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ABSTRACT

Background: Hypophosphatasia (HPP) is an autosomal recessive or dominate disease affecting bone mineralization, and adults with HPP are in risk to develop metatarsal stress fractures and femoral pseudofractures. Given to the scarce data on the bone quality and its association to the fracture risk in adults with HPP, this study aimed to evaluate bone turnover, bone strength and structure in adults with HPP.

Methods: In this cross-sectional study, we included 14 adults with genetically verified HPP and 14 sex-, age-, BMI-, and menopausal status-matched reference individuals. We analyzed bone turnover markers, and measured bone material strength index (BMSi) by impact microindentation. Bone geometry, volumetric density and bone microarchitecture as well as failure load at the distal radius and tibia were evaluated using a second-generation high-resolution peripheral quantitative computed tomography system.

Results: Bone turnover markers did not differ between patients with HPP and reference individuals. BMSi did not differ between the groups (67.90 [63.75–76.00] vs 65.45 [58.43–69.55], $p = 0.149$). Parameters of bone geometry and volumetric density did not differ between adults with HPP and the reference group. Patients with HPP had a tendency toward higher trabecular separation (0.664 [0.613–0.724] mm vs 0.620 [0.578–0.659] mm, $p = 0.054$) and inhomogeneity of trabecular network (0.253 [0.235–0.283] mm vs 0.229 [0.208–0.252] mm, $p = 0.056$) as well as lower trabecular bone volume fraction (18.8 [16.4–22.7] % vs 22.8 [20.6–24.7] %, $p = 0.054$) at the distal radius. In addition, compound heterozygous adults with HPP had a significantly higher cortical porosity at the distal radius than reference individuals (1.5 [0.9–2.2] % vs 0.7 [0.6–0.7] %, $p = 0.041$).

Conclusions: BMSi is not reduced in adults with HPP. Increased cortical porosity may contribute to the occurrence of femoral pseudofractures in compound heterozygous adults with HPP. However, further studies investigating

Abbreviations: ALP, alkaline phosphatase; *ALPL*, TNAP encoding gene; BALP, bone-specific alkaline phosphatase; BMD, bone mineral density; BMSi, bone material strength index; CHZ, compound heterozygous; Ct, cortical; Ct.Po, cortical porosity; Ct.Th, cortical thickness; CTX, carboxyterminal cross-linked telopeptide of type 1 collagen; CVs, coefficients of variation; DXA, dual energy X-ray absorptiometry; FEA, micro-finite element analysis; FGF-23, fibroblast growth factor 23; FSH, follicle stimulating hormone; HbA1c, glycated hemoglobin; HPLC, high pressure liquid chromatography; HR-pQCT, high-resolution peripheral quantitative computed tomography; HPP, hypophosphatasia; HZ, heterozygous; IMI, impact microindentation; IQR, interquartile range; IVA, instant vertebral assessment; LH, luteinizing hormone; LP, likely pathogenic; OC, osteocalcin; P, pathogenic; PINP, N-terminal propeptide of type 1 procollagen; PLP, pyridoxal-5′-phosphate; PTH, parathyroid hormone; Tb, trabecular; Tb.BV/TV, trabecular bone volume fraction; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; Tb.1/N.SD, inhomogeneity of trabecular network; TNAP, tissue non-specific alkaline phosphatase; TRAcP5b, tartrate-resistant acid phosphatase 5b; TSH, thyroid-stimulating hormone; Tt, total; vBMD, volumet ric bone mineral density.

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larger cohorts of adults with HPP using methods of bone histomorphometry are recommended to adequately assess the bone quality in adults with HPP.

1. Introduction

Hypophosphatasia (HPP) is a rare, autosomal recessive or dominant disorder caused by pathogenic variants in the tissue-nonspecific alkaline phosphatase (TNAP) encoding gene (*ALPL*) [1,2]. The disease is characterized by reduced activity of TNAP, a key player in mineralization of hard tissues [3–5]. Impaired function of TNAP leads to pathological mineralization and reduced construction of hydroxyapatite crystals in the bone [6–11]. Persistently low levels of alkaline phosphate (ALP) and elevated pyridoxal-5´-phosphate (PLP) in plasma are biochemical indicators of HPP [3,12]. The clinical presentation and severity are highly variable, depending on the inheritance mechanism and genotype [13]. Adults with HPP can develop multisystemic clinical features including chronic musculoskeletal pain, impaired physical function, fragility fractures and dental abnormalities [14–20]. Fragility fractures can occur at different sites in the skeleton, while metatarsal stress fractures with delayed healing and incomplete femoral fractures with thickened periosteum known as pseudofractures are well described clinical features in adults with HPP [16,19,21].

However, the bone phenotype in adults with HPP is complex and has not been well investigated. Previous studies have demonstrated variable bone mineral density (BMD) and T-scores assessed by dual energy X-ray absorptiometry (DXA) among adults with HPP [19,22,23]. In addition, lumbar spine T-scores tend to be high in adults with HPP and fractures [23,24]. Therefore, in contrast to bone diseases like osteoporosis, the evaluation of T-scores may not be a sensitive method to assess the fracture risk in HPP [22,23]. These findings suggest that additional factors contribute to altered bone strength in adults with HPP, including bone material properties as well as bone microarchitecture of trabecular and cortical compartments.

Impact microindentation (IMI) is used to directly measure mechanical characteristics of cortical bone on tissue level in vivo [25,26]. This technique assesses the resistance of bone to indentation, calculated as bone material strength index (BMSi) [25,27]. The IMI has been used in several studies, investigating the impact of bone material properties on fracture risk [26,28]. Moreover, it has been demonstrated that BMSi is decreased in individuals with low BMD and fragility fractures, independently of BMD measurements [29,30]. Further, high-resolution peripheral quantitative computed tomography (HR-pQCT) is a well-established method to assess bone geometry, volumetric BMD (vBMD) and bone microarchitecture at the distal radius and tibia. A single study evaluated bone structural parameters in adult patients with HPP using HR-pQCT, indicating that altered microarchitectural parameters of trabecular and cortical bone associate with risk of fractures [19]. We hypothesize that adults with HPP have altered bone strength and microarchitecture. The aim of this study was to assess bone turnover markers, bone material strength using IMI as well as bone geometry, microstructure and finite element analysis by HR-pQCT in adults with HPP and healthy matched reference individuals.

2. Methods

2.1. Study design and population

This cross-sectional study was conducted between October 2019 and September 2021. Patients with HPP were recruited from the Departments of Endocrinology at Copenhagen University Hospital Hvidovre and Aarhus University Hospital, the Departments of Clinical Genetics at Odense University Hospital and Vejle Hospital, and the Center for Inherited Metabolic Diseases Rigshospitalet, Denmark. Reference individuals were recruited trough online advertisement and from a population list including residents from the Capital Region and the Region Zealand of Denmark, randomly selected using the Danish Civil Registration System.

Patients with HPP were ≥ 18 years of age, compound heterozygous (CHZ) or heterozygous (HZ) for a pathogenic variant in *ALPL* according to the ACMG guidelines [31], had persistently low ALP ≤ 35 U/L (reference range 35–105 U/L) and at least one of the following symptoms: (1) dental manifestations; (2) musculoskeletal pain; (3) history of fracture(s). At inclusion, reference individuals had to have no ALP measurements ≤45 U/L and ≥ 50% of all ALP measurements ≥55 U/L, normal parathyroid hormone (PTH) and PLP as well as no severe vitamin D deficiency (25-hydroxyvitamin D ≤ 25 nmol/L).

Exclusion criteria for all participants included pregnancy, skin infection or severe skin affection in the measurement area of microindentation, known allergy to lidocain, former or current medical treatment influencing bone metabolism (oral corticosteroid >12 weeks, former or current anti-osteoporosis treatment at any time (regardless drug holiday), all kind of sex steroids (excluding oral contraception), anticonvulsants) and current malignant disorders. In addition, reference individuals could not participate when they had a family history of genetic metabolic bone disease (HPP, osteogenesis imperfecta), rickets in childhood, former or current osteoporosis or osteomalacia, known diabetes, chronic liver or gallbladder disease, former or current thyrotoxicosis (T4 over normal range ≥ 6 months) or Cushing's disease. Fourteen adults with HPP were matched 1:1 by sex, age (±5 years), BMI (±3 kg/m^2), and the duration of menopause (±2 years) in postmenopausal women with reference individuals. Participants were barefoot, wearing indoor clothes, when body weight was assessed to the nearest 0.1 kg on a calibrated scale (Seca, Hamburg, Germany). Standing height was measured to the nearest 0.1 cm using a calibrated Harpeden stadiometer (Holtain Limited, Crymych, UK).

Informed consent was provided by all participants and the study was performed in accordance with the Helsinki Declaration. The present study was registered at cliniclatrials.gov (NCT04181164) and was approved by the Ethics Committee (registration no.: H-19000730) and the Data Protection Agency (registration no.: VD-2019-102) for the Capital Region of Denmark.

2.2. Biochemical analyses

Blood samples were drawn after overnight fasting between 7:30 and 10:00 am. All participants had to pause vitamin B6 and calcium supplements for at least two weeks prior blood sampling. All analyses were done with serum (s-)/plasma (p-) as the sample material. For each assay the sample aliquots were kept frozen at −80 degrees Celsius until the day of analysis. Samples for bone turnover marker measurements were analyzed using one single batch of each assay. Carboxyterminal cross-linked telopeptide of type 1 collagen (s-CTX) was measured using the IDS-iSYS CTX (CrossLaps®) assay (Immunodiagnostic Systems, plc, Tyne and Wear, UK), and propeptide of type 1 procollagen (s-PINP) was analyzed using the IDS-iSYS intact PINP assay (Immunodiagnostic Systems). The bone-specific alkaline phosphatase (s-BALP) was measured using the IDS-iSYS Ostase® BALP assay (Immunodiagnostic Systems). Osteocalcin (s-OC) and tartrate-resistant acid phosphatase 5b (p-TRAcP5b) were analyzed using the N-Mid Osteocalcin assay and the BoneTRAP® assay, respectively (Immunodiagnostic Systems) on the automated iSYS analyzer (Immunodiagnostic Systems). The p-sclerostin was measured using the TECOMedical Sclerostin HS EIA assay (Quidel Corporation, San Diego, CA) and determinations were made in duplicate. Fibroblast growth factor 23 (p-FGF-23) was measured using the Liaison FGF-23 assay (Diasorin, Saluggia, Italy) on the automated

Liaison XL analyzer (Diasorin). Assay performance was verified using the manufacturers' control specimens and patient pools.

The p-vitamin B6/p-PLP was determined using the Chromsystems Vitamin B6 assay (Chromsystems, Munich, Germany) on a Dionex high pressure liquid chromatography (HPLC) system (Fischer Scientific, Roskilde, Denmark). The COBAS 8000 from Roche Diagnostics GmbH, Mannheim, Germany was used to analyze p-ALP, and all other biochemical parameters were measured by standard methods at the certified laboratory at Copenhagen University Hospital Hvidovre.

The intermediary precisions expressed as coefficients of variation (CVs) for s-CTX were 5.3% (at s-CTX concentration 213 ng/L), 3.4% (869 ng/L), and 3.5% (2113 ng/L) for iSYS. For s-PINP the intermediary precisions were 5.4% (18.96 μg/L), 6.5% (48.48 μg/L), and 6.1% (122.10 μg/L) for iSYS, and for s-BALP the intermediary precisions were 8.5%, 7.1%, 3.7%, and 6.3% at levels of 4.5, 13.2, 20.1, and 52.1 μg/L, respectively. In addition, CVs for s-OC were 3.0% (at an s-OC concentration of 8.73 μg/L), 3.6% (27.6 μg/L), and 3.5% (68.7 μg/L) and for p-TRAcP5b the intermediary precision was 10.9% (3.2 U/L), 4.8% (6.2 U/L), and 5.4% (9.0 U/L). For p-sclerostin, the intra-assay precision was <10% at both the 0.2 ng/mL and 1.9 ng/mL levels. Finally, for both p-FGF-23 and p-PLP the intermediary precision was <10% (all levels).

2.3. Fracture assessment and DXA

Information on fracture history in adults with HPP and reference individuals was obtained by a structured interview and from the electronical medical records as well as from documented radiographic test results. T-scores of the lumbar spine (L1-L4) and left hip were evaluated by DXA (Hologic Horizon™ QDR™ Series, Hologic, Inc., Bedford, MA, USA). Screening for vertebral fractures was performed by instant vertebral assessment (IVA) [32]. Fractures were categorized as low- (caused by fall from standing height or daily activities) and high-energy (related to a relevant trauma) fractures.

2.4. Bone impact microindentation testing

BMSi was assessed by IMI using a handheld indenter device (OsteoProbe® RUO, Active Live Scientific, Santa Barbara, CA, USA). Measurements were performed by two experienced physicians (LF, SM) at Odense University Hospital, Denmark. After skin disinfection and local analgesia with lidocaine (1%), the indenter device was used to create a microscopic indentation in the non-dominant mid-tibia bone by applying a dynamic impact. In brief, the 90-degree indenter device with a microscopic conic 10-μm edge was penetrated through the skin once and an initial preload of 10 N was applied to anchor the indenter into the bone and to ensure it had pierced the periosteum. Once the preload force had been reached, an impact with a peak force of 30 N was initiated to create the indentation. Ten accepted measurements in a defined radius (at least 2 mm away from previous site) were concluded. In addition, ten measurements were performed on a polymethylmethacrylate calibration phantom. Measurement stability was evaluated for bone tissue (<6 = excellent, 6–8 = good, 8–10 = adequate, >10 = could be improved) and reference material (<1 = excellent, 1–1.5 = good, 1.5–1.0 = adequate, 2–3 = could be improved, >3 = needs improvement). The system measures the indentation distance at peak force, translates it to a computerized unit and calculates an average value. Possible outliers were removed by the manufacturer's software. The indentation units, expressed as BMSi are defined as 100 times the ratio of the indentation distance from the impact into the calibration material, divided by the indentation distance from the impact into the bone [25,33].

2.5. High-resolution peripheral quantitative computed tomography

Bone geometry, vBMD, microarchitecture and estimated bone strength were evaluated using a second-generation HR-pQCT system (Xtreme CT II, Sanco Medical AG, Brüttisellen, Switzerland) according to the manufacturer's standard protocol [34,35]. The non-dominant distal radius and tibia were scanned, unless previous fractures or metal from surgeries were present at these sites, otherwise the contralateral limbs were examined. All scans were performed using the same HR-pQCT system at Odense University Hospital, Denmark. The scan regions were selected by the relative offset distance method using an %-of-length offset of 4.0% at the distal radius and 7.0% at the distal radius in patients with HPP and reference individuals [35]. The image quality was evaluated for motion artefacts by a five-step scale (1 = best; 5 = worst) [36], and images graded >3 were not included to data analysis.

Parameters of bone geometry including total, cortical and trabecular area (Tt.Area, Ct.Area and Tb.Area, respectively) were determined as mean cross-sectional area in all image slides [35,37]. Total, cortical and trabecular vBMD (Tt.vBMD, Ct.vBMD and Tb.vBMD, respectively) were calculated directly from grey scale image data and expressed as mg HA/cm^3 [35,37]. Microarchitectural parameters were assessed from the segmented bone structure in trabecular and cortical compartments applying direct 3D morphological measurement techniques. Trabecular number (Tb.N) was evaluated applying the ridge extraction technique, and trabecular thickness (Tb.Th) as well as trabecular separation (Tb.Sp) were measured using the distance transformation method [35]. Inhomogeneity of trabecular network (Tb.1/N.SD) was calculated as the standard deviation of the spacing between mid-axis (ridges) of the trabeculae [35]. Trabecular bone volume fraction (Tb.BV/TV) was calculated as the ratio of voxels in the mineralized bone segment to the total number of voxels in the trabecular section [35,38]. Cortical thickness (Ct.Th) was assessed by the distance transformation method, and cortical porosity (Ct.Po) was measured using automated dual threshold segmentation as described by others [39,40]. Threshold values for bone volume segmentation included 320 mg HA/cm^3 for trabecular- and 450 mg HA/cm^3 for cortical bone [35]. Failure load was estimated by a micro-finite element analysis (FEA) using the manufacturers software. Material properties of bone tissue included a Poisson's ratio of 0.3 and a Young modulus of 10 GPa [41], and failure load was estimated using the yield criterion of 0.7% critical strain and 2% critical volume [42]. Information on short term precision is important to interpret statistical differences between results. Precision error ranges of the second-generation HR-pQCT system has been assessed by Manske et al. [38]. The calculated root-mean-square coefficient of variation has ranged from 0.4% to 1.2% for geometrical areas and 0.4% to 2.4% for volumetric BMD [38,43]. Microarchitectural measurements has shown a variance from 0.8% to 2.7% for trabecular- and 1.3% to 13.7% for cortical parameters [38,43].

2.6. Statistical analysis

Categorial variables were stated as numbers and continuous variables were expressed as median (interquartile range [IQR]), as data were not normally distributed (evaluated for normal distribution using histogram and quantile-quantile plots). Differences between the groups were calculated using the Wilcoxon's rank-sum test. The Spearman's correlation was applied for association analyses. P values <0.05 were considered as statistically significant. All analyses were done using the R studio statistical software (version 3.6.1) and GraphPad Prism 9 (GraphPad Software Inc., CA, USA).

3. Results

3.1. Anthropometric, biochemical and clinical characteristics

Ten adults with HPP were HZ for a pathogenic variant in *ALPL* and classified as adult-onset HPP, and four patients were CHZ and diagnosed in childhood (pediatric-onset HPP). Genotypes of adults with HPP are specified in Supplemental Table 1. Basic characteristics including age, BMI, body weight, standing height and the duration of menopause did not differ between adults with HPP and reference individuals (Table 1).

Table 1
Basic characteristics.

	Adults with HPP (n = 14)	Reference individuals (n = 14)
Age (years)	44.5 (37.5–50.5)	46.00 (35.5–50.5)
Sex (female/male)	12/2	12/2
BMI (kg/m^2)	23.5 (22.6–30.3)	24.4 (22.5–30.8)
Body weight (kg)	65.0 (60.6–89.4)	67.5 (62.6–91.2)
Standing height (cm)	168.2 (161.5–169.5)	165.0 (162.1–168.8)
Menopause (post/pre)	3/9	3/9
Duration of menopause (years)	9.0 (5.0–17.0)	8.0 (4.5–19.0)
Calcium supplements (yes/no)	3/11	5/9
Vitamin D supplements (yes/no)	8/6	10/4
T-score lumbar spine	−0.2 (−1.1–0.7)	−0.3 (−0.9–0.2)
T-score left hip	−0.3 (−1.1–0.2)	−0.1 (−0.8–0.8)

Data er presented as median (IQR) or total numbers as appropriate.

Biochemical results are presented in Table 2. Median p-ALP was lower and p-PLP higher in adults with HPP, which we had expected as reference individuals had to have normal p-ALP and p-PLP measurements for inclusion. In addition, p-phosphate was significantly higher and s-BALP significantly lower in patients with HPP than reference individuals. Thyroid-stimulating hormone (p-TSH), p-PTH and p-25-hydroxyvitamin D did not differ statistically significant between the groups. All participants had normal kidney function and glycated hemoglobin (HbA1c) as well as negative M-component. Included male subjects had normal follicle stimulating hormone (p-FSH) and luteinizing hormone (p-LH). Levels of p-FSH, p-LH and p-estrogen were in accordance with pre- or postmenopausal conditions among all female participants.

Bone markers including s-PINP, s-CTX, s-OC, p-TRAcP5b and p-sclerostin did not differ between patients with HPP and reference individuals. Correlation analyses showed significantly positive associations between p-ALP and s-CTX (rho = 0.687, p = 0.007) as well as s-BALP and s-CTX (rho = 0.656, p = 0.011) in patients with HPP. Measurements of p-ALP correlated with p-TRAcP5b (rho = 0.630, p = 0.016), and p-PLP with p-sclerostin (rho = 0.551, p = 0.041) in the HPP group. In the reference group, p-phosphate correlated significantly with s-PINP (rho = 0.631, p = 0.015) as well as s-CTX (rho = 0.847, $p \leq 0.001$), and p-ALP correlated significantly with p-TRAcP5b (rho = 0.535, p = 0.049) and p-sclerostin (rho = 0.667, p = 0.009). Median p-FGF-23 was numerically lower among patients with HPP, though not statistically significant. Among patients with HPP and reference individuals, we did not observe significant correlations between p-phosphate and p-FGF-23. Spearman's correlations of bone markers and p-FGF-23 are shown in Supplementary Table 2.

Table 2
Biochemical parameters and bone markers.

	Adults with HPP (n = 14)	Reference individuals (n = 14)	p-Value
p-Calcium ion (1.18–1.32 mmol/L)	1.22 (1.20–1.24)	1.21 (1.20–1.22)	0.389
p-Phosphate (0.76–1.41 mmol/L)	1.23 (1.12–1.33)	0.93 (0.88–0.98)	0.001*
p-Magnesium (0.71–0.94 mmol/L)	0.87 (0.83–0.92)	0.84 (0.80–0.87)	0.062
p-Zinc (10–19 μmol/L)	11.0 (10.8–12.0)	13.0 (11.8–13.3)	0.073
p-25-hydroxyvitamin D (≥ 50 nmol/L)	68.0 (53.5–85.8)	81.5 (71.0–95.5)	0.085
p-TSH (0.65–4.80 *10^{-3} IU/L)	1.68 (1.19–2.11)	1.90 (1.33–2.41)	0.646
p-PTH (1.1–7.1 pmol/L)	3.7 (3.1–4.3)	3.8 (3.6–5.0)	0.231
p-PLP (15–73 nmol/L)	206 (107–874)	41 (34–60)	–
p-ALP (35–105 U/L)	22 (7–25)	65 (59–67)	–
s-BALP (μg/L)	5.3 (2.1–6.3)	14.7 (13.7–16.4)	<0.001*
s-PINP (μg/L)	53.0 (36.8–67.3)	45.5 (38.5–54.2)	0.667
s-CTX (ng/L)	290.6 (231.5–406.9)	288.9 (259.4–463.1)	0.910
s-Osteocalcin (μg/L)	16.8 (13.1–21.9)	13.25 (11.30–15.1)	0.081
p-TRAcP5b (U/L)	3.4 (2.8–3.8)	3.4 (2.9–3.7)	0.890
p-Sclerostin (ng/mL)	0.710 (0.603–0.768)	0.650 (0.623–0.703)	0.520
p-FGF-23 (pg/mL)	31.8 (27.1–35.0)	43.7 (36.6–50.9)	0.055

Values are median (IQR). Statistically significant results are marked with *. ALP = alkaline phosphatase; PLP = pyridoxal-5′-phosphate; PTH = parathyroid hormone; TSH = thyroid-stimulating hormone; BALP = bone specific alkaline phosphatase; CTX = C-terminal telopeptide of type 1 collagen; PINP = N-terminal propeptide of type 1 collagen; PTH = parathyroid hormone; TRAcP5b = tartrate-resistant acid phosphatase 5b; FGF-23 = fibroblast growth factor 23.

Results of fracture assessment are listed in Table 3. A history of fracture(s) was a possible inclusion criterion and a higher number of adults with HPP had non-vertebral, low-energy fractures as well as metatarsal fractures and fractures of toes compared with reference individuals. Two CHZ adults with HPP experienced bilateral femoral pseudofractures. Among all participants, no significant vertebral fracture (> 20% decrease in height) was identified.

3.2. Bone material strength

Median BMSi did not differ between adults with HPP and the reference group (median [IQR]) (67.90 [63.75–76.00] vs. 65.45 [58.43–69.55], p = 0.149). In addition, HZ and CHZ patients and reference individuals (HZR and CHZR) had similar BMSi: HZ vs. HZR (median [IQR]) (67.70 [61.65–75.63] vs. 63.75 [56.38–69.55], p = 0.190); CHZ vs. CHZR (median [IQR]) (70.85 [66.05–82.25] vs. 68.15 [66.43–71.60], p = 0.886). Further, BMSi did not differ between patients with HPP and metatarsal fractures (Met) as well as HPP patients without metatarsal fractures (NonMet) and reference individuals (MetR and NonMetR): Met vs. MetR (median [IQR]) (70.85 [62.70–79.95] vs. 67.30 [57.65–69.90], p = 0.393); NonMet vs. NonMetR (median [IQR]) (67.70 [63.50–73.75] vs. 64.45 [58.40–70.33], p = 0.382). Results of BMSi are visualized in Fig. 1. Measurements of BMSi did not correlate significantly with p-ALP, p-PLP, s-BALP, age, BMI, the number of non-vertebral fractures and non-vertebral, low-energy fractures, nor BMD at the lumbar spine or left hip (Table 4).

3.3. HR-pQCT measurements of the distal radius and tibia

Results of HR-pQCT measurements are shown in Table 5. Geometrical parameters at the distal radius and tibia (Tt. Area, Ct.Area and Tb. Area) did not differ between adults with HPP and the reference group. Measurements of Tt.vBMD and Tb.vBMD at the distal radius were slightly lower in HPP patients than reference individuals, though not statistically significant. Patients with HPP had a tendency toward higher Tb.Sp and Tb.1/N.SD as well as lower TB.BV/TV at the distal radius, though not statistically significant compared with reference individuals.

Table 3
Prevalence of non-vertebral fractures.

	Adults with HPP (n = 14)	Reference individuals (n = 14)
Any previous non-vertebral fracture (yes/no)	12/2	7/7
Any previous non-vertebral, low-energy fracture (yes/no)	11/3	3/11
Any previous non-vertebral, high-energy fracture (yes/no)	6/8	6/8
Fractures of the upper limps (yes/no)	6/8	4/10
Femoral fractures (including pseudofractures) (yes/no)	2/12	0/14
Fractures of tibia and fibula (yes/no)	5/9	2/12
Metatarsal fractures (yes/no)	6/8	0/14
Fractures of toes (yes/no)	4/10	1/13

Values are numbers of individuals with a certain type of fracture.

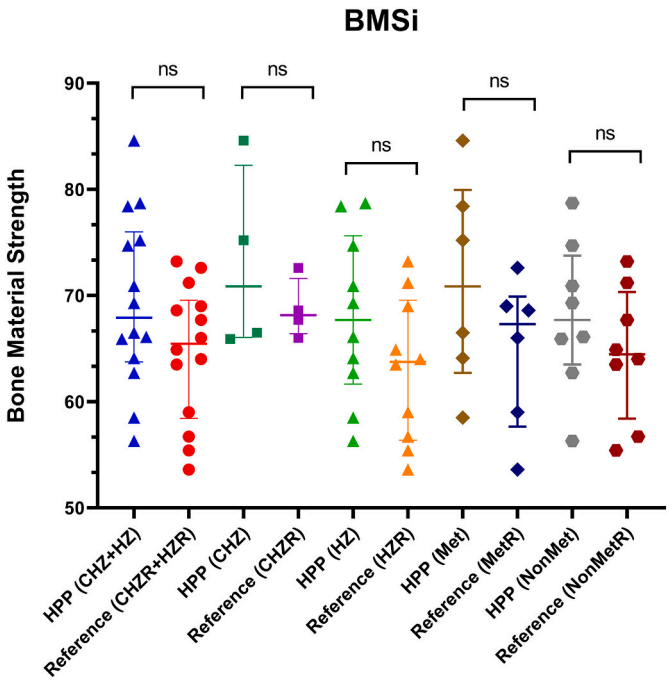

Fig. 1. Measurements of BMSi in HPP patients and reference individuals. BMSi was distinguished between adults with HPP (n = 14) and reference individuals (CHZR and HZR) (n = 14). In addition, BMSi of CHZ (n = 4) and HZ (n = 10) patients with HPP was compared with reference individuals (CHZR, n = 4; HZR, n = 10), respectively. Among patients with HPP, BMSi was distinguished between patients with metatarsal fractures (Met; n = 6) and reference individuals (MetR; n = 6) as well as between patients who did not have metatarsal fractures (NonMet; n = 8) and reference individuals (NonMetR; n = 8). Data are expressed as individual measurements, median and interquartile range. BMSi = bone material strength index; ns = not significant.

At both measured sites, Tb.N, Tb.Th, Ct.Th, Ct.Po and failure load did not differ between adults with HPP and reference individuals. Further, we performed subanalyses comparing HR-pQCT data between HZ as well as CHZ patients and matched reference individuals, respectively (Supplemental Tables 3 and 4). Parameters of bone geometry, vBMD and microarchitecture as well as failure load did not differ between HZ patients with HPP and reference individuals. The same observations were made when comparing these parameters between CHZ patients with HPP and reference individuals, with the exception that CHZ adults with HPP had significantly higher Ct.Po at the distal radius than reference individuals (median [IQR]) (1.5 [0.9–2.2] % vs 0.7 [0.6–0.7] %, p = 0.041). We performed correlation analyses between BMSi and all HR-pQCT measurements. In the HPP group, there were no significant correlations between BMSi and geometrical parameters, volumetric BMD or microarchitectural parameters and failure load at the distal radius and tibia (data not included in the manuscript). In comparison, BMSi measurements of reference individuals correlated significantly with Tt.vBMD (*rho* = 0.622, p = 0.020), Tb.vBMD (*rho* = 0.600, p = 0.023) and failure load (*rho* = 0.560, p = 0.040) at the distal tibia. Among reference individuals, all other parameters from HR-pQCT analyses did not correlate significantly with BMSi (data not included in the manuscript).

4. Discussion

In this study we assessed bone turnover markers, bone material strength and bone structural parameters in a cohort of adults with HPP and healthy matched reference individuals. In our cohort, a higher number of patients with HPP had non-vertebral, low-energy fractures, while bone turnover markers, BMSi as well as measurements of bone geometry and vBMD did not differ between the groups. However, our

Table 4
Spearman's coefficients of correlations between biochemical parameters, age, BMI, number of fractures, BMD and BMSi in patients with HPP (n = 14) and reference individuals (n = 14).

	p-ALP (U/L)	p-PLP (nmol/L)	s-BALP (µg/L)	Age (years)	BMI (kg/m^2)	NV fractures (number)	NV-LE fractures (number)	BMD lumbar spine	BMD left hip
BMSi									
Adults with HPP									
rho	−0.451	0.331	−0.444	0.658	−0.046	0.499	0.339	0.367	0.411
p-Value	0.106	0.248	0.111	0.129	0.875	0.069	0.276	0.197	0.146
Reference individuals									
rho	0.000	0.411	−0.075	−0.289	0.174	−0.502	−0.324	0.407	0.367
p-Value	1.000	0.144	0.800	0.317	0.552	0.068	0.259	0.151	0.197

Data are presented as *rho* and *p*-values. ALP = alkaline phosphatase; BALP = bone specific alkaline phosphatase; BMD = bone mineral density. PLP = pyridoxal-5′-phosphate; NV = non-vertebral, LE = low-energy.

Table 5
Bone geometry, volumetric density, microarchitecture and strength evaluated by HR-pQCT at the distal radius and tibia.

	Radius			Tibia		
	Adults with HPP (n = 14)	Reference individuals (n = 14)	*p*-value	Adults with HPP (n = 14)	Reference individuals (n = 14)	*p*-Value
Geometry (mm^2)						
Tt.Area	372.3 (329.7–381.9)	345.0 (317.0–379.6)	0.370	727.5 (649.6–759.9)	735.4 (651.0–794.0)	0.603
Ct.Area	47.05 (41.02–52.05)	46.50 (42.83–55.80)	0.701	108.9 (93.17–117.3)	121.5 (107.0–125.5)	0.135
Tb.Area	328.1 (292.4–338.2)	291.6 (278.2–329.7)	0.291	613.2 (561.1–658.1)	630.9 (541.2–666.3)	0.667
Volumetric density ($mgHA/cm^3$)						
Tt.vBMD	212.7 (197.3–240.5)	248.0 (228.8–270.0)	0.085	258.5 (244.7–291.9)	296.6 (246.4–319.2)	0.491
Ct.vBMD	801.2 (731.0–811.0)	790.8 (761.1–805.9)	0.982	878.2 (817.8–906.1)	893.6 (859.1–899.4)	0.730
Tb.vBMD	138.2 (124.5–157.4)	161.3 (148.7–176.4)	0.081	158.2 (149.4–167.6)	181.2 (157.7–194.8)	0.190
Microarchitecture						
Tb.N (1/mm)	1.460 (1.353–1.540)	1.536 (1.460–1.620)	0.085	1.405 (1.277–1.496)	1.409 (1.333–1.471)	0.769
Tb.Sp (mm)	0.664 (0.613–0.724)	0.620 (0.578–0.659)	0.054	0.688 (0.642–0.763)	0.681 (0.627–0.726)	0.581
Tb.Th (mm)	0.223 (0.218–0.227)	0.227 (0.216–0.236)	0.519	0.248 (0.235–0.265)	0.255 (0.238–0.274)	0.448
Tb.1/N.SD (mm)	0.253 (0.235–0.283)	0.229 (0.208–0.252)	0.056	0.279 (0.246–0.325)	0.275 (0.244–0.294)	0.783
Tb.BV/TV (%)	18.8 (16.4–22.7)	22.8 (20.6–24.7)	0.054	23.2 (22.3–24.8)	26.4 (23.3–28.6)	0.154
Ct.Th (mm)	0.647 (0.587–0.703)	0.693 (0.597–0.745)	0.280	1.183 (1.039–1.364)	1.321 (1.190–1.465)	0.285
Ct.Po (%)	0.8 (0.4–1.0)	0.7 (0.6–0.8)	0.710	2.3 (1.9–3.7)	2.2 (1.7–2.6)	0.433
Bone strength (*N*)						
Failure load	2378 (1805–2886)	2810 (2509–3083)	0.210	8193 (7607–9203)	9686 (8394–10,937)	0.137

Data are median (IQR).

results may indicate a tendency toward altered trabecular microarchitecture in adults with HPP, and subanalyses revealed significantly increased Ct.Po in CHZ adults with HPP.

Data on bone turnover makers in adults with HPP compared to a reference group are rare in the literature. In contrast to our results, Desborough et al. found significantly higher TRAcP5b and CTX in 20 adults with HPP compared with controls of which 61.9% had received treatment suppressing bone turnover [22]. This may be explained by the different reference groups, as we only included reference individuals who did not receive medicine suppressing nor increasing bone turnover markers. In comparison, significantly lower levels of PINP and CTX were reported in a Spanish study comparing bone turnover markers between 42 adults with low ALP activity (21 patients with pathogenic *ALPL* variant and 21 subjects with negative genetic screening) and a control group [44]. The inconsistent outcome compared to our study may be caused by the different selection of participants. The study from Spain identified patients through low levels of ALP, and half of the participants did not reveal pathogenic variants in *ALPL*, while we strictly included symptomatic adults with genetically verified HPP. Moreover, we speculate that diverse genotypes result in variable impairment of TNAP activity and thus bone turnover may cause inconsistent outcomes between studies investigating different cohorts of adults with HPP. Fibroblast growth factor 23 regulates phosphate homeostasis by supressing phosphate reabsorption in the kidney [45]. Interestingly, we found significantly higher p-phosphate levels, though lower p-FGF-23 values in patients with HPP than reference individuals. Possible explanations for this observation could be a dysfunction of FGF-23 synthesis in the bone or a disturbed feedback mechanism between p-phosphate concentrations and FGF-23 expression in patients with HPP.

This is the first study assessing BMSi in adult patients with HPP. BMSi has been demonstrated to be significantly lower in patients with low bone mass and fragility fractures than in non-fracture controls, regardless of BMD and fracture site [29,30]. A Norwegian case control study found significantly lower BMSi in 30 women with previous stress fractures, compared with 30 controls [46]. Opposite to these studies, correlations analyses showed a tendency that a higher number of fractures may associate with higher BMSi in adults with HPP. Interestingly, among reference individuals we observed a tendency that low BMSi may correlate with a higher number of fractures. Further, BMSi was significantly related to Tt.vBMD and failure load in this group. In contrast, a significant association between BMSi and fractures was not observed in patients with acromegaly nor patients with chronic kidney disease [47,48]. Our results indicate that BMSi may not be characteristically reduced among adults with HPP and therefore, low BMSi may not explain the increased fracture risk in these patients.

Bone structural parameters measured by HR-pQCT in adults with HPP were previously evaluated by Schmidt et al. [19]. In this study, patients with HPP and fractures ($n = 8$) had significantly lower Tt.BMD and Ct.Th at the right distal radius and the left distal tibia, compared with patients without fractures ($n = 6$) [19]. The same study has demonstrated significantly lower Tr.BMD and Tb.Th in adult HPP patients with fractures, though only at the right radius [19]. This study used another generation of HR-pQCT scanner than we did, and scanned the left tibia and right radius, as we examined the non-dominant limbs. On the other hand, a significant increase of Tb.N and a significant decrease of Tb.Sp as well as Tb.Th has been demonstrated in a study investigating bone histomorphometry by bone biopsies of the iliac crest from eight adult patients with HPP [49]. The method used in this study is a histological examination assessing bone structure on a quantitative level. In addition, this study used bone from the iliac crest and HR-pQCT analyses are performed at the distal radius and tibia. Consequently, results on bone microarchitecture in HPP are scarce and in parts inconsistent with our results, which may be explained by the different methods and reference populations used in our study and the studies described above. Furthermore, a murine study assessed trabecular and cortical parameters by quantitative nano computed tomography in bone marrow stroma cell collagen implants (ossicles) from both global TNAP knock out mice ($ALPL^{-/-}$) and $ALPL^{+/+}$ controls, and found significantly decreased trabecular BMD, bone volume fraction, Tb.Th, Tb.N, as well as increased Tb.Sp in $ALPL^{-/-}$ mice [50].

This study does have strengths. To reduce bias, we matched adults with HPP by sex, age, BMI and menopausal status with healthy reference individuals, and only included subjects who did not receive medicine altering bone metabolism. In addition, the presence of diseases affecting bone health including thyroid- and parathyroid disorders, diabetes, hypogonadism and multiple myeloma was excluded by biochemical analyses. We examined symptomatic and genetic verified patients with HPP of which the majority experienced fractures. Nevertheless, the skeletal manifestations in the adults with HPP here may not be severe enough to prove significant differences when compared with healthy

individuals. Adults with HPP were, except for two participants not family related, which lessens bias such as similar environmental factors and genetic makeup, that can affect bone health. The main weakness of the current study is the small sample size of adults with HPP and reference individuals, which has reduced power of statistical analyses. HPP is a rare condition, and we were not able to recruit a higher number of adults with HPP nor reference individuals for the current study. To achieve a larger sample size of adults with HPP, international collaborations would strengthen future studies. Further limitations include the lack of information on lifestyle factors such as smoking, alcohol consumption, physical activity and differences of the amount of calcium, vitamin D and phosphate intake. However, our study provides important information to better understand the bone phenotype and its pathology in adult patients with HPP.

5. Conclusions

Bone turnover markers and BMSi are not altered in our cohort of adults with HPP when compared with reference individuals. In addition, low BMSi does not correlate with fracture prevalence in adults with HPP. Our results indicate that altered trabecular bone structural parameters may contribute to the fracture risk in adults with HPP and suggest that increased Ct.Po could play a role in the development of femoral pseudofractures in CHZ HPP patients. Further studies assessing bone quality histologically and quantitatively by bone histomorphometry in larger cohorts of adults with HPP and reference individuals are required to explore the increased fracture risk in adults with HPP.

CRediT authorship contribution statement

NH: Project administration, Conceptualization, Methodology, Investigation, Data curation, Formal analyses, Writing - original draft. LF and SM: Investigation, Resources, Data curation, Writing - review & editing. ALF and MD: Supervision, Writing - review & editing. NRJ and APH: Resources, Writing - review & editing. JEBJ: Conceptualization, Methodology, Supervision, Writing - review & editing.

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Declaration of competing interest

LF, SM, ALF, MD, NRJ and APH have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. NH has received research funding from Alexion, AstraZeneca Rare Disease and lecturer fees from Gedeon Richter. JEBJ is a board member in Eli Lilly, Amgen, Gedeon Richter and UCB, received funding from Eli Lilly and Amgen and consulting fees from UCB, Giliad and Amgen.

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Appendix A. Supplementary data

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