Journal of Steroid Biochemistry and Molecular Biology 261 (2026) 107018

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Age-specific centiles for fibroblast growth factor 23 and its associations with mineral and bone metabolism in healthy children☆

Isabelle Piec [a,b,*,1], Andre Chu [a,c], Ben Nichols [d], Carianne Lewis [e], Christopher W. Thoroughgood [f], Konstantinos Gerasimidis [g], Inez Schoenmakers [a], Rachel Dunn [a], Jonathan CY Tang [a,b,2], Allison Chipchase [a,b], William D. Fraser [a,b], Emma A. Webb [a,c,*]

[a] Medical School, University of East Anglia, Norwich NR4 7UQ, UK
[b] Biochemistry department, Norfolk and Norwich University Hospital NHS Foundation Trust, Colney Lane, Norwich NR4 7UY, UK
[c] Jenny Lind Children's Hospital, Norfolk and Norwich University Hospital NHS Foundation Trust, Colney Lane, Norwich NR4 7UY, UK
[d] James Watt School of Engineering, Glasgow, Scotland G12 8QQ, UK
[e] Neonatal Intensive Care Unit, Norfolk and Norwich University Hospital, UK
[f] The Queen Elizabeth Hospital Kings Lynn NHS Foundation trust, Kings Lynn PE30 4ET, UK
[g] Human Nutrition, School of Medicine, Dentistry and Life Sciences, University of Glasgow, New Lister Building, Glasgow Royal Infirmary, Glasgow G31 2ER, UK

ARTICLE INFO

Keywords:
Fibroblast growth factor 23
Intact FGF23
C-terminal FGF23
Pediatric reference ranges
Centiles
Phosphate

ABSTRACT

Background: Fibroblast Growth Factor 23 (FGF23) regulates phosphate and vitamin D metabolism and is implicated in pediatric disorders such as X-linked hypophosphatemia and CKD–mineral and bone disorder. Accurate interpretation in children requires age-adjusted centile charts.
Objective: To construct age- and sex-specific centile charts for intact (iFGF23) and C-terminal (cFGF23) FGF23 in healthy UK children and examine associations with mineral and bone metabolism markers.
Methods: In a cross-sectional cohort of 430 children aged 0–16 years, plasma iFGF23 and cFGF23 were measured using DiaSorin and Quidel immunoassays. Age-specific centiles (2.5th–97.5th) were generated using GAMLSS modeling. Serum phosphate, albumin-adjusted calcium (AdCa), vitamin D metabolites, PTH, and bone turnover markers (PINP, CTX, NTX, BAP) were assessed. Associations were evaluated using LN-transformed regression and Pearson correlations.
Results: FGF23 concentrations declined with age, with highest values in infancy. At 2 years, median iFGF23 was 43.0 pg/mL (2.5–97.5th centiles: 20.8–81.6 pg/mL), while by 16 years, the median remained 42.9 pg/mL (20.8–81.5 pg/mL). For C-terminal FGF23 (cFGF23), the median was 98.2 RU/mL (2.5–97.5th centiles: 47.0–225.4 RU/mL) at 2 years and 80.2 RU/mL (38.4–184.1 RU/mL) at 16 years ($p < 0.001$). Correlation analyses showed weak but statistically significant associations of cFGF23 with PINP ($r = 0.199$, $p < 0.001$) and NTX ($r = 0.150$, $p = 0.005$), while iFGF23 exhibited similar weak correlations, with PINP ($r = 0.120$, $p < 0.016$) and BAP ($r = 0.121$, $p = 0.013$).
Conclusion: We constructed age-specific centile charts for iFGF23 and cFGF23 in healthy children required for the interpretation of FGF23 in clinical practice and to provide a foundation for improved management of pediatric disorders in phosphate and vitamin D metabolism. Phosphate was the strongest predictor of both iFGF23 and cFGF23. Age and 25OHD predicted iFGF23 but not cFGF23. Both were correlated with markers of bone metabolism.

☆ This manuscript is submitted posthumously on behalf of Pr. William D. Fraser, who made significant contributions to the work prior to their passing. We honor their memory and acknowledge their role in the research.

* Corresponding authors at: Medical School, University of East Anglia, Norwich NR4 7UQ, UK.
E-mail addresses: i.piec@uea.ac.uk (I. Piec), andre.chu@uea.ac.uk (A. Chu), Ben.Nichols@glasgow.ac.uk (B. Nichols), Carianne.Lewis@nnuh.nhs.uk (C. Lewis), Christopher.Thoroughgood@nnuh.nhs.uk (C.W. Thoroughgood), Konstantinos.Gerasimidis@glasgow.ac.uk (K. Gerasimidis), i.schoenmakers@uea.ac.uk (I. Schoenmakers), Rachel.Dunn@uea.ac.uk (R. Dunn), Jonathan.tang@uea.ac.uk, Jonathan.tang@ed.ac.uk (J.C. Tang), allison.chipchase@nnuh.nhs.uk (A. Chipchase), w.fraser@uea.ac.uk (W.D. Fraser), emma.webb@nnuh.nhs.uk (E.A. Webb).
[1] School of Biological Sciences, University of East Anglia, Norwich.
[2] Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, EH4 2XU, UK.

https://doi.org/10.1016/j.jsbmb.2026.107018
Received 31 December 2025; Received in revised form 1 April 2026; Accepted 7 April 2026
Available online 15 April 2026

1. Introduction

Fibroblast Growth Factor 23 (FGF23) is a hormone that plays a crucial role in phosphate and vitamin D homeostasis. It inhibits phosphate reabsorption in renal tubules and indirectly reduces intestinal phosphate absorption [1–3] by lowering $1,25(OH)_2D$. FGF23 suppresses vitamin D activation by inhibiting 1α-hydroxylase (which converts 25OHD to $1,25(OH)_2D$) and stimulates $1,25(OH)_2D$ catabolism [2]. Lower $1,25(OH)_2D$ concentrations further contribute to reduce phosphate absorption in the intestines [4]. FGF23 is primarily produced by osteocytes and osteoblasts in response to elevated serum phosphate and $1,25(OH)_2D$ [5]. Its role in bone metabolism includes inhibition of mineralization. Excessive FGF23 can lead to urinary phosphate loss and hypophosphatemia, particularly when kidney and parathyroid function are normal.

Genetic mutations in FGF23 or related pathways can lead to disorders like autosomal dominant hypophosphatemic rickets (ADHR) [6–8] and X-linked hypophosphatemia (XLH) [9–11]. Acquired disorders with elevated FGF23 include tumor induced osteomalacia (TIO). These conditions are characterized by abnormal phosphate homeostasis. In case of chronic kidney disease, increased serum phosphate and dysregulated FGF23 contributes to bone and mineral metabolism abnormalities, impacting pediatric bone health [12,13]. Elevated FGF23 concentrations during childhood lead to osteomalacia and ricket-like deformities.

In plasma, FGF23 circulates as an intact 32 kDa glycoprotein and as a mostly inactive C-terminal fragments generated by proteolytic cleavage. Post-translational O-glycosylation at Thr178 (via GALNT3) stabilizes the intact hormone, whereas cleavage at Arg176-X-X-Arg179 by furins produces fragments lacking biological activity. Plasma concentrations of FGF23s are measured by immunoassays. Intact (iFGF23) assays use dual antibodies targeting N- and C-terminal domains, while C-terminal (cFGF23) assays detect both intact and cleaved fragments, reflecting total FGF23.

FGF23 concentrations serve as a key diagnostic marker for phosphate metabolism. Given its distinct forms, establishing pediatric centiles for both intact and C-terminal FGF23 is critical for accurate interpretation of phosphate and vitamin D metabolism in health and disease

This study aimed to establish age-specific reference intervals for iFGF23 and cFGF23 using the 2.5th to 97.5th centiles, aligning with CLSI EP28 guidelines for pediatric populations and established growth chart methodologies.

2. Material and methods

2.1. Sample collection and storage

The study was approved by the Health Research Authority Health and Derby Research Ethics Committee (REC 20/EM/0270; IRAS# 287349). Written informed consent was obtained from parents or guardians of all participants. A total of 563 children were initially recruited from the Jenny Lind Children's Hospital (Norfolk and Norwich University Hospital, UK) into the study. Of these, several were excluded prior to analysis based on predefined criteria. Participants with no available demographic or biochemical data were removed (n = 22), as well as those who had no research blood results available (n = 66). Children who did not have samples processed for the bone marker panel were excluded (n = 15). Participants with biochemically abnormal calcium (n = 15) or abnormal phosphate values (n = 1), based on age specific pediatric reference ranges, were removed to ensure inclusion of only physiologically healthy individuals. A further two children were excluded because of a past medical history incompatible with the definition of a "healthy" reference population, and two additional cases were excluded following review due to unclear suitability. Finally, 10 children lacked EDTA samples required for FGF23 measurement, preventing inclusion in the analysis (Fig. 1).

After all exclusions, 430 children remained and were included in the final dataset for construction of reference intervals and determinant analyses. Due to sample type or volume limitations, some participants had only intact FGF23 measured, while others had only Cterminal FGF23. These participants were included in whichever analyses corresponded to the FGF23 assay available, accounting for the different sample sizes for iFGF23 and cFGF23.

Participants completed a five-item food frequency questionnaire (FFQ) informed from previous methodology [44], to estimate the daily calcium intake. Intake was evaluated against UK national dietary guidelines [45]. If intake was more than 1.5 standard deviation (SD) below the age-specific reference range, families received dietary advice on calcium-rich foods. Anthropometric data, including height and weight, were also collected using a calibrated weight scale. Body mass index (BMI) was calculated by using the formula: BMI = weight (kg)/height $(m)^2$. Age- and sex-specific SD scores (SDS) for height, weight, and BMI were calculated according to United Kingdom reference data [14].

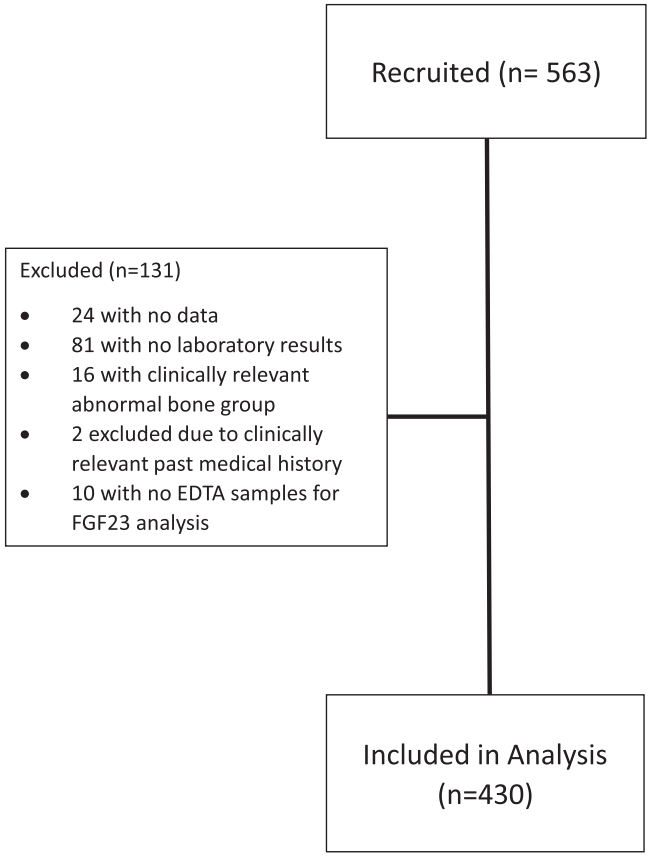

Fig. 1. Participant Flow Diagram for Eligibility, Recruitment, Exclusion and Final Sample Included in Analysis. This flow diagram illustrates participant progression through the study from initial screening to final inclusion in the analytical dataset. A total of 563 children were recruited. Participants were excluded for the following reasons: no demographic or clinical data available (n = 22), no laboratory research results (n = 81), absence of a bone marker panel (n = 16), biochemically abnormal calcium or phosphate values based on pediatric reference ranges (n = 15 and n = 1, respectively), past medical history inconsistent with inclusion criteria (n = 2), additional exclusions following eligibility review (n = 2), and absence of EDTA plasma required for FGF23 measurement (n = 10). After exclusions, 430 children were included in the final analysis. Due to sample availability, some participants contributed only intact FGF23 measurements, some only C-terminal FGF23 measurements, and others contributed both.

2.2. Biochemistry

Blood samples were collected under standardized conditions. Venous blood was collected into serum and EDTA tubes, with 2 mL obtained from children under five years and 5 mL from those aged five years or older. Samples were centrifuged immediately at 3000 ×g for 10 min and aliquoted into separate polypropylene tubes and stored at −20°C.Urine was collected into a sterile white universal tube, with 2 mL obtained from children under two years and 5 mL from those aged two years or older To minimize bone turnover marker variability blood and urine samples were taken in children fasted ≥ 6 h [15].

Serum calcium (albumin-adjusted, AdCa), phosphate, alkaline phosphatase (ALP), plasma parathyroid hormone (PTH), and bone turnover markers (plasma procollagen type I N-propeptide [PINP], cross-linked C-telopeptide of type I collagen [CTX], serum bone-specific alkaline phosphatase [BAP]) were measured using Roche Diagnostics (Basel, Switzerland) platforms and ELISA kits (Quidel Corporation, San Diego, CA, USA). Plasma intact FGF23 (iFGF23) was measured using the DiaSorin (Saluggia, Italy) Liaison XL immunoassay, and C-terminal FGF23 (cFGF23) using the Quidel ELISA. All assays were performed as per manufacturer's instructions. Assay performance was verified and were per manufacturer specifications (intra-assay CVs all <6%, inter-assay CVs all <16%, and limits of detection and quantification). Vitamin D metabolites (25OHD and 1,25(OH)$_2$D) were quantified in serum by in-house assay by LC-MS/MS [16,17].

Multivariable models relied on complete-case analysis, and some younger children had fewer available biochemical measurements owing to limited blood volumes and sample exclusions. This selective loss of cases is a common constraint in pediatric biochemical studies and may have modestly reduced statistical power, particularly in infancy. However, exploratory checks showed similar FGF23 distributions in included and excluded participants, suggesting that missingness did not introduce systematic bias.

2.3. Statistical analysis and reference range determination

No formal power calculation was performed, consistent with the nature of observational centile estimation studies. Recruitment followed the LMS method guidance [18] commonly used in pediatric reference interval generation. We targeted 20 children per year between ages 2–16, and 30 per year between 0–2 years, as previously used for pediatric IGF-1 and IGFBP-3 reference ranges. When emerging age trends suggested additional resolution was needed, targeted 'top up' recruitment was performed.

The generalized additive models for location, scale and shape (GAMLSS) package [19] was used in R (version 4.5.1 and ggplot2 [20,21]) to plot age-dependent centile charts and calculate Z-scores for each biochemical parameter, for sexes combined and males and females separately. Models were trialed using different combinations of input to the GAMLSS function pertaining to model distribution and smoothing method, with one degree of freedom. The Akaike information criterion (AIC) and root mean square error (RMSE) were used to select the optimal model in each case and in instances where these values differed by less than 5% between models, the simpler model was selected.

Centiles (2.5, 10, 25, 50, 75, 90, and 97.5) were selected to depict the full distribution, with the 2.5th and 97.5th centiles representing the

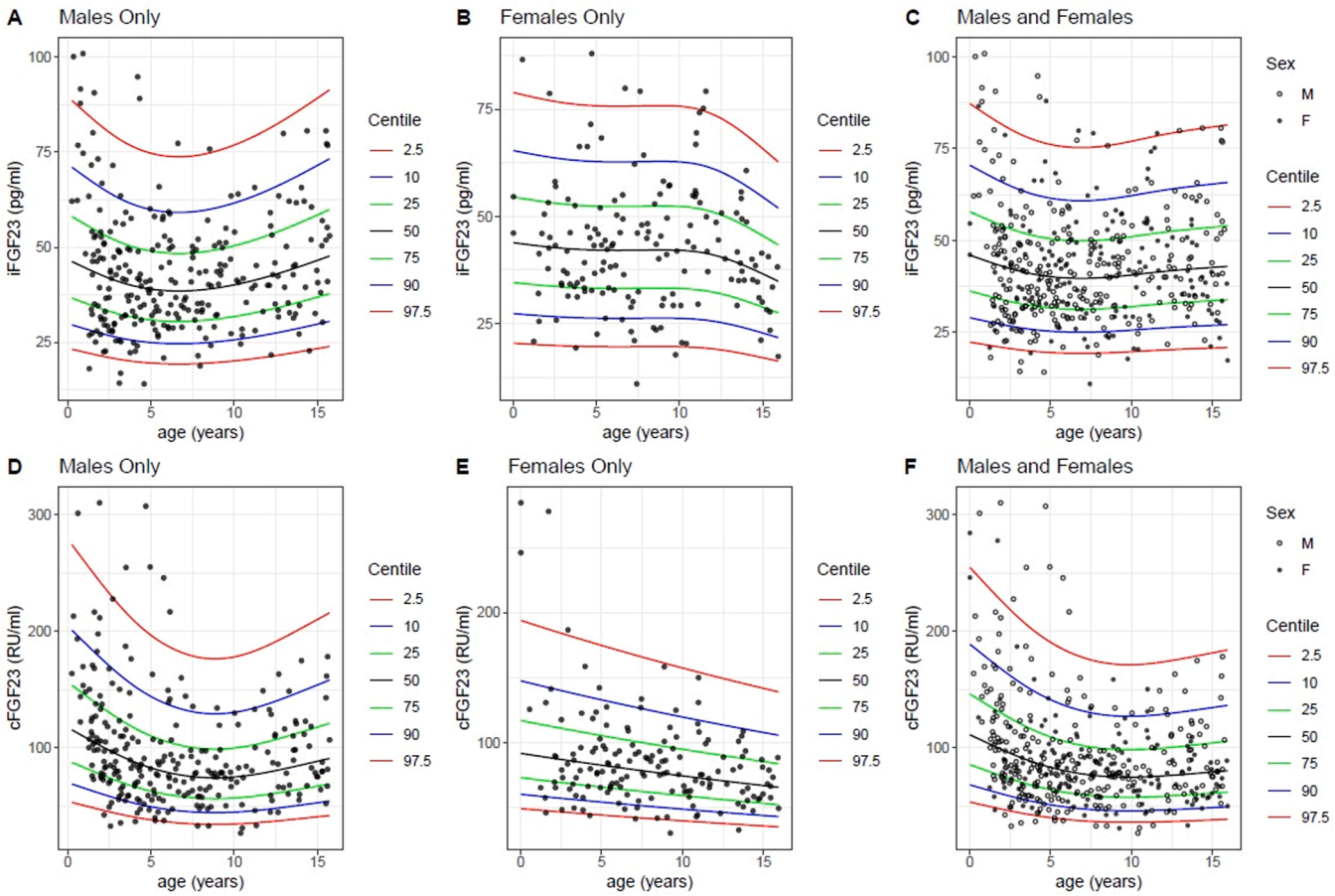

Fig. 2. Age-specific centile charts for iFGF23 and cFGF23. Panels A–C show intact FGF23 (iFGF23, pg/mL) and Panels D–F show C-terminal FGF23 (cFGF23, RU/mL) from birth to 16 years. Age-stratified centile curves (2.5th, 10th, 25th, 50th, 75th, 90th, and 97.5th) are presented for boys (A, D), girls (B, E), and all children combined (C, F).

central 95% interval recommended for pediatric reference ranges following CLSI EP28-A3c [22]. Intermediate centiles provide clinically relevant benchmarks and facilitate visualization of age-related trends, consistent with approaches used in pediatric growth charts [23,24]. These centiles are presented graphically (Fig. 2) and in supplementary table 1.

Further statistical analysis was performed with SPSS® (IBM Corporation, version 29.0.1.1). Non-normally distributed data were converted to natural logarithms (LN). Analyses were performed on LN-transformed iFGF23 and cFGF23. Statistical outliers in LN(cFGF23) were identified using Grubbs' test (using GraphPad Prism version 10 (Boston, MA, U.S.A.)). Five samples met the outlier criterion for LN(cFGF23) and were excluded from analyses.

Univariate associations were examined using simple linear regression without covariate adjustment, as these analyses were used to identify predictors eligible for inclusion in multivariable models. Variables showing a p-value < 0.20 in these univariate analyses were then considered for inclusion in the multivariate model along with age, sex and BMI-SDS or Height-SDS as factors a priori. Backward multivariate analysis with elimination of the least-significant variable was used to identify determinants of iFGF23 and cFGF23. The relationship with bone turnover markers (PINP, CTX, BAP, uNTX) were analyzed separately by Spearman correlation since these are not considered to be regulators of FGF23. Statistical significance was set at $p < 0.05$.

For LN-transformed variables, results were back-converted to the original scale by exponentiating the mean and confidence limits of the log-transformed values. This yields the geometric mean and corresponding 95% confidence interval, which better represent central tendency for skewed data.

3. Results

A total of 430 children (270 boys, 160 girls) were included in the analysis. Sex distribution, body mass index Standard Deviation Scores (BMI-SDS) and results of biochemical assessments are presented in Table 1; normally distributed data are presented as mean ± SD and non-normally distributed data are presented as median and interquartile range [25].

3.1. FGF23 Reference ranges

Reference intervals for intact FGF23 (iFGF23, pg/mL) and C-terminal FGF23 (cFGF23, RU/mL) derived from GAMLSS models and expressed as age-specific 2.5th 97.5th centiles are given in Fig. 2 and supplemental table 1 for boys (1 A and D), girls (1B and E) and sexes combined (1 C and F).

The distribution of iFGF23 narrowed with age, with higher values observed in infancy and early childhood, followed by stabilization through adolescence. At 2 years, the median (50th percentile) was 43.0 pg/mL, with a 95% reference interval spanning 20.8–81.6 pg/mL; by 16 years, the median was 42.9 pg/mL (95% RI: 20.8–81.5 pg/mL) (Fig. 2A, boys, Fig. 2B girls, Fig. 2C, all children). Concentrations of cFGF23 declined progressively with age across childhood. At 2 years, the median was 98.2 RU/mL, with a 95% reference interval spanning 47.0–225.4 RU/mL for all gender combined; by 16 years, the median was 80.2 RU/mL (95% RI: 38.4–184.1 RU/mL). Sex-specific analysis showed similar trends, with boys exhibiting a steeper decline and slightly higher upper centiles in early childhood (Fig. 2D, boys; Fig. 2E, girls, Fig. 2F, all children).

Table 1
Characteristics of study population (all children aged 0–16 included in this analysis).

	All children
Age, years [range]	7.4 ± 4.5 [0–16]
Sex (n, %)	270 boys (62.8%), 160 girls (37.2%)
BMI-SD scores	0.31 ± 1.31
Height-SD scores	0.34 ± 1.24
Weight-SDS	0.30 [-0.42 – 1.11]
25(OH)D, nmol/L	63.9 ± 22.1
1,25(OH)$_2$D, pmol/L	131.9 [98.6–147.0]
Phosphate, mmol/L	1.49 ± 0.23
AdCa, nmol/L	2.36 ± 0.08
ALP, U/L	208 [173.5–258.5]
BAP, U/L	78.3 [61.3–99.0]
PTH, pmol/L	3.8 [2.6–5.1]
CTX, ng/L	1.52 ± 0.48
PINP, ng/L	503.6 [376.2–665.9]
uNTX	85.2 [55.6–117.0]

Values are presented as mean ± standard deviation (SD) or median [interquartile range (IQR)] as appropriate. Abbreviations: P, phosphate; AdCa, albumin-adjusted calcium; ALP, alkaline phosphatase; BAP, bone-specific alkaline phosphatase; PTH, parathyroid hormone; CTX, C-terminal telopeptide; PINP, procollagen type I N-terminal propeptide; uNTX:cre, urine N-telopeptide to creatinine ratio.

3.2. Determinants of FGF23

Univariable linear regression analyses were performed to examine associations between LN(iFGF23) and individual predictors (Table 2). Serum phosphate demonstrated the strongest positive association with LN(iFGF23) ($B = 0.297$, $R^2 = 0.036$, $p < 0.001$). Serum 25OHD was also positively associated ($B = 0.003$, $R^2 = 0.026$, $p < 0.001$). In contrast, age ($p = 0.650$), sex ($p = 0.640$), BMI-SDS ($p = 0.895$), height-SDS ($p = 0.858$), adjusted calcium ($p = 0.160$), ALP ($p = 0.517$), 1,25

Table 2
Univariate associations of regulators with LN-transformed FGF23.

Predictor	B	SE	β	R^2	p
LN(iFGF23)					
Age	-0.002	0.004	-0.022	0.000484	0.650
Sex	-0.017	0.036	-0.023	0.000529	0.640
BMI-SDS	0.002	0.013	0.007	0.000049	0.895
Height-SDS	0.003	0.014	0.009	0.000081	0.858
AdCa (mmol/L)	**0.319**	**0.227**	**0.068**	**0.004624**	**0.160**
Phosphate (mmol/L)	**0.297**	**0.074**	**0.190**	**0.036100**	**< 0.001**
ALP (U/L)	-0.000	0.000	-0.032	0.001024	0.517
25OHD (nmol/L)	**0.003**	**0.001**	**0.160**	**0.025600**	**< 0.001**
1,25(OH)$_2$D (pmol/L)	0.000	0.000	-0.017	0.000289	0.731
PTH (pmol/L)	0.005	0.009	0.028	0.000784	0.579
BAP (U/L)	0.000	0.000	-0.015	0.000225	0.758
CTX (ng/mL)	0.059	0.036	0.080	0.006400	0.103
PINP (ng/mL)	*0.000*	*0.000*	*0.176*	*0.030976*	*< 0.001*
uNTX	0.000	0.000	0.093	0.008649	0.082
LN(cFGF23)					
Age	-0.019	0.005	-0.197	0.038809	**< 0.001**
Sex	-0.073	0.44	-0.081	0.006561	0.096
BMI-SDS	0.018	0.016	0.053	0.002809	0.288
Height-SDS	-0.003	0.017	-0.008	0.000064	0.870
AdCa (mmol/L)	**0.889**	**0.273**	**0.156**	**0.024336**	**0.001**
Phosphate (mmol/L)	**0.627**	**0.087**	**0.331**	**0.109561**	**< 0.001**
ALP (U/L)	*0.000*	*0.000*	*0.109*	*0.011881*	*0.029*
25OHD (nmol/L)	0.000	0.001	-0.010	0.000100	0.831
1,25(OH)$_2$D (pmol/L)	**0.001**	**0.001**	**0.072**	**0.005184**	**0.140**
PTH (pmol/L)	**0.018**	**0.010**	**0.086**	**0.007396**	**0.086**
BAP (U/L)	*0.000*	*0.000*	*0.096*	*0.009216*	*0.048*
CTX (ng/mL)	0.050	0.044	0.056	0.003136	0.0259
PINP (ng/mL)	*0.000*	*0.000*	*0.223*	*0.049729*	*< 0.001*
uNTX	*0.002*	*0.000*	*0.266*	*0.070756*	*< 0.001*

This table presents univariate associations between LN-transformed FGF23 concentrations. LN(iFGF23) = intact FGF23 and LN(cFGF23)= (C-terminal FGF23 and biochemical regulators. For each predictor, the table reports the unstandardized regression coefficient (B), standard error (SE), standardized coefficient (β), coefficient of determination (R^2) and p-value. Analyses were performed using pairwise complete cases. LN-transformation was applied to normalize FGF23 distributions. Statistical significance: $p < 0.05$; $p < 0.01$; $p < 0.001$ (two-tailed). Variables in bold were included in multivariable models. Variables in italics were significant in univariate analyses but not included because they are not considered physiological regulators of FGF23.

$(OH)_2D$ (p = 0.731), PTH (p = 0.579), BAP (p = 0.758), CTX (p = 0.103), and uNTX (p = 0.082) showed no evidence of association. PINP showed a small but statistically significant association (p < 0.001); however, bone turnover markers were not considered physiological regulators of iFGF23 and were therefore not progressed to multivariable modelling.

Backward regression analyses were then performed for LN(iFGF23), including age, sex and BMI-SDS or height-SDS as a priori covariates, together with predictors meeting the univariate entry threshold (p < 0.20): phosphate, 25OHD and adjusted calcium. In the final model (N =406), phosphate, 25OHD and age remained significant independent predictors, whereas sex, BMI-SDS or height-SDS and adjusted calcium were removed (p > 0.05) (Table 3). The model explained 5.8% of the variance in LN(iFGF23) (R^2 = 0.058; adjusted R^2 = 0.051; F(3,402) = 8.291; p < 0.001). Higher phosphate was associated with greater LN (iFGF23) (B = 0.365, SE = 0.093, p < 0.001), such that a 0.1 mmol/L increase corresponded to an estimated 3.7% rise in geometric mean iFGF23 (95% CI: +1.8% to +5.6%). Serum 25(OH)D was also independently associated (B = 0.003, SE = 0.001, p = 0.001), with a 25 nmol/L increment predicting a 7.8% increase (95% CI: +2.6% to +13.2%). Age contributed to a smaller but significant effect (B = 0.015, SE = 0.005, p = 0.002), corresponding to approximately a 1.5% increase in iFGF23 per year of age (95% CI: +0.5% to +2.5%). No independent associations were observed for sex, BMI-SDS or adjusted calcium after adjustment (Table 4).

Univariable regression analyses indicated that LN(cFGF23) was most strongly associated with serum phosphate (B = 0.627, SE = 0.087, R^2 = 0.110, p < 0.001; Table 2). Serum adjusted calcium also showed a positive association (B = 0.889, SE = 0.273, R^2 = 0.024, p = 0.001). Weak but statistically significant associations were observed for ALP (p = 0.029), BAP (p = 0.048), CTX (p = 0.026), and strong associations were found with PINP (p < 0.001) and uNTX (p < 0.001); however, bone turnover markers were not considered physiological regulators of cFGF23 and were therefore not progressed to multivariable modelling. No meaningful associations were detected for 25(OH)D or $1,25(OH)_2D$ (p > 0.14). Age was negatively associated with LNc (B = –0.019, SE = 0.005, R^2 = 0.039, p < 0.001), whereas BMI-SDS showed a small, non-significant positive association (p = 0.288). Height-SDS showed no association (p = 0.870).

In multivariable models using backward selection, phosphate, adjusted calcium, and PTH remained in the final model, while ALP, BAP, age, sex, 25(OH)D, and $1,25(OH)_2D$ were removed (p ≥ 0.10). Age, sex and BMI-SDS or height-SDS were used as a priori covariates. The final model (N =382, Figure 3) explained 12.6% of the variance in LN (cFGF23) (R^2 = 0.126; adjusted R^2 = 0.118; F(3,378) = 16.79; p < 0.001). Phosphate was the strongest independent predictor (B = 0.574, SE = 0.095, p < 0.001), corresponding to an estimated 3.7% increase in geometric mean cFGF23 per 0.1 mmol/L increment (95% CI: 1.8–5.6%). Adjusted calcium also contributed independently (B = 0.800, SE = 0.282, p = 0.005), predicting a 3.7% rise per 0.05 mmol/L increment (95% CI: 0.8–6.7%). Finally, PTH showed a small but statistically significant independent contribution (B = 0.026, SE = 0.010, p = 0.010), corresponding to approximately a 2.4% increase per 1 pmol/L (95% CI: 0.7–4.9%). Sex, age, BMI-SDS or height-SDS and $1,25(OH)_2D$ were excluded during backward selection (p > 0.05).

Table 3
Multivariate determinants of LN-transformed FGF23 with age, sex and BMI-SDS as covariates.

Predictor	B	95% CI (B)	SE	β	R^2	p
LN(iFGF23)						
Model fit: F(3,402)= 8.291, p < 0.001						
Age (years)	0.015	0.005–0.025	0.005	0.190	0.0361	0.002
Phosphate (mmol/L)	0.365	0.184–0.548	0.093	0.231	0.0534	< 0.001
25(OH)D (nmol/L)	0.003	0.001–0.005	0.001	0.164	0.0269	0.001
LN(cFGF23)						
Model fit: F(3,378)= 16.790, p < 0.001						
Phosphate (mmol/L)	0.574	0.388–0.760	0.095	0.293	0.0858	< 0.001
AdCa (mmol/L)	0.800	0.247–1.353	0.282	0.139	0.0180	0.005
PTH	0.026	0.006–0.046	0.010	0.128	0.0137	0.010

This table summarizes multivariate linear regression models identifying independent determinants of LN-transformed FGF23 concentrations. Separate models were fitted for LN(iFGF23) and LN(cFGF23) using backward stepwise selection. Candidate variables included age, sex, BMI-SDS, and predictors with univariate p < 0.10. For each retained predictor, the table reports the regression coefficient (B), 95% confidence interval (CI), and p-value. Model fit statistics (n, R^2, adjusted R^2) are provided for each outcome. Outcomes were natural-log transformed to normalize distributions. Significance levels: $p < 0.05$; $p < 0.01$; $p < 0.001$ (two-tailed).

Table 4
Correlations Between LN-transformed FGF23 and bone turnover markers in children.

Marker	LN(iFGF23)		LN(cFGF23)	
	r	p	r	p
PINP	0.120	p < 0.016 **	0.199	p < 0.001 ***
CTX	0.042	p = 0.387	0.07	p = 0.152
BAP	0.121	p = 0.013 **	0.092	p = 0.059
uNTX	0.025	p = 0.345	0.150	p = 0.005 **

This table presents Spearman correlation coefficients (r) and corresponding p-values for associations between LN(iFGF23) (intact FGF23) and LN(cFGF23) (C-terminal FGF23) and markers of bone turnover (PINP, CTX, BAP and uNTX). Analyses were performed using pairwise complete cases. Statistical significance: p < 0.05; p < 0.01; p < 0.001 (two-tailed).

3.3. Correlations with bone turnover markers

Pearson correlation analyses were performed between LN(cFGF23) and LN(iFGF23) and biochemical markers of bone turnover (PINP, uNTX, CTX, and BAP). For LN(cFGF23), the strongest positive correlation was observed with PINP (r = 0.199, p < 0.001), followed by uNTX (r = 0.150, p = 0.005). BAP showed a borderline association (r = 0.092, p = 0.059), whereas CTX was not associated with LN(cFGF23) (r = 0.070, p = 0.152). For LN(iFGF23), weak but statistically significant correlations were observed with PINP (ρ = 0.120, p = 0.016) and BAP (ρ = 0.121, p = 0.013). No significant associations were found with CTX (ρ = 0.042, p = 0.387) or uNTX (ρ = 0.025, p = 0.345).

Among the bone turnover markers themselves, strong intercorrelations were evident, particularly between PINP and BAP (ρ = 0.604, p < 0.001), PINP and CTX (ρ = 0.576, p < 0.001), and PINP and uNTX (ρ = 0.511, p < 0.001). Additional correlations were observed between BAP and CTX (ρ = 0.376, p < 0.001), BAP and uNTX (ρ = 0.276, p < 0.001) and uNTX and CTX (ρ=0.219, p < 0.001).

4. Discussion

Our findings address a critical gap in age-specific FGF23 centiles that support the interpretation of research in FGF23 and mineral metabolism in children. Given FGF23's role in phosphate and vitamin D regulation, and abnormalities in conditions such as XLH, accurate interpretation of FGF23 in children is essential. This study demonstrates that intact FGF23 concentrations vary significantly with age, particularly in infancy, and that serum phosphate is the primary biochemical determinant of both intact and C-terminal FGF23 in healthy children. In addition, 25OHD and age independently predicted intact FGF23, whereas C-terminal FGF23 was influenced by phosphate, adjusted calcium, and a small but significant contribution from PTH. Together, these findings highlight overlapping but distinct regulatory profiles for intact and C-terminal FGF23 and highlight the importance of developmental and mineral metabolism factors when interpreting FGF23 concentrations in childhood.

Our centile distributions for iFGF23 and cFGF23 broadly align with published pediatric reference ranges, such as those reported by Brescia et al. (2022) [26], who identified an upper reference limit of 61.2 pg/mL for iFGF23 in 115 healthy children aged 1–18 years using the DiaSorin assay. However, unlike Stanczyk et al. (2021) [27], who found no significant age dependency, our data reveal a clear elevation of iFGF23 in infants ≤ 1 year ($n = 115$). This observation is consistent with Baroncelli et al. (2023) [28], who demonstrated significantly higher iFGF23 concentrations in infants compared to older children and adolescents ($n = 282$), reinforcing the concept that infancy represents a distinct physiological phase for FGF23 regulation. Collectively, these findings support the need for age-stratified reference intervals, particularly in early childhood, where developmental changes in mineral metabolism and bone turnover may influence FGF23 secretion.

Notably, their study did not detect significant sex or age differences, whereas our data revealed elevated iFGF23 in infants ≤ 1 year. Moreover, Baroncelli et al. (2023) [28] reported significantly higher iFGF23 concentrations in infants compared to older children and adolescents. In contrast with previous studies [26–28] which reported no sex differences, our data indicate sex-specific trends in cFGF23, particularly during early childhood. This discrepancy may reflect differences in assay type (C-terminal vs intact), sensitivity, or population characteristics (sample number for the study cited can be found in Supplemental Table 2). While the biological basis for sex-related variation in FGF23 remains unclear, potential contributors include differences in skeletal growth velocity, hormonal milieu (testosterone and estradiol) [29], and iron status [30], all of which can modulate FGF23 production. These findings highlight the importance of considering sex as a potential covariate in pediatric FGF23 research, even if current reference intervals do not routinely account for it.

We measured both iFGF23 and cFGF23. iFGF23 assays only detect the biologically active hormone. This measurement is therefore preferable for endocrine assessment. In contrast, cFGF23 assays detect both intact hormone and C-terminal fragments. Unlike intact assays, C-terminal assays reflect FGF23 production and processing, as fragments arise from proteolytic cleavage. Traditionally, these fragments were considered biologically inactive; however, emerging evidence [31–33] suggests they may exert effects such as antagonizing FGF23 signaling or influencing iron metabolism, adding complexity to interpretation in clinical and research settings. Detection of a broader range of molecules can be useful for identifying alterations in FGF23 processing and clearance but may complicate clinical interpretation. FGF23 processing is influenced by iron status, with iron deficiency and inflammation increasing transcription and cleavage, leading to elevated C-terminal fragments [32,34]. Additionally, cFGF23 is cleared via the kidney; therefore, concentrations rise in renal disease, reflecting impaired clearance rather than increased production [35,36]. Furthermore, in adults, associations with markers of renal function, iron status, and inflammation differ between iFGF23 and cFGF23 [32,35], highlighting that assay choice influences interpretation of pathophysiological relationships and the importance of considering iron status and kidney function when interpreting cFGF23 values. Moreover, iFGF23 has a higher degree of intra-individual biological variability (18.3%) and significant diurnal rhythm compared to cFGF23 (intra-individual variability 8.3%), and lower diurnal variation [37]. These relationships are mostly unknown in children. Interpretation is further complicated by the lack of assay harmonization: commercial platforms (e.g., Kainos, Millipore, Immutopics) use different antibodies, calibration materials, and protocols resulting in significant inter-assay bias and poor agreement [38,39], limiting compatibility between studies, laboratories and clinical centers. Taken together, reference intervals and diagnostic thresholds should be established and applied specifically for the assays used.

In adults, FGF23 is strongly associated with parathyroid hormone, 25OHD and $1,25(OH)_2D$, particularly in CKD and phosphate-wasting disorders, in which secondary hyperparathyroidism and impaired 1α-hydroxylase expression and reduced phosphate clearance increase FGF23 concentrations [12,40]. In our pediatric cohort, phosphate remained the dominant determinant of both iFGF23 and cFGF23. For iFGF23, 25OHD and age were also independent predictors, while $1,25(OH)_2D$ and PTH showed no independent association. For cFGF23, phosphate remained the strongest determinant, but adjusted calcium and a small but statistically significant effect of PTH were also observed, whereas 25OHD and $1,25(OH)_2D$ were not independent predictors. These findings highlight that, in healthy children, FGF23 regulation is driven primarily by phosphate and developmental influences, with additional contributions to cFGF23 from calcium homeostasis and growth-related factors, rather than classical endocrine feedback loops alone. The Royal College of Paediatrics and Child Health recommends phosphate reference ranges of 1.3–2.6 mmol/L in infants (<1 year) and 0.9–1.8 mmol/L in children aged 1–16 years. In our cohort (manuscript in preparation), phosphate peaked during infancy and declined after age 2, while PTH increased during puberty. These trends support the stratification of FGF23 centiles by developmental stage and explain the elevated FGF23 concentrations observed in infants compared to older children. Despite including all recognized biochemical regulators of FGF23 (i.e. serum phosphate, 1,25-dihydroxyvitamin D, and parathyroid hormone (PTH), the principal physiological stimuli of FGF23 secretion in adults), the final models explained only 5.8% of the variance in LN(iFGF23) and 12.6% for LN(cFGF23). Although initially surprising, these modest R^2 values likely reflect the broader complexity of FGF23 regulation in early life. First, FGF23 physiology during infancy and childhood may be strongly influenced by growth-related hormonal axes, including IGF-1, growth hormone, sex steroids, and osteocyte-derived signals, which undergo rapid developmental change and were not measured in this study. This is consistent with recent pediatric studies showing developmental variation in FGF23 concentrations, including higher iFGF23 in infancy and differences across pubertal stages [28] and the lack of robust associations with mineral markers in healthy cohorts [27]. However, in our data, neither BMI-SDS nor height-SDS—used as indirect indicators of adiposity and maturational tempo—were associated with iFGF23 or cFGF23 in univariate analyses, and their inclusion in multivariable models produced no change in parameter estimates. This suggests that linear growth, somatic size, and pubertal tempo are unlikely to be major determinants of FGF23 in healthy children. Second, healthy children exhibit greater physiological heterogeneity than adults, where many established FGF23–biochemical relationships were observed, and this may reduce the explanatory power of standard mineral metabolism predictors. Studies in acute paediatric illness further demonstrate that FGF23 can vary independently of phosphate, responding to inflammation and iron status [41]. Although additional analyses using age-based developmental categories and height-SDS did not suggest major pubertal or maturational effects on FGF23, the absence of formal Tanner staging remains a limitation. Puberty is associated with marked changes in bone turnover, phosphate handling and endocrine signalling, and direct pubertal assessment would allow these influences to be evaluated more precisely. Together, these observations suggest that in healthy children, FGF23 is regulated by a broader, developmentally dynamic set of hormonal and osteogenic influences that extend beyond the classical mineral metabolism regulators, and that incorporating GH/IGF1 axis markers, sex steroids, bone turnover dynamics, and formal pubertal staging would likely explain a greater proportion of variance in future studies.

Correlation analyses demonstrated weak but statistically significant associations between cFGF23 and bone turnover markers, particularly PINP and uNTX, supporting the concept that osteocyte-derived FGF23 is modestly related to bone remodeling rate in healthy children. For iFGF23, correlations with bone turnover markers were even weaker, with only PINP and BAP showing small but significant associations. Together, these results indicate that bone turnover contributes to, but does not drive, circulating FGF23 concentrations in health. These findings align with Papastergiou et al. (2024) [41], who reported

associations between iFGF23 and mineral metabolism markers in children with acute infections, and contrast with Baroncelli et al. (2024) [28], who found no correlation between iFGF23 and phosphate in healthy children but an inverse relationship in XLH. Emerging evidence suggests that FGF23 may serve as an early biomarker in metabolic bone disease of prematurity, preceding detectable changes in phosphate or ALP. However, direct evidence in preterm infants remains limited [28, 42,43].

5. Strengths and limitations

This study benefits from a large, well-characterized pediatric cohort spanning infancy through adolescence, enabling detailed age stratification. The use of GAMLSS modeling allowed construction of age-specific centile charts for both intact and C-terminal FGF23, providing clinically relevant interpretation tools. Comprehensive biochemical profiling, including mineral metabolism and bone turnover markers, adds depth to the analysis, However, the cross-sectional design limits insight into longitudinal changes in FGF23 across growth and puberty. The sample size was relatively small in certain strata, particularly sex-specific categories for infants, reducing precision of early-life centiles. There is currently no patient data available to evaluate the sensitivity and specificity of these measures in distinguishing normal values from those observed in children with abnormalities. The absence of formal Tanner staging is a limitation of this study. Although additional analyses using age based developmental categories and inspection of centile trajectories suggested that puberty did not materially influence FGF23 concentrations, formal pubertal assessment would strengthen future studies, particularly when investigating hormonal regulators across adolescence.

6. Conclusion

Our findings demonstrate that FGF23 centiles and determinants in childhood are distinct from adult patterns, emphasizing the need for age-specific centile charts and caution when extrapolating adult data. Our results indicate that concentrations of FGF23 should be interpreted in the context of simultaneous measurements of serum phosphate and 25OHD (for iFGF23) or adjusted calcium and PTH (for cFGF23), and in consideration of age and sex and BMI-SDS. These data support the integration of FGF23 measurement into diagnostic panels for the management of conditions such as X-linked hypophosphatemia, CKD-MBD and metabolic bone disease. Future longitudinal studies should explore FGF23 dynamics across growth and puberty in healthy children and establish the sensitivity and specificity of these measures in children with abnormalities in FGF23 and phosphate metabolism. However, the cross-sectional design of this study limits insights into intra-individual developmental trajectories of FGF23 during childhood and adolescence. Longitudinal data would be valuable to track individual changes over time, assess the impact of growth and puberty, and evaluate responses to nutritional and hormonal interventions.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the author(s) used Microsoft Copilot (GPT-based AI tool) to assist with language refinement and improving clarity. After using this tool, the author(s) thoroughly reviewed, verified, and edited all AI-generated content to ensure accuracy, originality, and alignment with the author(s)' own analysis and interpretation. The author(s) take full responsibility for the content of the published article.

The author(s) confirms that the use of this AI tool complied with all relevant data privacy and intellectual property requirements. No confidential, proprietary, or personally identifiable information was shared with the AI tool during the manuscript preparation process.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jsbmb.2026.107018.

References

[1] T. Shimada, et al., Cloning and characterization of FGF23 as a causative factor of tumor-induced osteomalacia, Proc. Natl. Acad. Sci. USA 98 (2001) 6500–6505.
[2] T. Shimada, et al., FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis, J. Bone Miner. Res. 19 (2004) 429–435.
[3] L.D. Quarles, Endocrine functions of bone in mineral metabolism regulation, J. Clin. Invest 118 (2008) 3820–3828.
[4] S. Christakos, Vitamin D: a critical regulator of intestinal physiology, JBMR 5 (2021) e10554.
[5] S. Ubaidus, et al., FGF23 is mainly synthesized by osteocytes in the regularly distributed osteocytic lacunar canalicular system established after physiological bone remodeling, J. Electron Microsc. 58 (2009) 381–392.
[6] K.E. White, et al., Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23, Nat. Genet 26 (2000) 345–348.
[7] T. Shimada, et al., Mutant FGF-23 responsible for autosomal dominant hypophosphatemic rickets is resistant to proteolytic cleavage and causes hypophosphatemia in vivo, Endocrinology 143 (2002) 3179–3182.
[8] E.A. Imel, S.L. Hui, M.J. Econs, FGF23 concentrations vary with disease status in autosomal dominant hypophosphatemic rickets, J. Bone Miner. Res. 22 (2007) 520–526.
[9] F. Francis, et al., Genomic organization of the human PEX gene mutated in X-linked dominant hypophosphatemic rickets, Genome Res 7 (1997) 573–585.
[10] K.B. Jonsson, et al., Fibroblast growth factor 23 in oncogenic osteomalacia and X-linked hypophosphatemia, N. Engl. J. Med. 348 (2003) 1656–1663.
[11] Y. Yamazaki, et al., Increased circulatory level of biologically active full-length FGF-23 in patients with hypophosphatemic rickets/osteomalacia, J. Clin. Endocrinol. Metab. 87 (2002) 4957–4960.
[12] M. Leifheit-Nestler, D. Haffner, How FGF23 shapes multiple organs in chronic kidney disease, Mol. Cell. Pedia 8 (2021) 12.
[13] P. Wahl, M. Wolf, FGF23 in chronic kidney disease, Adv. Exp. Med. Biol. 728 (2012) 107–125.
[14] RCPCHGrowth and Research - RCPCH Digital Growth Charts. ⟨https://growth.rcpch.ac.uk/researchers/introduction/⟩.
[15] J.A. Clowes, et al., Effect of feeding on bone turnover markers and its impact on biological variability of measurements, Bone 30 (2002) 886–890.
[16] J.C.Y. Tang, et al., Reference intervals for serum 24,25-dihydroxyvitamin D and the ratio with 25-hydroxyvitamin D established using a newly developed LC–MS/MS method, J. Nutr. Biochem. 46 (2017).
[17] J.C.Y. Tang, , 2025, Measurement of 1,25-dihydroxyvitamin D in serum by LC-MS/MS compared to immunoassay reveals inconsistent agreement in paediatric samples. 63, 962–971 632025962971.
[18] T.J. Cole, The LMS method for constructing normalized growth standards, Eur. J. Clin. Nutr. 44 (1) (1990) 45–60.
[19] R.A. Rigby, D.M. Stasinopoulos, Generalized Additive Models for Location, Scale and Shape, J. R. Stat. Soc. Ser. C. Appl. Stat. 54 (2005) 507–554.
[20] R Core Team. R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria.* ⟨https://www.R-project.org/⟩. (2018).
[21] H. Wickham, Ggplot2: Elegant Graphics for Data Analysis, Springer-Verlag New York, 2016.
[22] P. Wayne, 2010, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition (EP28-A3c). (2010).
[23] Royal College of Paediatrics and Child Health Growth Chart Expert Group, UKWHO Growth Chart. Guid. Health Prof. Chart.Des. (2009).
[24] Royal College of Paediatrics and Child Health Growth Chart Expert Group, CEL 20 Introd. Sch. -Age (218 Years) Growth Charts (2012).
[25] F. Habibzadeh, How to report the results of public health research, J. Public Health Emerg. 1 (2017) 90.
[26] V. Brescia, et al., Determination of iFGF23 Upper Reference Limits (URL) in healthy pediatric population, for its better correct use, Front. Endocrinol. (Lausanne) 13 (2022) 1–13.
[27] M. Stanczyk, S. Chrul, K. Wyka, M. Tkaczyk, Serum intact fibroblast growth factor 23 in healthy paediatric population, Open Med. (Pol.) 16 (2021) 1022–1027.

[28] G.I. Baroncelli, et al., Intact FGF23 concentration in healthy infants, children, and adolescents, and diagnostic usefulness in patients with X-linked hypophosphatemic rickets, J. Endocrinol. Invest 47 (2024) 873–882.
[29] O. Ogunmoroti, et al., Associations between endogenous sex hormones and FGF-23 among women and men in the Multi-Ethnic Study of Atherosclerosis, PLoS One 17 (2022).
[30] J.A. Wheeler, E.L. Clinkenbeard, Regulation of Fibroblast Growth Factor 23 by Iron, EPO, and HIF, Curr. Mol. Biol. Rep. 5 (2019) 8–17.
[31] R. Goetz, et al., Isolated C-terminal tail of FGF23 alleviates hypophosphatemia by inhibiting FGF23-FGFR-Klotho complex formation, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 407–412.
[32] G. Courbon, et al., Bone-derived C-terminal FGF23 cleaved peptides increase iron availability in acute inflammation, Blood 142 (2023) 106–118.
[33] B.B. Ho, C. Bergwitz, FGF23 signalling and physiology, J. Mol. Endocrinol. 66 (2021) R23–R32.
[34] G. Courbon, V. David, Fibroblast growth factor 23 is pumping iron: C-terminal-fibroblast growth factor 23 cleaved peptide and its function in iron metabolism, Curr. Opin. Nephrol. Hypertens. 33 (2024).
[35] S. Sharma, et al., Intact and C-Terminal FGF23 Assays—Do Kidney Function, Inflammation, and Low Iron Influence Relationships With Outcomes? J. Clin. Endocrinol. Metab. 105 (2020) e4875–e4885.
[36] S. Sharma, et al., Renal Clearance of Fibroblast Growth Factor-23 (FGF23) and its Fragments in Humans, J. Bone Miner. Res. 37 (2022) 1170–1178.
[37] E.R. Smith, M.M. Cai, L.P. McMahon, S.G. Holt, Biological Variability of Plasma Intact and C-Terminal FGF23 Measurements, J. Clin. Endocrinol. Metab. 97 (2012) 3357–3365.
[38] Smith, E.R., McMahon, L.P. & Holt, S.G. Method-specific differences in plasma fibroblast growth factor 23 measurement using four commercial ELISAs. **51**, 1971–1981 (2013).
[39] M.D. Sinha, C. Turner, D.J. Goldsmith, FGF23 concentrations measured using "intact" assays similar but not interchangeable, Int. Urol. Nephrol. 45 (2013) 1821–1823.
[40] P. Wahl, M. Wolf, FGF23 in Chronic Kidney Disease, in: M. Kuro-o (Ed.), Endocrine FGFs and Klothos, Springer US, New York, NY, 2012, pp. 107–125, https://doi.org/10.1007/978-1-4614-0887-1_8.
[41] E. Papastergiou, et al., Intact FGF23 and Markers of Iron Homeostasis, Inflammation, and Bone Mineral Metabolism in Acute Pediatric Infections, Biology 13 (2024).
[42] D. Yakubovich, et al., Factors associated with early phosphate levels in preterm infants, Eur. J. Pedia 179 (2020) 1529–1536.
[43] S. Llorente-Pelayo, et al., Role of fibroblast growth factor-23 as an early marker of metabolic bone disease of prematurity, BMC Pedia 24 (2024).
[44] M. Zheng, K.J. Campbell, E. Scanlan, S.A. McNaughton, Development and evaluation of a food frequency questionnaire for use among young children, PLOS ONE 15 (3) (2020).
[45] (PHE). Public Health England. Government Dietary Recommendations for Energy and Nutrients for Males and Females Aged 1–18 Years and 19+ Years, Public Health England, London, 2016.