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The effects of maternal iron deficiency on infant fibroblast growth factor-23 and mineral metabolism

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ABSTRACT

Fibroblast growth factor-23 (FGF23), a phosphate(Phos)-regulating hormone, is abnormally elevated in hypophosphataemic syndromes and an elevated FGF23 is a predictor of mortality in kidney disease. Recent findings suggest iron deficiency as a potential mediator of FGF23 expression and murine studies have shown *in utero* effects of maternal iron deficiency on offspring FGF23 and phosphate metabolism.

Our aim was to investigate the impact of maternal iron status on infant FGF23 and mineral metabolites over the first 2 years of life. Infants born to mothers with normal (**NI** $n = 25$,) and low (**LI** $n = 25$) iron status during pregnancy, from a mother-infant trial (ISRCTN49285450) in rural Gambia, West Africa, had blood and plasma samples analysed at 12, 24, 52, 78 and 104 weeks (wk) of age.

Circulating intact-FGF23 (I-FGF23), Phos, total alkaline phosphatase (TALP) and haemoglobin (Hb) decreased and estimated glomerular filtration rate increased over time [all $P \leq 0.0001$)]. C-terminal-FGF23 (C-FGF23) and TALP were significantly higher in **LI** compared with **NI,** from 52 wk for C-FGF23 [Beta coefficient (SE) 18.1 (0.04) %, $P = 0.04$] and from 24 wk for TALP [44.7 (29.6) U/L, $P = 0.04$]. Infant Hb was the strongest negative predictor of C-FGF23 concentration [−21% (4%) RU/mL, $P \leq 0.0001$], Phos was the strongest positive predictor of I-FGF23 [32.0(3.9) pg/mL, $P \leq 0.0001$] and I-FGF23 did not predict C-FGF23 over time [−0.5% (0.5%), $P = 0.3$].

In conclusion, this study suggests that poor maternal iron status is associated with a higher infant C-FGF23 and TALP but similar I-FGF23 concentrations in infants and young children. These findings further highlight the likely public health importance of preventing iron deficiency during pregnancy. Whether or not children who are born to iron deficient mothers have persistently high concentrations of these metabolites and are more likely to be at risk of impaired bone development and pre-disposed to rickets requires further research.

1. Introduction

An elevated concentration of circulating fibroblast growth factor-23 (FGF23) has been shown to be involved in an ever growing set of diseases since its discovery as the cause of certain forms of bone mineralisation disorders such as hereditary hypophosphataemic rickets [1]. These include renal disease and arterial calcification, and an elevated FGF23 concentration is a predictor of mortality in these patients [2]. FGF23, mainly expressed in osteocytes, is primarily a regulator of phosphate (Phos) homeostasis, acting predominantly at the kidney to increase Phos excretion by regulating sodium-phosphate co-transporters, but it also regulates CYP27B1 and CYP24A1 enzyme activity and thus has effects on vitamin D metabolism [3,4].

The regulatory mechanisms of *FGF23* gene expression and subsequent protein processing in the osteocyte are unclear but a role for iron in FGF23 regulation has been identified. A growing body of evidence has shown inverse associations between markers of iron status with FGF23 concentration in animals [5,6] and human studies in Africa [7–10], North America [11] and Poland [12]. A murine study has indicated that maternal iron status during pregnancy influences FGF23 and Phos regulation in infancy [6]. The study showed that pups born to iron deficient dams had significantly higher serum C-terminal and Intact- FGF23 concentrations and correspondingly lower Phos and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) concentrations compared with pups born to iron replete dams [6]. The impact of prenatal iron status on FGF23 and Phos regulation and the corresponding impact on bone health have not yet been investigated in humans. This may be especially relevant in populations with high rates of iron deficiency.

The aim of the current study was to investigate the influence of maternal iron status on circulating FGF23 and mineral homeostasis during early life in rural Gambians; a population in which iron deficiency is

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common. To this end, the changes in circulating FGF23 and markers of iron and Phos metabolism from 12 weeks (wk) to 2 years of age were measured longitudinally in Gambian children born to mothers with normal and low iron status during pregnancy.

2. Materials & methods

2.1. Participants

The West Kiang province of The Gambia, West Africa, is a subsistence agricultural region with a high prevalence of iron deficiency anaemia particularly among children and in pregnant women [13]. In addition, elevated FGF23 concentrations have been detected in children with rickets-like bone deformities in this area [14]. Participants were recruited from the MRC (Medical Research Council) Keneba field station, as part of the Early Nutrition and Immune Development trial of pregnant women and their infants (ISRCTN49285450) in which women were randomised to 4 intervention groups from <20 wk gestation [15]. All groups received iron and folate (as per standard clinical care) with or without additional multiple micronutrients and or protein energy throughout pregnancy [15]. At 6 to 18 months of age, the corresponding children were then further randomised to a lipid-based nutritional supplement, with or without additional multiple micronutrients forming a total of 8 different supplement groups. Ethical approval was given by The Gambian Government/MRC Unit Joint Ethics Committee and written informed consent was obtained from participants or their parents/guardians. The study was conducted in accordance with the Declaration of Helsinki.

2.2. Sample collection and biochemical analysis

An overnight-fasted venous blood sample was collected in mothers at "booking" (~14 wk of pregnancy) and at 20 and 30 wk of pregnancy and in infants at 12, 24, 52, 78 and 104 wk of age. Blood samples were collected into lithium heparin (LiHep) and EDTA-coated tubes. Haemoglobin (Hb) was measured in maternal and infant whole blood samples by haemoglobinometer (Medonic CA 530 Haematology Analyser, Boule Medical AB, Stockholm, Sweden). Blood samples were then separated by centrifugation after which the plasma was frozen at −70 °C until analysis. Maternal LiHep samples and infant samples at 24 wk were analysed for ferritin (Ferr), iron (Fe), unbound iron binding capacity (UIBC) and transferrin (Tf). Soluble transferrin receptor (sTfR) and c-reactive protein (CRP) were measured in maternal samples only (Cobas, Integra 400 Plus, Roche Diagnostics, Indiana, USA) at all time points. Infant LiHep and EDTA plasma samples were transported on dry ice to MRC Human Nutrition Research (HNR), Cambridge, UK, for analysis of calcium (Ca), phosphate (Phos), albumin (Alb), magnesium (Mg), total alkaline phosphatase (TALP), and cystatin C (Cys C) (Kone Analyser 20i, Finland) and C-terminal and intact- fibroblast growth factor-23 (C-FGF23 & I-FGF23; Immutopics, CA, USA and Kainos, Japan respectively) at all time points. Assay accuracy and precision were monitored across the working range of the assays using kit controls supplied by the manufacturer. In addition, an aliquot of a pooled plasma sample was assayed in each batch to monitor possible drift over time. Intra and inter- assay coefficients of variation were <12% for I-FGF23, <7% for Cys C, <4% for C-FGF23, and <2% for the remaining analytes.

2.3. Anthropometry and characteristics

Infant length, weight and head-circumference were measured at all time points, using standard protocols and with regularly calibrated equipment. Weight-for-age, length-for-age, weight-for-length and head-circumference-for-age sex specific Z-scores were calculated using WHO reference data [16].

All infants were breast-fed into the second year of life. The infants were exclusively breastfed until a mean age of 5.3 months. This timing did not differ significantly by group 5.3 (1.3) vs. 5.4 (1.1) months ($P = 0.8$) (Moore S.E, Personal Communication).

2.4. Sample selection for analysis

To detect a difference of ~67 RU/mL (or 0.75 of a standard deviation based on existing Gambian data (not published)) in plasma C-FGF23 between two groups at $\beta = 80\%$ power $\alpha = 0.05$ it was estimated that each group needed 25 infants. Samples were selected on the basis of maternal iron status at 20 wk gestation ($n = 400$). All maternal samples with a CRP <5 mg/L and a measurement of Ferr and sTfR at 20 wk together with infants who had sufficient EDTA plasma at all 5 time points were eligible for selection ($n = 85$). Women were then ranked by sTfR/logFerr index and grouped into those with an sTfR (mg/L)/logFerr (μg/L) >2.0 for low iron status [17] (**LI**, $n = 25$) and sTfR/logFerr <1.5 for normal iron status (**NI**, $n = 25$) and the corresponding infant samples were then analysed at all 5 time points. There was a similar distribution of the 8 supplement groups among the **LI** and **NI** women (data not shown).

2.5. Statistical analysis & calculations

Statistical analysis was performed using DataDesk 6.3.1 (Data Description Inc., Ithaca, NY, USA) and all figures were drawn using GraphPad Prism 5.0 (GraphPad Software, San Diego CA, USA). Data are reported as mean and standard deviation (SD) for normally distributed or geometric mean (+1SD, −1SD) for negatively skewed data. Hierarchical linear models, adjusted for participant ID (identification code/study number) and age (and infant weight-for-age Z-score for infant biochemistry models only), were used to identify differences in variables over time. To identify overall group differences, the same hierarchical model was used with the addition of participant ID nested in group. To identify specific time point differences by group an interaction term between group and time point was included and Fisher's Least Significant Difference (LSD) post-hoc tests were reported. To determine the extent of within individual tracking over time, hierarchical models were used adjusting for age and participant ID and the F-ratio and P-value reported for each variable. Backward step-wise elimination was used to determine the predictors of plasma FGF23 concentration over time. Glomerular filtration rate (eGFR) was not a significant predictor of C-FGF23 concentration but was a significant predictor of I-FGF23. When included in the predictive models of C- and I-FGF23, eGFR made no material difference to the relationships between Hb, I- and C-FGF23 and so was not included in the models. There was a similar distribution of males (M) and females (F) in the **LI** and **NI** children (F/M: **LI** = 10/15, **NI** = 13/12) and there was no sex difference in any of the biochemical variables. There were sex differences in anthropometry but these were accounted for with the calculation of age and sex adjusted Z-scores and so sex was not included as a variable in the models. $P < 0.05$ was considered as statistically significant.

eGFR (mL/min) was estimated using the following equation $\frac{74.835}{CysC\ (\frac{mg}{L})^{1.33}}$ [18]. Calcium was corrected for albumin (Ca-corr; mmol/L) by normalising to an albumin concentration of 36 g/L using a correction factor 0.0167 mmol Ca/g albumin [19]. This correction factor was calculated from the slope of the relationship between Ca and albumin using local reference data [7].

3. Results

3.1. Maternal factors during pregnancy

At 20 wk of pregnancy the mean (SD) age of the mothers was 29.4 (7.0) years of age and height was 1.61 (0.06) metres, neither of which differed significantly between the groups ($P = 0.9$ and $P = 0.4$ respectively). Mothers in the **LI** group tended to be lighter than the mothers in the **NI** group [53.0 (8.4) vs. 58.0 (11.8) kg, $P = 0.1$] and their body mass