase 5b (TRAP5b) levels at 3 months, with a significant decrease in N-terminal telopeptide of type 1 collagen (NTX) levels at 24 months. Lumbar spine BMD T scores continuously increased during treatment.

Conclusion Significant changes in bone turnover and mineral metabolism markers after asfotase alfa treatment suggest that treatment-mediated mineralization may enable remodeling and bone turnover on previously unmineralized surfaces. Urine PEA/Cr ratios may be a useful parameter in monitoring treatment during routine care.

Keywords Bone turnover · Hypophosphatasia · Enzyme replacement therapy · Alkaline phosphatase · Bone mineral density

Introduction

Hypophosphatasia (HPP) is a rare, inherited, metabolic disease caused by a tissue nonspecific alkaline phosphatase (TNSALP) deficiency, leading to the extracellular accumulation of TNSALP substrates and further resulting in impaired bone mineralization, skeletal defects, and systemic manifestations of the disease [1–3]. Adult patients with HPP can experience a high disease burden, including a wide range of dental and orthopedic problems such as fractures, pseudofractures, bone and joint pain, muscle weakness, and fatigue. Consequently, patients with HPP have reduced physical functioning and health-related quality of life [3–6].

The pathophysiology of HPP involves extracellular accumulation of inorganic pyrophosphate (PPi), a TNSALP substrate, which inhibits bone mineralization by blocking hydroxyapatite crystal formation [2]. Asfotase alfa is a human recombinant TNSALP enzyme replacement therapy that is approved for HPP treatment, typically for patients of all ages with pediatric-onset HPP (all adults regardless of onset in Japan) [7]. Prior research has shown that asfotase alfa treatment reduces the accumulation of TNSALP substrates, PPi, and pyridoxal 5′-phosphate (PLP), and promotes bone

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mineralization [8, 9]. However, assessment of PPi is not available as a routine clinical assay and is currently used in research settings only. Other established indicators of TNSALP activity, such as PLP and urine phosphoethanolamine (PEA) [1, 2, 10], are not implemented for monitoring asfotase alfa treatment in routine practice since clinically available PLP assays do not use an inhibitor of alkaline phosphatase (ALP) activity (e.g., levamisole) to prevent PLP decay in vitro by arresting asfotase alfa activity in serum samples of treated patients [9, 11], and PEA has not been evaluated in that context.

Along with reduced ALP activity and the accumulation of its substrates, poor bone mineralization and bone turnover appear to be hallmarks of HPP. Indeed, previous studies have indicated diminished bone turnover along with reduced serum ALP activity [12, 13], and elevated calcium and/or phosphate levels in patients with HPP may be considered sequelae of poor bone mineralization [14–16]. While case reports from adult patients with HPP suggest that asfotase alfa has a positive clinical effect on bone health [17, 18], limited data are available on how treatment with asfotase alfa affects mineral metabolism and bone turnover, and how these treatment effects can be biochemically monitored during routine clinical care. Therefore, this study characterized and evaluated parameters of bone turnover and *ALPL*-associated mineral metabolism in adult patients with pediatric-onset HPP who were treated with asfotase alfa in a routine clinical practice setting.

Methods

Study design

EmPATHY (Evaluate and monitor Physical Performance of Adults Treated with Asfotase Alfa for Pediatric-Onset Hypophosphatasia) is an observational study, consisting of retrospective chart review and prospective data collection, conducted at a single center in Germany (NCT03418389). The study design was reviewed and approved by the ethics committee of the University of Würzburg, Germany (No. 9/18).

Study population

Adult patients (aged ≥ 18 years) with a confirmed diagnosis of pediatric-onset HPP, who were receiving asfotase alfa (Strensiq®, Alexion Pharmaceuticals, Inc., Boston, MA, USA) treatment for 24 months during routine clinical practice at the Orthopedic Hospital König-Ludwig-Haus, Julius-Maximilians-Universität Würzburg, Germany, were included in the analysis. A confirmed diagnosis of pediatric-onset HPP was defined by the following: (1) low serum ALP activity for age and sex and/or an *ALPL* gene variant; and (2) clinical manifestations consistent with HPP. The genetic variants along with respective American College of Medical

Genetics and Genomics (ACMG) classifications are listed in Supplemental Table S1. The decision to initiate treatment with asfotase alfa was based on the treating physician's clinical judgment, as per the standard of care. The dose of asfotase alfa received by each patient was prescribed by the treating physician in accordance with the European Medicines Agency's Summary of Product Characteristics and applicable German label. The recommended dose of asfotase alfa is 6 mg/kg/week at 2 mg/kg three times per week or 1 mg/kg six times per week, notwithstanding individualized dose adjustments following clinical assessment of the risk-benefit ratio [19].

All participants provided signed informed consent before enrollment. Patients were not included if they were receiving asfotase alfa for an off-label use, currently participating in an Alexion Pharmaceuticals, Inc.-sponsored clinical trial, or receiving an experimental drug or treatment.

Data collection

Data were collected as previously described [20]. Briefly, demographic data, including age at HPP onset and HPP-related manifestations, were retrospectively collected from patients' medical records. Clinical and laboratory data relevant to HPP were routinely collected from patients at baseline (before initiating treatment with asfotase alfa) and 3, 6, 12, 18, and 24 months after treatment initiation. Data collection was based on available information from assessments commonly conducted at the investigator's site; no investigations outside the standard of care were performed.

Outcomes

Laboratory data on bone turnover and mineral metabolism markers relevant to HPP were collected through routine clinical tests performed at the laboratory of the Orthopedic Clinic (University of Würzburg) and collaborating laboratories. The parameters analyzed included ALP substrates: PLP (only at baseline) and urine PEA/creatinine (Cr) ratio; mineral metabolism markers: parathyroid hormone 1-84 (PTH), calcium, and phosphate; the bone-derived hormone marker: fibroblast growth factor-23 (FGF-23); and bone turnover markers: osteocalcin, procollagen type 1 N-propeptide (P1NP), tartrate-resistant acid phosphatase 5b (TRAP5b), and N-terminal telopeptide of type 1 collagen (NTx). Laboratory data on glomerular filtration rate (GFR) were also collected to assess renal function, which could impact the results of bone turnover and mineral metabolism markers.

Bone mineral density (BMD) assessments were conducted using dual-energy X-ray absorptiometry (DXA), including lumbar spine T score (L1-4), right and left hip total T scores, and right and left hip neck T scores.

Statistical analysis

Descriptive statistics were calculated for demographic and baseline characteristics of the study population. Outcome parameters were tested for normal distribution using the Shapiro-Wilk test. For laboratory markers of mineral metabolism and bone turnover, significant deviations from a normal distribution were observed; thus, nonparametric testing was performed using Wilcoxon-matched pairs tests to examine the change of values at subsequent visits when compared with baseline. For DXA results, which showed a normal distribution, matched pairs t tests (parametric) were applied. Laboratory and DXA results are reported graphically and depicted as median (first quartile, third quartile [Q1, Q3]) and minimum, maximum (min, max). DXA results are also reported as mean values (± standard deviation).

For each variable, comparisons between specific timepoints and baseline were calculated for subjects with data at both timepoints (i.e., for all comparisons, each patient served as their own control). For consistency, graphs representing longitudinal results are showing data only for those subjects with data available at all timepoints (i.e., pairwise calculations are typically based on a higher case number than depicted in the graphs). All tests were two-sided with a significance level of 5%. SPSS Statistics (SPSS Inc., an IBM company, Chicago, IL, USA) statistical software was used to conduct all data analyses.

Results

Baseline patient demographics and treatment characteristics are shown in Table 1. A total of 21 adult patients (n = 16, female) with pediatric-onset HPP, aged 19–78 years (median 50 years), were included in the analysis. All patients had genetically confirmed HPP (Supplemental Table S1) with consistently reduced serum ALP activity and bone manifestation of the disease, including a positive fracture history in 20 patients. Additional bone manifestations of HPP in the study population were bowing of the long bones (n = 9), craniosynostosis (n = 7), scoliosis (n = 5), and kyphosis (n = 3). Although all adult patients had a diagnosis of pediatric-onset HPP, 14 (67%) of 21 patients did not experience a first fracture until adulthood. Almost all patients (20 [95%] of 21 patients) reported further manifestations of HPP, such as pain (n = 20; 95%), muscle weakness that limited daily activities (n = 19; 90%), joint pain (n = 18; 86%), and early loss of primary (n = 17; 81%) and permanent (n = 9; 43%) dentition.

Serum ALP activity, urine PEA/Cr ratio, and GFR levels were assessed at every visit from baseline through 24 months of treatment; results are shown in Fig. 1A–C. As expected, serum ALP activity significantly increased when compared with baseline at every study timepoint following the start of treatment with asfotase alfa. Median (Q1, Q3) urine PEA/Cr ratio levels were increased at baseline (53.2 mmol/mol Cr [37.3, 78.3]) and reduced to near the normal range after treatment initiation with asfotase alfa. Compared with baseline, this decrease was statistically significant at every timepoint assessed (6, 12, 18, and 24 months); however, median urine PEA/Cr ratio levels remained above the upper limit of the normal range throughout the 24-month follow-up period.

Renal function was continuously monitored throughout the study, specifically since some patients had reduced baseline GFR levels equivalent to chronic kidney disease (CKD) grades 2 and 3. Although median GFR levels did not change significantly over the course of the study, individual GFR levels at certain timepoints dipped below 60 mL/min/1.73m^2; however, these decreases appeared to be transient, and severe renal compromise was not observed in any patient during the course of treatment.

Median (Q1, Q3) serum levels for markers of mineral metabolism (PTH, calcium, phosphate) and FGF-23 in study participants at baseline and 3, 6, 12, 18, and 24 months of treatment are shown in Fig. 2A–D. Significant increases in PTH were observed at 3 months (41.7 pg/mL [24.9, 51.5]; P = 0.008) and 6 months (38.1 pg/mL [26.6, 46.0]; P = 0.018) of treatment when compared with baseline (27.6 pg/mL [23.6, 36.9]). There were no significant changes in median serum levels of calcium and phosphate at any study timepoint when compared with baseline. Median serum PTH, calcium, and phosphate levels stayed within normal limits throughout the analysis. Similarly, median (Q1, Q3) serum levels for FGF-23, an essential regulator of serum phosphate homeostasis, did not significantly change from baseline (84 RU/mL [54, 103]) through 24 months of treatment with asfotase alfa and remained within normal limits. Outliers ranging between 1.5- and 3.0-fold above the upper limit of the normal range stem from one patient with CKD grade 3 and GFR levels consistently between 45 and 60 mL/min/1.73m^2.

Median (Q1, Q3) serum levels (osteocalcin, P1NP, TRAP5b, and NTx) for bone turnover markers are shown in Fig. 3A–D. Median serum osteocalcin levels increased significantly at 3 months (18.8 ng/mL [10.9, 23.4]; P = 0.001) and 6 months (16.3 ng/mL [11.5, 22.9]; P = 0.001) when compared with baseline (10.4 ng/mL [8.5, 14.4]), and then returned to near baseline levels after 12 months (12.4 ng/mL [10.4, 19.4]) and remained within normal limits through 24 months (13.7 ng/mL [9.3, 18.5]) of treatment with asfotase alfa. Similarly, median serum P1NP levels increased significantly at 3 months (96.7 μg/L [72.8, 127.0]; P = 0.002) and 6 months (70.1 μg/L [56.9, 98.2]; P = 0.035) compared with baseline (56.0 μg/L [46.0, 70.0]) and returned to near baseline levels at 12 months (56.8 μg/L [49.7, 72.9]), remaining within normal limits through 24 months (54.6 μg/L [43.6, 66.7]) of treatment.

In parallel, TRAP5b levels increased significantly at 3 months (4.2 U/L [2.8, 5.2]; P = 0.009) when compared with baseline (3.1 U/L [2.6, 4.1]) and returned towards baseline

Table 1 Baseline characteristics of the study population

Characteristic	Total patient population (N = 21)	
Sex, n (%)	Female, 16 (76)	Male, 5 (24)
Height, cm, mean (SD)	156 (7)	165 (21)
Weight, kg, mean (SD)	71 (19)	80 (28)
BMI, kg/m^2, mean (SD)	29 (7)	29 (7)
Age at therapy start, years, median (range)	50 (19–78)	
History of HPP-related manifestations, n (%) [a]		
Skeletal [b]	21 (100)	
Fracture	20 (95)	
Neurological	8 (38)	
Muscular	20 (95)	
Dental	20 (95)	
Renal	5 (24)	
Rheumatic	4 (19)	
Pain	20 (95)	
Fracture detail (n = patients assessed), total events (%)		
Patient with any fracture (n = 21)	20 (95)	
Total number of fractures (n = 20)	105 (100)	
Trauma fractures	65 (62)	
Insufficiency fractures	40 (38)	
Age category at first fracture (n = 20)	20 (100)	
Child (< 18 years old)	6 (30)	
Adult (≥ 18 years old)	14 (70)	

HPP, hypophosphatasia; *SD*, standard deviation

[a] Calculated as a percentage of the patients for whom data were available

[b] Excluding fractures

levels at 12 months (2.9 U/L [2.3, 4.7]), remaining within normal limits at all timepoints from baseline through 24 months (2.7 U/L [1.6,3.9]) of treatment. Compared with baseline NTx levels (11.8 nM BCE/L [10.0, 17.2]), there was a numerical increase in NTx levels at 3 months (13.2 nM BCE/L [9.9, 17.0]; $P = 0.435$) and 6 months (13.3 nM BCE/L [11.1, 16.0]; $P = 0.528$) that did not reach statistical significance. NTx levels decreased thereafter, with the decline being statistically significant at 24 months (9.5 nM BCE/L [8.2, 10.8]; $P = 0.022$) when compared with baseline. NTx levels remained within the normal range at all timepoints.

Mean lumbar spine T scores (Fig. 4) were in the upper range of normal at baseline and increased when compared with baseline after 6 and 12 months of treatment with asfotase alfa ($P < 0.05$). Assessments of DXA at the femoral region were valid for only a few patients, with the majority of them having metal implants, making statistical evaluations not feasible due to the small sample size.

During treatment with asfotase alfa, four bone fractures and three pseudofractures occurred in four patients with a history of fractures. One patient experienced metatarsal pseudofractures of two bones and two traumatic avulsion fractures at the thumb following injury of the hand; one patient experienced an insufficiency fracture at the medial malleolus 3 months after treatment initiation; and one patient had a solitary metatarsal pseudofracture detected. In one patient with a preexisting unstable femoral implant and progressively thinned femoral cortex, a femoral fracture occurred when the patient became more active after starting treatment.

Discussion

Results of this study show significant changes in bone turnover and mineral metabolism markers in adult patients with pediatric-onset HPP who were treated with asfotase alfa. As expected, serum ALP activity levels increased substantially following treatment with asfotase alfa. Though serum ALP activity may be useful in assessing treatment adherence, we cannot draw any conclusions concerning therapeutic ALP levels and treatment
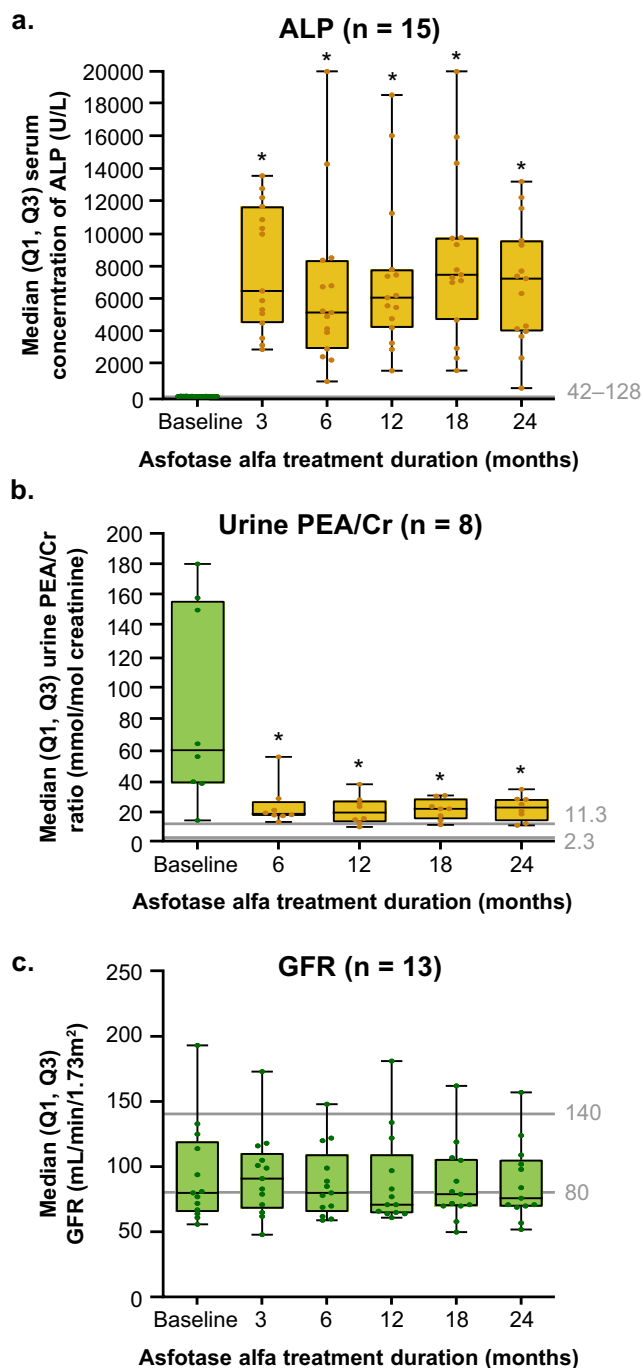
Fig. 1 A–C ALP, urine PEA/Cr, and GFR during 24 months of asfotase alfa treatment. **A** ALP normal range 42–128 U/L (males 53–128; females 42–98). **B** Urine PEA/Cr normal range 2.3–11.3 mmol/mol Cr. **C** GFR normal range 80–140 mL/min/1.73m². *Statistical significance ($P < 0.05$) vs baseline. Whiskers include values outside of Q1 and Q3 with extremes marking the minimum and maximum values. ALP, alkaline phosphatase; Cr, creatinine; GFR, glomerular filtration rate; PEA, phosphoethanolamine; Q, quartile

Fig. 2 A–D Mineral metabolism and hormone markers during 24 months of asfotase alfa treatment. **A** PTH normal range 14.9–56.9 pg/mL. **B** Calcium normal range 2.1–2.6 mmol/L. **C** Phosphate normal range 0.6–1.8 mmol/L. **D** FGF-23 normal range 34–140 RU/mL (males 34–97; females 44–140). *Statistical significance ($P < 0.05$) vs baseline. Whiskers include values outside of Q1 and Q3 with extremes marking the minimum and maximum values. Outliers include values 1.5-fold or greater above Q3. FGF-23, fibroblast growth factor-23; PTH, parathyroid hormone 1-84; Q, quartile

efficacy. In that regard, previous studies have focused on measuring PPi and PLP substrate levels [9, 11, 16, 21, 22].

Unfortunately, monitoring these substrates in a routine clinical setting is impractical because degradation of PPi and PLP after drawing blood must be prevented by the addition of

a.

Osteocalcin (n = 9)

b.

P1NP (n = 7)

c.

TRAP5b (n = 5)

d.

NTX (n = 11)
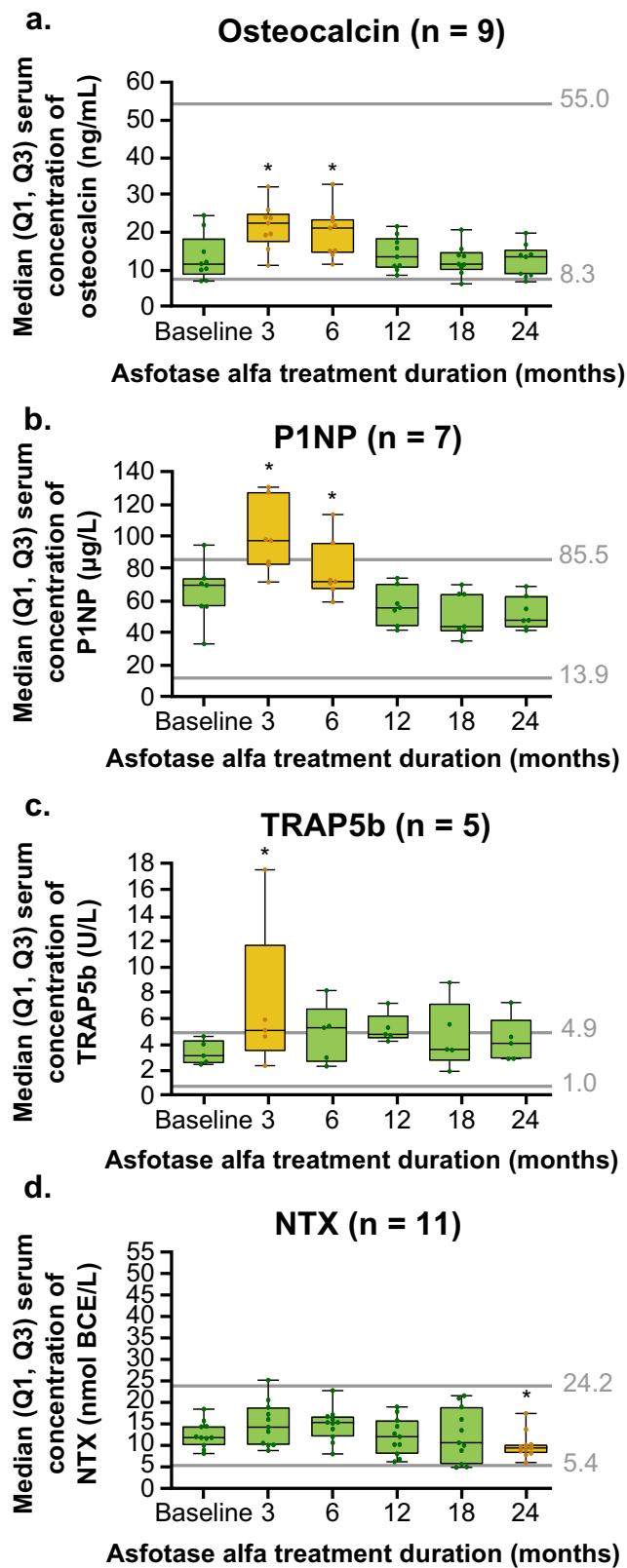
◀ **Fig. 3 A–D** Bone turnover markers during 24 months of asfotase alfa treatment. **A** Osteocalcin normal range 8.3–55.0 ng/mL (males 9.6–41.0; females premenopausal 8.3–34.0, postmenopausal 12.8–55.0). **B** P1NP normal range 13.9–85.5 µg/L (males 13.9–85.5; females premenopausal 15.1–58.6, postmenopausal 20.3–76.3). **C** TRAP5b normal range 1.0–4.9 U/L (males 1.9–4.8; females premenopausal 1.0–4.2, postmenopausal 1.5–4.9). **D** NTx normal range 5.4–24.2 nM BCE/L (males 5.4–24.2; females premenopausal 6.2–19.0, postmenopausal 12.9–22.7). *Statistical significance ($P < 0.05$) vs baseline. Whiskers include values outside Q1 and Q3 with extremes marking the minimum and maximum values. NTx, N-terminal telopeptide of type 1 collagen; P1NP, procollagen type 1 N-propeptide; Q, quartile; TRAP5b, tartrate-resistant acid phosphatase 5b

clinical setting [23, 24], but neither levamisole-treated test tubes nor clinically approved PPi assays are commercially available, yet.

Accordingly, as part of this observational study, we evaluated whether assessing levels of the glycophospholipid, PEA, normalized to Cr in urine (urine PEA/Cr ratio), was feasible and responsive to treatment with asfotase alfa in adults with HPP. In line with that hypothesis, urine PEA/Cr ratio levels were significantly reduced at all study timepoints (6, 12, 18, and 24 months) following treatment initiation when compared to baseline. However, median urine PEA/Cr ratio levels did not reach the normal range, which is similar to the findings in a previous report [25]; instead, median urine PEA/Cr ratio levels remained slightly above the upper limit of the normal range. Unfortunately, normal range values for urine PEA/Cr ratio levels are generally poorly defined with ranges varying among different laboratories. Therefore, drawing conclusions
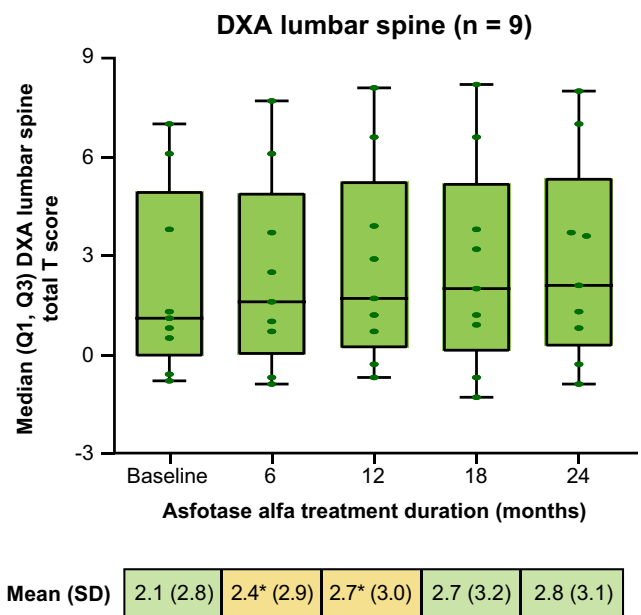
DXA lumbar spine (n = 9)

| Mean (SD) | 2.1 (2.8) | 2.4* (2.9) | 2.7* (3.0) | 2.7 (3.2) | 2.8 (3.1) |

Fig. 4 DXA lumbar spine total T score during 24 months of asfotase alfa treatment. *Statistical significance ($P < 0.05$) vs baseline based on paired t tests of the mean (SD) values. Whiskers include values outside of Q1 and Q3 with extremes marking the minimum and maximum values. DXA, dual-energy X-ray absorptiometry; Q, quartile; SD, standard deviation

a standardized concentration of an ALP inhibitor, such as levamisole, to test tubes. In addition, samples for PPi would have to be kept on ice and filtered immediately for use in a

from specific target values is difficult, specifically since there is no established target range for urine PEA/Cr ratio levels in adults with HPP. Accordingly, we cannot confirm whether the degree of reduction or absolute urine PEA/Cr ratio levels attained following the initiation of asfotase alfa are meaningful with regard to treatment response or efficacy. Even though PEA is considered a natural substrate of ALP [26], the metabolic pathway eventually leading to PEA accumulation in HPP is not fully understood [27, 28]. This is because large amounts of PEA are supposed to be derived from the phosphatidylinositol glycan moiety that couples proteins such as ALP to the cell surface, and the possibility that the majority of PEA is derived from the liver rather than bone [29]. Reflecting on these limitations, it appears premature to recommend urine PEA/Cr ratio levels as a pivotal marker for treatment efficacy or dose adjustments. Still, if additional studies can support the significance of this marker for monitoring treatment, this may become expedient in the future.

In this study, baseline serum levels for all bone and mineral metabolism markers were near normal. However, along with reduced serum ALP activity, baseline levels for PTH, osteocalcin, and NTx were detected toward the lower end of the normal range. These findings are consistent with previous reports indicating that there are reduced mineral turnover, diminished bone metabolism, and, consequently, compromised uptake of circulating minerals to the bone prior to treatment in patients with HPP [12, 13, 16]. Correspondingly, calcium and specifically phosphate levels tended to be at the higher end of the normal range. Importantly, none of the patients in this study was exposed to osteoporosis drugs for at least the last 5 years before initiation of ERT. Accordingly, a relevant impact of such drugs or their withdrawal on bone turnover markers in this study is highly unlikely.

Treatment initiation in this cohort elicited considerable alterations in mineral metabolism and bone turnover, likely reflecting corresponding changes in bone remodeling. Compared with baseline, there were significant increases in PTH at 3 and 6 months of treatment, while serum calcium, phosphate, and FGF-23 levels did not change significantly across 24 months of treatment with asfotase alfa. Building on the understanding that asfotase alfa degrades mineralization inhibitors such as PPi and potentially phospho-osteopontin, these changes may reflect a compensatory mechanism to address a transiently increased need for calcium, while on the other hand, the requirement for additional phosphate can be compensated by phosphate released from previously accumulated ALP substrates.

In contrast to other bone-targeted treatment strategies such as bisphosphonates, PTH/parathyroid hormone-related protein analogues, or antibodies against receptor activator of nuclear factor kappa-B ligand (RANKL) or sclerostin, enzyme replacement therapy with asfotase alfa is not known to directly and specifically interfere with cell differentiation, proliferation, activity, or apoptosis. However, even though asfotase alfa is essentially just reenabling and catalyzing a previously insufficient chemical process, our data clearly show that beyond merely facilitating degradation of phosphoric compounds, treatment with asfotase alfa eventually has an impact on osteoblast and osteoclast-mediated bone remodeling. Considering that bone turnover and remodeling is only feasible on mineralized bone surfaces, it appears reasonable to assume that mineralization of previously unmineralized osteoid (i.e., healing of osteomalacia following asfotase alfa treatment) could enable remodeling activity particularly on previously inaccessible bone surfaces. Specifically, there was a prolonged increase in TRAP5b levels, likely reflecting increased differentiation and formation of osteoclasts. Similarly, osteocalcin and P1NP were significantly elevated during the first 6 months of treatment with an increase in osteocalcin being suggestive of increased osteoblast activity and bone mineralization [30, 31], whereas elevated P1NP indicates increased type 1 collagen synthesis and deposition. In parallel, actual resorptive activity determined by NTx exhibited a nonsignificant trend to be transiently increased and eventually decreased, suggesting a progressively stable situation during the course of treatment without further need for enhanced remodeling.

In line with previous reports, lumbar spine BMD scores in this cohort of severely affected HPP patients were higher than femoral BMD scores and above average for the normal population [32, 33]. A potential explanation for this finding was the accumulation of osteoid and inappropriately mineralized, mechanically inferior bone tissue, specifically in axially loaded vertebrae as a consequence of underlying osteomalacia with compromised bone quality and reduced turnover. In addition, erroneous calcification of ligamentous structures may contribute to high lumbar spine BMD scores in patients with HPP. Following treatment initiation with reduction of mineralization inhibitors such as PPi, progressive mineralization of both these may have contributed to the observed further increase in lumbar spine BMD.

Because ALP activity reduces the level of mineralization inhibitors and elevated ALP activity is associated with vascular calcification in CKD, concerns regarding an increased risk of vascular calcification during treatment with asfotase alfa have been raised based on animal models of excessive ALP activity [34, 35]; however, no such findings have been documented in humans [36]. Notably, lateral assessments regularly done in addition to our BMD assessments did not reveal any signs of aortal calcification or increased mineral deposition within the longitudinal ligament. Accordingly, the increased BMD scores following initiation of asfotase alfa are most likely associated with bone healing, bone remodeling, and enhanced bone mineralization of preexisting mineralized tissue. Indeed, increases in lumbar spine BMD scores have previously been reported in children on enzyme replacement therapy, and in this setting, improvements in Rickets Severity

Score correlated significantly with increased lumbar spine $BMD_{ht}Z$ scores [37].

Regarding fracture incidence, our data provide a first impression on the incidence of fractures following the initiation of enzyme replacement therapy in patients with HPP. However, of the four fractures and three pseudofractures documented, only three metatarsal fractures in two patients and one fracture of the medial malleolar in another patient shortly after treatment initiation can possibly be associated with HPP-related bone quality. Thus, it is premature to draw conclusions related to fracture incidence following treatment initiation.

The main limitations of our study are its observational nature and the limited sample size, which restricts generalizability of our findings. Regardless, considering the rarity of adult HPP patients with severe pediatric-onset HPP and overt bone manifestations, results presented herein reflect the largest available cohort with consistent outcome data in that regard.

Taken together, the observed alterations in mineral metabolism and bone formation markers, together with changes in BMD, further support the understanding that asfotase alfa facilitates bone mineralization, while also providing some insight that treatment with asfotase alfa may elicit bone remodeling and potentially improve bone structure in adults with pediatric-onset HPP.

Conclusions

Results of this study indicate that asfotase alfa treatment is associated with significant changes in biochemical markers of bone and mineral metabolism in adult patients with pediatric-onset HPP. These changes suggest that treatment-mediated mineralization may enable bone remodeling and bone turnover on previously unmineralized and thus inaccessible bone surfaces. In addition, our findings suggest that the urine PEA/Cr ratio should be evaluated further as a potential biochemical marker for monitoring asfotase alfa treatment.

Declarations

References

1. Rockman-Greenberg C (2013) Hypophosphatasia. Pediatr Endocrinol Rev 10(Suppl 2):380–388
2. Whyte MP (2010) Physiological role of alkaline phosphatase explored in hypophosphatasia. Ann N Y Acad Sci 1192:190–200
3. Whyte MP (2016) Hypophosphatasia - aetiology, nosology, pathogenesis, diagnosis and treatment. Nat Rev Endocrinol 12:233–246
4. Weber TJ, Sawyer EK, Moseley S, Odrljin T, Kishnani PS (2016) Burden of disease in adult patients with hypophosphatasia: results from two patient-reported surveys. Metabolism 65:1522–1530

5. Kuehn K, Hahn A, Seefried L (2020) Mineral intake and clinical symptoms in adult patients with hypophosphatasia. J Clin Endocrinol Metab 105:e2982–e2992

6. Seefried L, Dahir K, Petryk A, Hogler W, Linglart A, Martos-Moreno GA, Ozono K, Fang S, Rockman-Greenberg C, Kishnani PS (2020) Burden of illness in adults with hypophosphatasia: data from the global hypophosphatasia patient registry. J Bone Miner Res 35:2171–2178

7. Alexion Pharmaceuticals, Inc. Strensiq (asfotase alfa) injection, for subcutaneous use [prescribing information]. New Haven, CT, USA. 2018.

8. Choida V, Bubbear JS (2019) Update on the management of hypophosphatasia. Ther Adv Musculoskelet Dis 11:1759720X19863997

9. Kishnani PS, Rockman-Greenberg C, Rauch F, Bhatti MT, Moseley S, Denker AE, Watsky E, Whyte MP (2019) Five-year efficacy and safety of asfotase alfa therapy for adults and adolescents with hypophosphatasia. Bone 121:149–162

10. Russell RG, Bisaz S, Donath A, Morgan DB, Fleisch H (1971) Inorganic pyrophosphate in plasma in normal persons and in patients with hypophosphatasia, osteogenesis imperfecta, and other disorders of bone. J Clin Invest 50:961–969

11. Seefried L, Kishnani PS, Moseley S, Denker AE, Watsky E, Whyte MP, Dahir KM (2021) Pharmacodynamics of asfotase alfa in adults with pediatric-onset hypophosphatasia. Bone 142:115664

12. Genest F, Seefried L (2018) Subtrochanteric and diaphyseal femoral fractures in hypophosphatasia-not atypical at all. Osteoporos Int 29:1815–1825

13. Lopez-Delgado L, Riancho-Zarrabeitia L, Garcia-Unzueta MT, Tenorio JA, Garcia-Hoyos M, Lapunzina P, Valero C, Riancho JA (2018) Abnormal bone turnover in individuals with low serum alkaline phosphatase. Osteoporos Int 29:2147–2150

14. Salles JP (2020) Hypophosphatasia: biological and clinical aspects, avenues for therapy. Clin Biochem Rev 41:13–27

15. Whyte MP (2017) Hypophosphatasia: an overview For 2017. Bone 102:15–25

16. Whyte MP, Greenberg CR, Salman NJ, Bober MB, McAlister WH, Wenkert D, van Sickle BJ, Simmons JH, Edgar TS, Bauer ML, Hamdan MA, Bishop N, Lutz RE, McGinn M, Craig S, Moore JN, Taylor JW, Cleveland RH, Cranley WR, Lim R, Thacher TD, Mayhew JE, Downs M, Millán JL, Skrinar AM, Crine P, Landy H (2012) Enzyme-replacement therapy in life-threatening hypophosphatasia. N Engl J Med 366:904–913

17. Freitas TQ, Franco AS, Pereira RMR (2018) Improvement of bone microarchitecture parameters after 12 months of treatment with asfotase alfa in adult patient with hypophosphatasia: case report. Medicine (Baltimore) 97:e13210

18. Remde H, Cooper MS, Quinkler M (2017) Successful asfotase alfa treatment in an adult dialysis patient with childhood-onset hypophosphatasia. J Endocr Soc 1:1188–1193

19. Alexion Europe SAS. Strensiq, 40 mg/mL and 100 mg/mL solution for injection [summary of product characteristics]. Levallois-Perret, France. 2020.

20. Genest F, Rak D, Petryk A, Seefried L (2020) Physical function and health-related quality of life in adults treated with asfotase alfa for pediatric-onset hypophosphatasia. JBMR Plus Published online August 4:e10395. https://doi.org/10.1002/jbm4.10395

21. Hofmann CE, Harmatz P, Vockley J, Högler W, Nakayama H, Bishop N, Martos-Moreno GÁ, Moseley S, Fujita KP, Liese J, Rockman-Greenberg C, ENB-010-10 Study Group (2019) Efficacy and safety of asfotase alfa in infants and young children with hypophosphatasia: a phase 2 open-label study. J Clin Endocrinol Metab 104:2735–2747

22. Whyte MP, Simmons JH, Moseley S, Fujita KP, Bishop N, Salman NJ, Taylor J, Phillips D, McGinn M, McAlister WH (2019) Asfotase alfa for infants and young children with hypophosphatasia: 7 year outcomes of a single-arm, open-label, phase 2 extension trial. Lancet Diabetes Endocrinol 7:93–105

23. Jansen RS, Duijst S, Mahakena S, Sommer D, Szeri F, Váradi A, Plomp A, Bergen AA, Oude Elferink RPJ, Borst P, van de Wetering K (2014) ABCC6-mediated ATP secretion by the liver is the main source of the mineralization inhibitor inorganic pyrophosphate in the systemic circulation-brief report. Arterioscler Thromb Vasc Biol 34:1985–1989

24. Tolouian R, Connery SM, O'Neill WC, Gupta A (2012) Using a filtration technique to isolate platelet free plasma for assaying pyrophosphate. Clin Lab 58:1129–1134

25. Akiyama T, Kubota T, Ozono K, Michigami T, Kobayashi D, Takeyari S, Sugiyama Y, Noda M, Harada D, Namba N, Suzuki A, Utoyama M, Kitanaka S, Uematsu M, Mitani Y, Matsunami K, Takishima S, Ogawa E, Kobayashi K (2018) Pyridoxal 5′-phosphate and related metabolites in hypophosphatasia: effects of enzyme replacement therapy. Mol Genet Metab 125:174–180

26. Fedde KN, Whyte MP (1990) Alkaline phosphatase (tissue-nonspecific isoenzyme) is a phosphoethanolamine and pyridoxal-5′-phosphate ectophosphatase: normal and hypophosphatasia fibroblast study. Am J Hum Genet 47:767–775

27. Calzada E, Onguka O, Claypool SM (2016) Phosphatidylethanolamine metabolism in health and disease. Int Rev Cell Mol Biol 321:29–88

28. Millan JL, Whyte MP (2016) Alkaline phosphatase and hypophosphatasia. Calcif Tissue Int 98:398–416

29. Millan JL, Whyte MP, Avioli LV, Fishman WH (1980) Hypophosphatasia (adult form): quantitation of serum alkaline phosphatase isoenzyme activity in a large kindred. Clin Chem 26:840–845

30. Owen TA, Aronow M, Shalhoub V, Barone LM, Wilming L, Tassinari MS, Kennedy MB, Pockwinse S, Lian JB, Stein GS (1990) Progressive development of the rat osteoblast phenotype in vitro: reciprocal relationships in expression of genes associated with osteoblast proliferation and differentiation during formation of the bone extracellular matrix. J Cell Physiol 143:420–430

31. Calvo MS, Eyre DR, Gundberg CM (1996) Molecular basis and clinical application of biological markers of bone turnover. Endocr Rev 17:333–368

32. Genest F, Claussen L, Rak D, Seefried L (2020) Bone mineral density and fracture risk in adult patients with hypophosphatasia. Osteoporos Int

33. Whyte MP, Zhang F, Wenkert D, McAlister WH, Mack KE, Benigno MC, Coburn SP, Wagy S, Griffin DM, Ericson KL, Mumm S (2015) Hypophosphatasia: validation and expansion of the clinical nosology for children from 25 years experience with 173 pediatric patients. Bone 75:229–239

34. Amadeu de Oliveira F, Narisawa S, Bottini M, Millan JL (2020) Visualization of mineral-targeted alkaline phosphatase binding to sites of calcification in vivo. J Bone Miner Res 35:1765–1771

35. Hui M, Tenenbaum HC (1998) New face of an old enzyme: alkaline phosphatase may contribute to human tissue aging by inducing tissue hardening and calcification. Anat Rec 253:91–94

36. Whyte MP, McAlister WH, Mumm S, Bierhals AJ (2019) No vascular calcification on cardiac computed tomography spanning asfotase alfa treatment for an elderly woman with hypophosphatasia. Bone 122:231–236

37. Simmons JH, Rush ET, Petryk A, Zhou S, Martos-Moreno GA (2020) Dual X-ray absorptiometry has limited utility in detecting bone pathology in children with hypophosphatasia: a pooled post hoc analysis of asfotase alfa clinical trial data. Bone 137:115413

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