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Sex and Iron Modify Fibroblast Growth Factor 23 (FGF23) Concentration in 1-Year-Old Children

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Short title: Sex and iron modify FGF23 in 1-year old children.

Precis: In this cross-sectional study of 721 1-year-old infants, girls had higher intact fibroblast growth factor 23 (FGF23) than boys, and iron level associated with intact and C-terminal FGF23 in both sexes

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Abstract:

*Context***:** Fibroblast growth factor 23 (FGF23) plays an important role in phosphate homeostasis but its regulation is inadequately characterized.

Objective: To examine regulators of FGF23, especially sex and iron status, in early childhood.

Design: A cross-sectional study involving 1-year-old children.

*Setting and participants***:** Healthy term infants with a birth weight appropriate for gestational age were recruited to an ongoing vitamin D trial at the Kätilöopisto Maternity Hospital, Helsinki, Finland. At 12-month follow-up visits serum FGF23, 25-hydroxyvitamin D (25OHD), phosphate, ionized calcium, parathyroid hormone (PTH) and iron status were measured. All 721 children (51% girls) with complete data were included.

Main Outcome Measures: Intact and C-terminal FGF23 concentrations and iron status at 1 year of age.

Results: Intact FGF23 was higher in girls than in boys, the medians (IQR) being 44.4 (36.8, 51.9) pg/mL and 40.9 (34.5, 49.0) pg/mL ($p<0.001$). C-terminal FGF23 was similar in boys and girls, 2.8 (2.1, 3.7) pmol/L and 2.9 (2.2, 3.7) pmol/L ($p=0.393$), respectively. Iron concentration associated positively with intact FGF23. Iron concentration was the strongest modifier of intact FGF23 (B 0.498, CI95% 0.333 to 0.663, $p<0.001$) with ferritin, season, ionized calcium, 25OHD and sex as other covariates. The association between iron and C-terminal FGF23 was inverse (B -0.072, CI95% -0.092 to -0.051, $p<0.001$).

Conclusions: At 1 year of age FGF23 status was different in girls and boys, the intact FGF23 concentrations being higher in girls. Iron modified FGF23 concentrations, the intact FGF23 being higher, and C-terminal lower, in those with higher iron concentration.

Keywords: FGF23, vitamin D, iron, sex, infant

1. Introduction

Fibroblast growth factor 23 (FGF23) is produced by osteocytes and participates in phosphate and vitamin D metabolism. Intact FGF23 reduces renal phosphate reabsorption by acting directly on the proximal tubules. FGF23 also reduces gut phosphate absorption indirectly by inhibiting renal synthesis of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). C-terminal FGF23 is a cleavage product of active intact FGF23, with no demonstrated effect on phosphate concentration (1-3).

Regulation of FGF23 is complex and not yet fully understood. Apart from regulation by phosphate, parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), also other factors are involved (4). We previously demonstrated that intact FGF23 concentrations in early infancy (at age 3 months) differ between sexes, girls having significantly higher concentrations than boys (5). Differences between sexes in C-terminal FGF23 concentrations have been demonstrated in older children, and age-related variation has also been suggested (6-8).

Effects of iron deficiency on FGF23 concentrations were first discovered when studying autosomal dominant hypophosphatemic rickets (ADHR), caused by gain of function mutations in the *FGF23* gene (9, 10). These mutations prevent cleavage of FGF23 and lead to increased circulating levels of intact FGF23, hyperphosphaturia and hypophosphatemia (11). In adults with ADHR and in knock-in ADHR mice, iron deficiency or low iron concentrations correlate inversely with both intact and C-terminal FGF23 (12, 13). In healthy adults, iron deficiency promotes increased expression and cleavage of FGF23, resulting in increased C-terminal FGF23 concentrations but normal intact FGF23 concentrations and normal phosphate levels (11, 14). In wild-type mice, iron deficiency results in elevated intact FGF23 in pups, but not adult mice (13), suggesting that FGF23 cleavage mechanisms could be immature and change with age.

The physiological regulators of FGF23 metabolism remain inadequately characterized. Our study aimed to further study these regulators, and especially the role of sex and iron status, on FGF23 concentrations in a large cohort of one-year old healthy infants.

2. Subjects and Methods

A. Participants

This study is a part of the Vitamin D intervention in Infants (VIDI), a controlled, double-blinded trial conducted at the Kätilöopisto Maternity Hospital in Helsinki, Finland. The study was conducted in accordance with the Declaration of Helsinki and approved by the Research Ethics Committee of the Hospital District of Helsinki and Uusimaa (107/13/03/03/2012). The trial protocol is registered in ClinicalTrials.gov (NCT01723852). Written informed consent was provided by the parents at recruitment.

Between January 2013 and June 2016, altogether 987 healthy, term infants with weight appropriate for gestational age were randomized to receive daily vitamin D_3 supplementation of either 10 µg (400 IU) or 30 µg (1200 IU) from 2 weeks to 2 years of age. We have previously described the study protocol and detailed inclusion and exclusion criteria(15). Data presented here represents participants in both VIDI intervention groups. In order to examine sex differences in FGF23 concentrations, we included 721 children (51% girls) with complete data on background factors and concentrations of FGF23, 25-hydroxyvitamin D (25OHD), ionized calcium, phosphate and parathyroid hormone (PTH) at age 1 years. When analyzing the impact of iron status on FGF23 concentration, the data analysis comprised 650 children (51% girls). Maternal and infant data were collected from medical records at recruitment. Detailed descriptions of baseline data and maternal and umbilical cord blood 25OHD levels, including analyses by season (winter, spring, summer, autumn), have previously been reported (16). Anthropometric measurements and venous blood samples were obtained at 12-month follow-up visits. Growth was evaluated using Finnish pediatric growth references (17). Plasma and serum aliquots were stored at -80°C until analysis.

B. Biochemical variables

Analyses of FGF23, 25OHD and PTH concentrations were carried out at the Pediatric Research Centre, University of Helsinki.

Concentrations of intact and C-terminal FGF23 were determined from plasma samples using commercially available enzyme-linked immunosorbent assay (ELISA) –kits by Kainos Laboratories (Tokyo, Japan) for intact FGF23 and Biomedica Medizinprodukte GmbH & Co KG (Vienna, Austria) for C-terminal FGF23, according to manufacturers' instructions. The assay used for determining C-terminal FGF23 concentrations measures both C-terminal and intact FGF23 fragments. Both assays have been shown to have good sensitivity and reliably measure intact FGF23 also at lower ferritin levels (18, 19). Duplicate analysis of randomly selected subgroups of 38 intact and 36 C-terminal FGF23 samples showed mean CV 2% and 7%, respectively. The detection range was 0 to 800 pg/mL for intact and 0 to 20 pmol/L for C-terminal FGF23.

Serum concentrations of 25OHD and PTH were analyzed using an IDS-iSYS fully automated immunoassay system (Immunodiagnostic Systems Ltd., Bolton, UK) with chemiluminescence detection. All samples were analyzed in the same batch. Intra-assay variation for S-25OHD was 7% and for PTH <6%. Quality and accuracy of S-25OHD analyses were validated by participation in the vitamin D External Quality Assessment Scheme (DEQAS, Charing Cross Hospital, London, UK*)*. The current method showed 6% positive bias compared to NIST standards in international comparisons. PTH concentrations that were undetectably low (n=19) were coded as 4.4 pg/mL (lower detection limit 4.5 pg/mL).

Laboratory analyses of ionized calcium, phosphate, alkaline phosphatase (ALP), creatinine, hemoglobin, mean corpuscular volume (MCV), iron, ferritin, transferrin and transferrin-saturation (TSAT) were performed at the Central Laboratory of Helsinki University Hospital (HUSLAB) using standard, accredited photometric, impedance and flow-cytometric methodology. HUSLAB is an accredited laboratory adhering to international (T055) SFS-EN ISO 15189 and SFS-EN ISO/IEC 17025 standards.

C. Statistical analysis

Results for groups are given in median and interquartile range (IQR). Independent samples T-test with normally distributed variables, Mann-Whitney U-test with those variables lacking normal distribution after logarithmic transformation, or Pearson Chi-Square test with categorical variables

enabled comparison between sexes. Factors with an effect on FGF23 concentration were examined in linear regression models. The final models included independent modifying variables with a p-value <0.05. When analyzing the impact of iron concentration (presented in quartiles), or season, on other continuous variables, analysis on variance (ANOVA) was performed, and Tukey served as a post-hoc test, with Bonferroni correction for multiple comparisons. P-value <0.05 was considered statistically significant. For data analyses, we utilized IBM SPSS Statistics 22 (IBM, Armonk, USA).

3. Results

A. Cohort characteristics

The study cohort comprised 721 1-year-old children (0.98-1.01 years; 51% girls) who participated in the VIDI study. In accordance with the inclusion criteria, children were born at term and birth sizes were appropriate for gestational age (Table 1.). At birth and at the 1 year follow-up visit, boys were heavier (p<0.001) and longer (<0.001) than girls (Table 1.). Due to the original recruitment schedule, sample collection occurred most often during spring (44%).

B. Biochemical findings

Median (IQR) intact FGF23 concentration was 40.9 (34.5, 49.0) pg/mL in boys and 44.4 (36.8, 51.9) pg/mL in girls, the concentration being 8% higher in girls (p<0.001) (Table 1). The median (IQR) C-terminal FGF23 concentrations were similar in boys and girls, 2.8 (2.1, 3.7) pmol/L and 2.9 (2.2, 3.7) pmol/L (p=0.393), respectively. These findings persisted after adjustment for weight, length Z-score, weight-to-length ratio, hemoglobin and ferritin.

Most children were vitamin D sufficient, the median (IQR) serum 25OHD concentrations being 95 nmol/L (78, 118) in boys and 99 nmol/L (79, 118) in girls (p=0.356). Moreover, median PTH concentration was normal in both sexes (23 and 24 pg/mL, respectively, normal range 15-65 ng/L). Ionized calcium concentration was higher in girls than in boys (1.34 vs. 1.33 mmol/L, p=0.002, normal range 1.16-1.39 mmol/L) and serum ALP concentration was higher in boys than in girls (286 vs. 268 U/L, p=0.039, normal range 115-460 U/L). Phosphate concentrations were within the normal age-appropriate range (1.3-2.2 mmol/L) in all, and similar in boys and girls.

Median hemoglobin concentration was higher in boys than in girls (120 vs. 118 g/L, p=0.007), the range being 81 to 142 g/L. Altogether 92 (15%) children had hemoglobin below the age-specific reference (111-142 g/L), and 61% of these were girls. Iron concentration was below the reference (≤7 µmol/L) in 151 children (23%) without any sex difference (p=0.826). The range for iron concentration was 2 to 31 µmol/L. Ferritin was lower in boys than in girls (18 vs. 26 µg/L, p<0.001), the range being 3 to 254 µg/L. The number of children with ferritin below the lower reference (6 µg/L) was 12 (2%). Only 4 boys and 1 girl had mild iron-deficiency anemia defined as concurrently low hemoglobin and ferritin concentration.

C. Key modifiers of FGF23

The key modifiers of intact FGF23 at 1 year were iron, season, sex, 25OHD, ionized calcium and ferritin (Table 2). After stratifying the analyses by sex, iron and season remained significant factors in both (Table 3). Higher iron concentration and winter, compared with other seasons, associated with higher intact FGF23. In the whole group and separately in girls, 25OHD and ionized calcium concentrations associated positively, and ferritin inversely, with intact FGF23. Phosphate was not a significant modifier of intact FGF23.

As to C-terminal FGF23, iron concentration and weight at 1 year were modifying factors; the higher the iron concentration, the lower the C-terminal FGF23 (Table 2).

When participants were divided into quartiles according to iron concentration, both intact and C-terminal FGF23 differed between the quartiles (Table 4). In the *highest* iron quartile, intact FGF23 was higher than in other quartiles. Respectively, in the *lowest* iron quartile, C-terminal FGF23 was higher than in other quartiles. Phosphate concentration associated positively with iron concentration. In contrast, 25OHD and PTH did not differ between iron quartiles. Bonferroni correction did not affect the results.

To summarize, iron was an independent modifier of both intact and C-terminal FGF23 concentration and correlated with phosphate concentration. High iron concentration associated with high serum intact FGF23, low C-terminal FGF23 and high phosphate concentration.

4. Discussion

This is the first study to evaluate FGF23 concentration and its regulators, especially the role of sex and iron status in this interplay in healthy one-year old children. The key findings of the study were: firstly, there is a significant difference in intact FGF23 concentrations between boys and girls at the age of 1 year. This difference was smaller than in our previous study on 3-month-old infants but still clearly evident. Secondly, iron had a significant impact on FGF23 concentrations at age one year. Iron concentration was the most important modifying factor for intact FGF23 concentration, along with sex, season, 25OHD, ionized calcium and ferritin. Higher iron concentration associated with higher intact FGF23. Interestingly, season and ionized calcium levels were associated with FGF23 concentrations in girls, but not in boys, and serum phosphate was not a significant modifier of FGF23 status.

Recently some studies in different populations, mostly in school-age children or adults, have highlighted the regulatory role of iron in FGF23 metabolism (8, 11, 12, 14). In small children, the regulatory mechanisms may be different, and there may be regulating factors no longer seen in adulthood. For example, among healthy 3-month old infants, we previously observed that girls presented with almost 50% higher intact FGF23 concentrations than boys (5). This was a novel finding, which is confirmed also in the current large cohort, where the difference between boys and girls at age 1 year was less marked, 8%, but still similar in direction. We have hypothesized that minipuberty, the activation of the hypothalamic-pituitary-gonadal axis (20), and especially high testosterone concentrations in boys during infancy, could regulate FGF23 metabolism. The most significant endocrine manifestations of minipuberty occur during the first 6 months of life and it is thus unlikely that minipuberty alone could explain the persistent difference in FGF23 concentrations between girls and boys. Growth velocity is lower in girls than in boys especially during the first 6 months of life (21). Growth velocity decreases from birth, and plateaus before the age of 1 year. Our observation of sex-related differences in intact FGF23 concentrations could promote phosphate

sparing in boys compared with girls, and this could be a physiological adjustment for higher phosphate need in boys during the rapid growth period.

Fetal phosphate levels are relatively high, and decrease within a few days after birth concurrently with cessation of placental phosphate influx and resolution of transient hypoparathyroidism. Rodent models and infant data have shown that intact FGF23 concentration increases rapidly after birth (22, 23) and thereby FGF23 may have an important role in regulating phosphate balance in early infancy. Since iron status and FGF23 are associated already in cord blood (24) iron is likely to have a regulatory role already then. The physiological regulators of phosphate homeostasis during early childhood still remain inadequately understood and should be explored in longitudinal studies.

The present study shows an association between iron and intact and C-terminal FGF23. This observation is in line with some other studies in older children and adults, but has not previously been evaluated in young children. In an iron deficient population, low iron concentration leads to stimulation of *FGF23* expression, and further to increased cleavage of intact FGF23 resulting in increased C-terminal FGF23 (14). Unlike previous studies, we also observed an association between iron and intact FGF23 concentrations: lower iron concentration was associated with lower intact FGF23 levels. Our cohort is large, and thus enabled us to detect associations that may not be evident in smaller studies. Furthermore, our cohort was mainly iron sufficient. We observed that in higher iron quartiles, the ratio between intact and C-terminal FGF23 increased, thus the relative rate of cleavage was lower. It is possible that the importance of iron as an FGF23 regulator varies depending on the subject's state of iron sufficiency.

Age-dependent regulation patterns could also play a role in our cohort and be specific to this age group. In ADHR, the incomplete penetrance of the disease and the observation that disease onset may coincide with iron deficiency, has led to several studies examining the role of iron in FGF23 metabolism. In a rodent model, iron deficiency resulted in increased *Fgf23* expression in osteocytes (10). In iron deficient mice with the ADHR mutation in *Fgf23*, intact and C-terminal FGF23 were increased, while iron deficient wild-type mice presented merely with increased C-terminal FGF23. On the other hand, iron deficiency led to increased intact and C-terminal FGF23 in both wild-type and

ADHR mice during the neonatal period (13), and an inverse association between ferritin and C-terminal FGF23 was seen already in cord blood (24). Thus, the regulatory role of iron in FGF23 metabolism, and the regulation of FGF23 cleavage may change with age.

The concentration of the iron-binding protein ferritin reflects the iron stores of the body (25). The total amount of body iron and its distribution varies during infancy (26). Compared with iron concentration, ferritin changes more slowly. Our finding of a lower ferritin concentration in boys could be due to their higher demand of iron during the period of rapid growth. The prevalence of iron-deficiency anemia was low in our cohort. It is worth noting that children who were small for gestational age or preterm, and thus at elevated risk for iron deficiency, were excluded from the studied cohort.

Our study was limited to a cross-sectional evaluation of FGF23 and its regulators. Examination of longitudinal changes in FGF23 concentrations was not possible in the present cohort due to limited blood volume. On the other hand, our previous cohort of 3-month-old infants was recruited at the same maternity hospital in Helsinki, and in both studies the inclusion criteria were identical. Thus, our previous and current results are likely to be comparable and allow some conclusions regarding the age-related changes in FGF23 and its regulators during infancy.

In conclusion, at 1 year of age, girls presented with significantly higher intact FGF23 concentrations than boys. Compared with 3-month old children, the difference between the sexes was considerably smaller. Iron concentration had an impact on both intact and C-terminal FGF23 concentrations; in fact, iron was the strongest modifier of intact FGF23 concentration. High iron concentration associated with high serum intact FGF23, and low C-terminal FGF23. Further, in addition to iron concentration, sex-related factors participate in the regulation of FGF23 metabolism. These findings are novel and indicate that differences between boys and girls in mineral homeostasis are evident at age 1 year. Further studies are warranted to understand the sex-dependent differences seen here in FGF23 regulation. It also remains to be explored in future studies, whether optimization of iron status in early childhood has wider bone health implications.

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Author contributions

EHS, MEC, SA and OM designed the study, EHS, MEC, SA and OM were responsible for conducting the research, EHS and MEC analysed the data, EHS and MEC wrote the first draft of the manuscript; EHS, MEC, SV, HHA, JR, OH, TH, HV, SA and OM took part in the writing and final editing of the report, with EHS, MEC and OM having the primary responsibility for the final content. All authors read and approved the final version.

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Table 1

Table 1. Child characteristics at 1 year of age			
	BOYS	GIRLS	T-test p value
Number of participants	353	368	
Maternal age at delivery (years)	31 (29, 35)	32 (29, 35)	0.653
Weight at birth (kg)	3.6 (3.3, 3.8)	3.5 (3.2, 3.8)	0.020
Length at birth (cm)	50 (49, 52)	50 (49, 51)	<0.001
Duration of gestation (days)	282 (275, 285)	282 (278, 287)	0.018
Decimal age at follow-up	1.0 (0.99, 1.01)	1.0 (0.98, 1.01)	0.548*
Weight at follow-up (kg)	10.0 (9.4, 10.8)	9.4 (8.7, 10.0)	<0.001
Length at follow-up (cm)	76.0 (74.5, 77.5)	74.5 (73.0, 76.0)	<0.001
Season at follow-up			0.583**
winter	17.6% (n=62)	16.3% (n=60)	
spring	42.2% (n=149)	46.5% (n=171)	
summer	20.1% (n=71)	20.4% (n=75)	
autumn	20.1% (n=71)	16.8% (n=62)	
Biochemical variables of the child			
Intact FGF23 (pg/mL)	40.9 (34.5, 49.0)	44.4 (36.8, 51.9)	<0.001
C-terminal FGF23 (pmol/L)	2.8 (2.1, 3.7)	2.9 (2.2, 3.7)	0.393†
25OHD (nmol/L)	95 (78, 118)	99 (79, 118)	0.490
PTH (pg/mL)	23 (16, 31)	24 (16, 35)	0.356†
Ionized calcium (mmol/L)	1.33 (1.31, 1.35)	1.34 (1.32, 1.36)	0.002
Phosphate (mmol/L)	1.9 (1.8, 2.0)	1.9 (1.8, 2.0)	0.410
Hemoglobin (g/L)[a]	120 (114, 125)	118 (113, 123)	0.007
MCV (fL)[a]	77 (74, 79)	78 (76, 79)	<0.001
Iron (µmol/L)	11 (7, 14)	11 (7, 14)	0.826
Ferritin (µg/L)	18 (12, 29)	26 (17, 40)	<0.001†
Transferrin (g/L)	2.9 (2.7, 3.1)	2.7 (2.5, 3.0)	<0.001
TSAT (%)	14 (10, 19)	15 (10, 20)	0.279
ALP (U/L)	286 (240, 345)	268 (219, 344)	0.039*
Creatinine (µmol/L)	25 (22, 28)	25 (22, 29)	0.786†
Values are medians (IQR), IQR=interquartile range (25%, 75%)			
*Mann-Whitney U, **Pearson Chi-Square exact 2-sided, †after logarithmic transformation			
MCV=mean corpuscular volume, TSAT=transferrin saturation, ALP=alkaline phosphatase			
[a]N=624			

Table 2

Table 2. Key modifiers of intact and C-terminal FGF23 concentrations				
	β	B	95% CI	p value
Intact FGF23				
Iron (µmol/L)	0.220	0.498	0.333, 0.663	<0.001
Season (1=winter, 2=others)	-0.192	-5.839	-8.042, -3.637	<0.001
Sex (1=boy, 2=girl)	0.130	2.944	1.285, 4.604	0.001
25OHD (nmol/L)	0.122	0.047	0.019, 0.076	0.001
Ionized Ca (mmol/L)	0.107	37.646	11.703, 63.590	0.005
Ferritin (µg/L)	-0.106	-0.059	-0.100, -0.018	0.005
C-terminal FGF23				
Iron (µmol/L)	-0.259	-0.072	-0.092, -0.051	<0.001
Weight (kg)	0.106	0.135	0.040, 0.229	0.005
Analysis conducted with linear regression model with forward method, β=standardized regression coefficient, B=regression coefficient, 95% CI=95 % confidence interval for B				

Table 3

Table 3. Key modifiers of intact FGF23 concentration according to sex				
BOYS	β	B	95% CI	p value
Iron (µmol/L)	0.298	0.713	0.467, 0.959	<0.001
Season (1=winter, 2=others)	-0.284	-8.835	-12.030, -5.641	<0.001
GIRLS	β	B	95% CI	p value
25OHD (nmol/L)	0.191	0.069	0.032, 0.107	<0.001
Iron (µmol/L)	0.173	0.364	0.147, 0.580	0.001
Ionized Ca (mmol/L)	0.159	51.187	17.734, 84.640	0.003
Season (1=winter, 2=others)	-0.116	-3.415	-6.413, -0.416	0.026
Ferritin (µg/L)	-0.104	-0.047	-0.094, -0.001	0.047
Analysis conducted with linear regression model with forward method using ionized calcium, 25OHD, Fe, ferritin and season as independent confounders, β=standardized regression coefficient, B=regression coefficient, 95% CI=95 % confidence interval for B. All factors with p-value <0.05 are reported.				

Table 4

Table 4. Biochemical and growth parameters at 1 year according to iron quartiles (Q1-Q4)					
	Q1	Q2	Q3	Q4	ANOVA p value
N					
all	165	157	166	162	-
boys	80	76	80	80	-
girls	85	81	86	82	-
Iron (µmol/L)					
all	5.3 (3.9, 6.4)	9.1 (8.2, 9.9)	12.3 (11.6, 13.1)	17.0 (15.4, 19.2)	-
boys	5.7 (4.0, 6.7)	9.3 (8.3, 9.9)	12.2 (11.5, 13.3)	17.1 (15.7, 18.9)	-
girls	5.0 (3.8, 6.3)	9.0 (8.2, 9.9)	12.4 (11.8, 13.1)	17.0 (15.3, 19.5)	-
Intact FGF23 (pg/mL)					
all	39.4 (32.8, 47.0)	42.9 (35.7, 49.3)	42.8 (35.6, 50.5)	47.1 (38.5, 55.3)	<0.001[a]
boys	38.9 (30.0, 44.7)	41.2 (34.1, 48.6)	40.9 (34.4, 49.6)	43.4 (38.7, 55.3)	<0.001[b]
girls	40.9 (33.3, 48.0)	44.0 (38.0, 50.8)	43.5 (36.7, 51.5)	49.9 (38.4, 55.6)	0.001[c]
C-terminal FGF23 (pmol/L)					
all	3.4 (2.4, 4.5)	2.9 (2.1, 3.7)	2.7 (2.2, 3.4)	2.6 (2.2, 3.1)	<0.001†[d]
boys	3.4 (2.4, 4.4)	2.9 (2.1, 3.9)	2.7 (2.2, 3.4)	2.5 (2.1, 3.0)	<0.001†[c]
girls	3.3 (2.4, 4.6)	2.9 (2.0, 3.7)	2.7 (2.1, 3.1)	2.7 (2.3, 3.2)	<0.001†[d]
25OHD (nmol/L)					
all	98 (77, 120)	96 (79, 114)	95 (77, 114)	101 (79, 122)	0.195†
boys	97 (78, 121)	93 (75, 108)	99 (81, 117)	96 (78, 129)	0.191†
girls	98 (75, 120)	102 (81, 121)	90 (76, 107)	104 (79, 120)	0.065†
PTH (pg/mL)					
all	25 (16, 34)	24 (16, 34)	24 (17, 33)	21 (14, 32)	0.140†
boys	24 (16, 33)	25 (15, 34)	24 (17, 29)	20 (13, 29)	0.185†
girls	27 (16, 36)	24 (18, 34)	25 (16, 37)	21 (14, 34)	0.711†
Phosphate (mmol/L)					
all	1.85 (1.75, 1.93)	1.93 (1.83, 2.01)	1.92 (1.82, 2.03)	1.94 (1.84, 2.04)	<0.001[d]

boys	1.83 (1.76, 1.92)	1.94 (1.84, 2.01)	1.91 (1.84, 2.05)	1.95 (1.83, 2.03)	<0.001[d]
girls	1.87 (1.74, 1.95)	1.91 (1.79, 2.02)	1.93 (1.80, 2.03)	1.94 (1.85, 2.04)	<0.001[c]
Length (cm)					
all	75.0 (73.8, 77.0)	75.4 (73.5, 77.0)	75.5 (73.5, 77.0)	75.2 (73.5, 77.0)	0.869
boys	76.5 (74.6, 77,5)	76.0 (75.0, 77.5)	76.0 (74.1, 78.0)	76.0 (74.4, 77.0)	0.895
girls	74.4 (73.1, 75.5)	74.0 (72.5, 75.8)	75.0 (73.0, 76.1)	74.3 (73.0, 76.0)	0.774
Weight (kg)					
all	9.6 (9.0, 10.4)	9.7 (9.1, 10.4)	9.8 (9.0, 10.6)	9.7 (8.9, 10.4)	0.529
boys	10.1 (9.3, 10.7)	10.0 (9.6, 10.9)	10.2 (9.3, 10.9)	9.9 (9.3, 10.7)	0.548
girls	9.3 (8.6, 9.9)	9.3 (8.7, 10.0)	9.3 (8.8, 10.2)	9.4 (8.7, 10.2)	0.576
Values are medians (IQR), IQR=interquartile range (25%, 75%)					
Iron concentration ranges in quartiles: Q1 1.8-7.4, Q2 7.5-10.7, Q3 10.8-14.3 and Q4 14.4-30.6 µmol/L					
Post hoc test Tukey: significant differences between quartiles at p≤0.010					
[a]Q4 vs. Q1, Q2 and Q3, [b]Q4 vs. Q1 and Q2 vs. Q4, [c]Q1 vs. Q4, [d]Q1 vs Q2, Q3 and Q4					
†Variables with non-normal distribution: ANOVA was performed after logarithmic transformation					

Figure 1

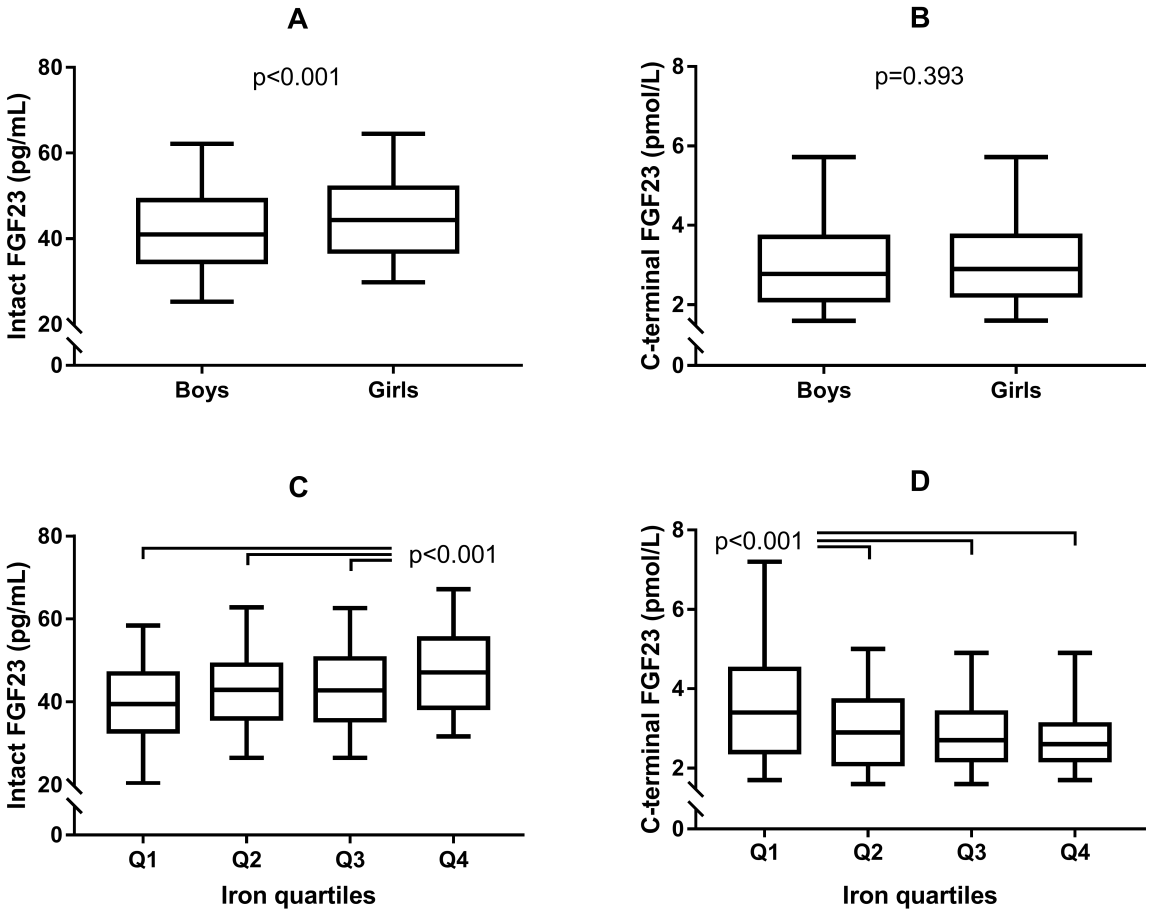

Figure 1. Differences in mean intact and C-terminal FGF23 concentrations between sexes and iron quartiles. (A) Intact FGF23 was higher in girls than in boys ($p<0.001$) while (B) C-terminal FGF23 was similar in boys and girls ($p=0.393$). (C) Iron associated positively with intact FGF23: in the highest iron quartile, intact FGF23 was higher than in other quartiles ($p<0.001$). (D) Iron associated inversely with C-terminal FGF23; in the lowest iron quartile, C-terminal FGF23 was higher than in other quartiles ($p<0.001$).