ORIGINAL ARTICLE

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Effects of Iron Deficiency Anemia and Its Treatment on Fibroblast Growth Factor 23 and Phosphate Homeostasis in Women

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ABSTRACT

Fibroblast growth factor 23 (FGF23) is an osteocyte-derived hormone that regulates phosphate and vitamin D homeostasis. Through unknown mechanisms, certain intravenous iron preparations induce acute, reversible increases in circulating FGF23 levels that lower serum phosphate in association with inappropriately low levels of calcitriol, similar to genetic diseases of primary FGF23 excess. In contrast, studies in wild-type mice suggest that iron deficiency stimulates *fgf23* transcription but does not result in hypophosphatemia because FGF23 is cleaved within osteocytes by an unknown catabolic system. We tested the association of iron deficiency anemia with C-terminal FGF23 (cFGF23) and intact FGF23 (iFGF23) levels in 55 women with a history of heavy uterine bleeding, and assessed the longitudinal biochemical response over 35 days to equivalent doses of randomly-assigned, intravenous elemental iron in the form of ferric carboxymaltose (FCM) or iron dextran. Iron deficiency was associated with markedly elevated cFGF23 (807.8 ± 123.9 relative units [RU]/mL) but normal iFGF23 (28.5 ± 1.1 pg/mL) levels at baseline. Within 24 hours of iron administration, cFGF23 levels fell by approximately 80% in both groups. In contrast, iFGF23 transiently increased in the FCM group alone, and was followed by a transient, asymptomatic reduction in serum phosphate <2.0 mg/dL in 10 women in the FCM group compared to none in the iron dextran group. Reduced serum phosphate was accompanied by increased urinary fractional excretion of phosphate, decreased calcitriol levels, and increased parathyroid hormone levels. These findings suggest that iron deficiency increases cFGF23 levels, and that certain iron preparations temporarily increase iFGF23 levels. We propose that intravenous iron lowers cFGF23 in humans by reducing *fgf23* transcription as it does in mice, whereas carbohydrate moieties in certain iron preparations may simultaneously inhibit FGF23 degradation in osteocytes leading to transient increases in iFGF23 and reduced serum phosphate. © 2013 American Society for Bone and Mineral Research.

KEY WORDS: PHOSPHATE; FGF23; IRON; PTH; VITAMIN D

Introduction

Ferric carboxymaltose (FCM) is a parenteral form of iron that can be used to treat iron deficiency when oral iron is either ineffective or contraindicated.[1] Several randomized controlled trials demonstrated the efficacy and safety of intravenous FCM for treating iron deficiency associated with chronic kidney disease, inflammatory bowel disease, heavy uterine bleeding, and during the postpartum period.[2–10] In these populations, several patients who received FCM developed transient and asymptomatic reductions in serum phosphate that typically appeared within 2 to 4 weeks of treatment and resolved spontaneously within 6 to 12 weeks.[9]

Reduced serum phosphate also occurs following intravenous administration of saccharated ferric oxide and iron polymaltose.[11–18] Most studies suggest that isolated phosphaturia rather than global, proximal tubular dysfunction accounts for reduced serum phosphate levels following intravenous iron treatment. Hypophosphatemia due to an isolated renal phosphate leak is a cardinal feature of syndromes of primary excess of fibroblast growth factor 23 (FGF23), which is a phosphate-regulating peptide hormone secreted by osteocytes.[19] Although the mechanism is poorly understood, recent studies suggest that reduced serum phosphate in response to intravenous iron is mediated by an acute increase in FGF23, which induces phosphaturia and suppresses levels of 1,25-dihydroxyvitamin D.[20]

Conversely, a recent animal study suggested that iron deficiency stimulates FGF23 transcription in osteocytes.[21] In iron-deficient animals, excess FGF23 was cleaved within osteocytes, and its inactive, C-terminal fragments were detected in the circulation, whereas levels of biologically active, intact

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hormone remained unchanged. Indirect support for conservation of this mechanism in humans came from a single study that demonstrated an inverse correlation between iron and FGF23 levels when FGF23 was measured with a C-terminal immunoassay that detects both full-length FGF23 and its C-terminal fragments, but no correlation when FGF23 was measured with an assay that is specific for the intact peptide.[22] Because previous studies that reported changes in FGF23 levels in response to intravenous iron therapy measured either C-terminal or intact FGF23 levels but not both, our understanding of the effects of iron therapy on FGF23 metabolism in humans with iron deficiency is incomplete. The goals of the current study were to examine the effects of iron deficiency and its rapid correction on C-terminal and intact FGF23 levels in women with iron deficiency anemia secondary to heavy uterine bleeding, to investigate the mechanism of reduced serum phosphate levels following intravenous iron administration, and to compare the relative effects of equivalent doses of iron in the form of FCM versus iron dextran.

Subjects and Methods

Study population

This was an open-label, multicenter, 5-week, prospective, randomized trial that compared single, equivalent doses of intravenous elemental iron in the form of FCM versus iron dextran in women with iron deficiency anemia due to heavy uterine bleeding. Women were eligible to participate if they were age 18 years or older and met the following laboratory criteria at screening: hemoglobin <12 g/dL; and serum ferritin $\leq$100 ng/mL or ferritin $\leq$300 ng/mL in combination with a transferrin saturation (TSAT) $\leq$ 30%. Exclusion criteria included hypersensitivity to any component of FCM or iron dextran; serum phosphate <2.6 mg/dL at screening; history of hemochromatosis, untreated primary hyperparathyroidism, gastrointestinal malabsorption, malignancy within the previous 5 years, chronic kidney disease, end-stage renal disease, or kidney transplantation; treatment with intravenous iron within the previous 10 days; treatment with erythropoiesis stimulating agents, red blood cell transfusion, radiotherapy, chemotherapy, or surgical procedures requiring general anesthesia within the previous 30 days; aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels greater than 1.5 times normal; pregnancy; and active alcohol or drug abuse. The study was approved by a central Institutional Review Board (Integreview, Austin, TX, USA), and registered at Clinical Trials.gov (http://clinicaltrials.gov/show/NCT01307007; Hypophosphatemia With Intravenous Ferric Carboxymaltose Versus Iron Dextran in Women With Iron Deficiency Secondary to Heavy Uterine Bleeding). All participants provided written informed consent.

Design

The design of the study and flow of participants are presented in Fig. 1*A*, *B*. After informed consent was obtained, participants who were found to be eligible entered the treatment phase of the study. Randomization occurred centrally via a fax-based system. Participants who were randomized to FCM (Luitpold

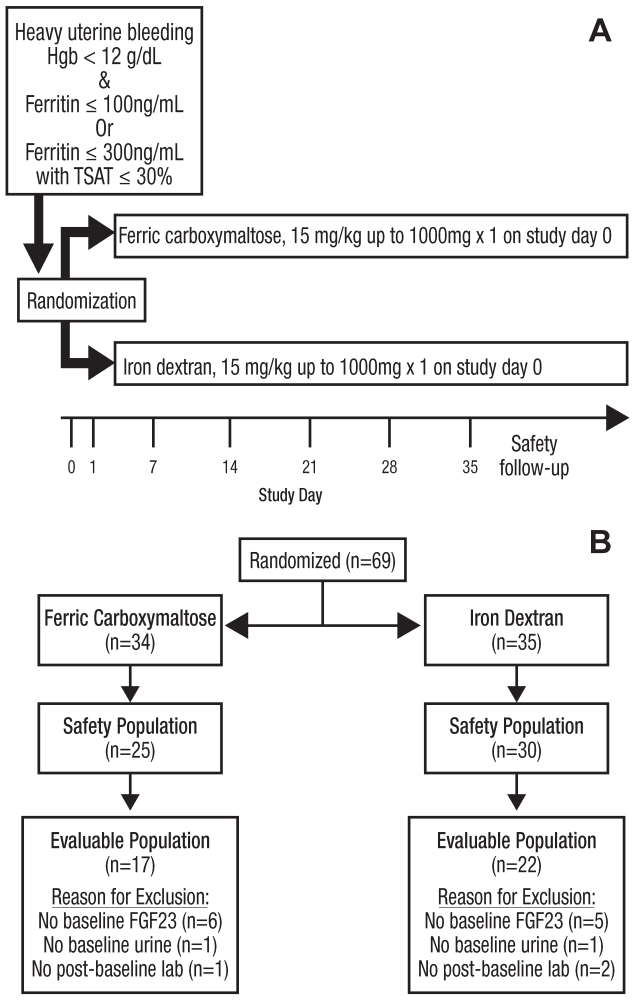

Fig. 1. Schematic of study design and flow of participants. (*A*) Study design; (*B*) CONSORT diagram of flow of participants. The safety population included all participants who received study drug regardless of subsequent laboratory testing. The evaluable population included participants with baseline and at least one postrandomization set of FGF23 and blood and urinary phosphate levels. Hgb, hemoglobin; TSAT, transferrin saturation.

Pharmaceuticals Inc., Shirley, NY, USA) received an intravenous infusion of 15 mg/kg up to a maximum of 1000 mg diluted in 250 mL normal saline solution. The complete dose was infused over 15 minutes on day 0. Participants who were randomized to iron dextran received Dexferrum (Luitpold Pharmaceuticals Inc.) on day 0 using a standard protocol. An initial test dose of 25 mg was administered slowly over 5 minutes. After a 1-hour period of clinical observation, participants who demonstrated no adverse reaction received the remainder of the dose, up to 15 mg/kg or 1000 mg including the test dose.

Laboratory testing of blood and three-hour urine collections was performed at the screening visit and on study day 0 (when iron was infused), day 1 (24 hours after iron infusion), day 7, day 14, and day 35 (Fig. 1*A*). Any participant who experienced a reduction in serum phosphate below the normal range after day 0 underwent additional phosphate measurements at 14 day intervals following day 35 until the level returned to the normal reference range. Safety evaluations of all participants included ascertainment of adverse events, physical examinations at all

study visits, and laboratory assessments. Any participant who withdrew from the study received a follow-up phone call to ascertain adverse events 30 days after they received study drug.

Endpoints

The prespecified primary endpoints were changes in blood and urine markers of phosphate and bone metabolism among the evaluable population, defined as those participants with baseline and at least one postrandomization set of FGF23 and blood and urinary phosphate levels. Secondary endpoints included the proportion of participants achieving a hemoglobin increase $\geq$2 g/dL anytime between baseline and day 35, and the change from baseline to highest hemoglobin, ferritin, and TSAT anytime during the 35-day study period. Additional safety endpoints included incidence, severity, and seriousness of adverse events, and incidence of treatment-emergent abnormal clinical laboratory values in the safety population, defined as all participants who received the study drug regardless of subsequent laboratory testing.

Laboratory testing

Blood and urine samples were sent to a central laboratory for analysis (Covance Central Laboratory Service, Indianapolis, IN, USA). Hemoglobin, blood counts, iron indices, electrolytes, and serum and urinary phosphate and creatinine were measured with standard, automated, multianalyte techniques. Urinary fractional excretion of phosphate (FEPi) was calculated from the 3-hour, morning urine collections as urinary phosphate $\times$ serum creatinine/serum phosphate $\times$ urinary creatinine. Serum levels of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D were measured in duplicate by radioimmunoassay (DiaSorin, Stillwater, MN, USA) with intraassay coefficients of variation (CV) <11.6%. Plasma C-terminal FGF23 (cFGF23) was measured in duplicate in EDTA-plasma samples using a human FGF23 immunometric assay capable of detecting both the intact peptide and its C-terminal fragments (Immutopics, San Clemente, CA, USA) with intraassay CV $\leq$3.8%. Because cFGF23 testing was performed in parallel with rolling enrollment and follow-up, all cFGF23 assay plates tested combinations of baseline and postintervention samples from multiple study participants. Serum intact FGF23 (iFGF23) was measured in batches of baseline and postintervention samples from multiple study participants at study end using an immunometric assay that detects the intact peptide exclusively (Kyowa Medex Company, Shizuoka, Japan) with intraassay CV <2.7%. Serum hepcidin levels were measured on day 0, day 7, and day 35 using an enzyme-linked immunosorbent assay (Intrinsic LifeSciences, La Jolla, CA, USA) with intraassay CV $\leq$19%. To test for the possibility of global proximal tubular dysfunction, urinary amino acids, glucose, albumin, and beta 2 microglobulin were measured. To assess bone turnover, we measured urinary deoxypyridinoline crosslink, and serum osteocalcin and bone-specific alkaline phosphatase.

Power

It was estimated that a sample size of 20 participants per arm would provide >90% power to detect a within-group change in serum phosphate from baseline of at least 0.8 times the standard deviation of mean baseline phosphate. This sample would provide >90% power to detect a between-group difference in change from baseline in serum phosphate of at least 1.1 times the standard deviation of the mean change.

Statistical analysis

Baseline characteristics are presented as mean $\pm$ SEM for continuous variables and as proportions for categorical variables. The association between iron indices and baseline and change in FGF23 levels was assessed with Spearman correlations. For the primary analyses of mean changes from baseline in continuous markers of phosphate homeostasis, we used restricted maximum likelihood-based repeated measures analyses (linear mixed models) that considered the effects of treatment, visit, and treatment $\times$ time interaction, with baseline values treated as a fixed covariate. The models employed unstructured covariance structures without imputing missing values. All outcome variables that were not normally distributed were analyzed after natural log-transformation. Changes from baseline to each scheduled evaluation and to the minimum, maximum, and final value within treatment groups were compared to zero with Wilcoxon signed-ranks tests, and between-group differences were compared with Wilcoxon rank-sum tests.

To test the secondary endpoint of whether the proportion of participants who achieved a hemoglobin increase $\geq$2 g/dL at any time after the baseline visit differed by iron preparation, we calculated the two-sided, 95% confidence interval (CI) for the absolute difference in the proportions between the FCM and iron dextran groups. This was an intention-to-treat analysis such that any participants who withdrew from the study prior to having a postbaseline hemoglobin assessment were considered to have failed to achieve a $\geq$2 g/dL increase. We used paired *t* tests to analyze whether the intravenous iron formulations significantly increased continuous measures of hemoglobin, TSAT, ferritin, and hepcidin from baseline to the highest value among evaluable participants within each treatment group.

For the categorical safety endpoints, we summarized the number and proportion of participants who experienced an adverse event in the safety population, and compared rates between the iron treatment groups using Fisher's exact test. Analyses were conducted using SAS version 9 (SAS Institute, Inc., Cary, NC, USA). All statistical tests were two-tailed, and p values <0.05 were considered statistically significant.

Results

Sixty-nine women were randomized at four participating clinical centers, 34 to FCM and 35 to iron dextran (Fig. 1*B*). Of these, 25 women in the FCM group and 30 in the iron dextran group who received study drug were included in the safety population (the four centers contributed 12, 3, 10, and 30 participants, respectively). The evaluable population for the phosphate homeostasis analyses included 17 participants in the FCM group and 22 in the iron dextran group (11, 2, 8, and 18 participants from the four centers, respectively).

Baseline assessment

Baseline characteristics were similar between the groups in the evaluable population except for a greater proportion of African Americans in the FCM arm (Table 1). Serum phosphate, FEPi, and iFGF23 levels were normal, but cFGF23 levels were markedly elevated in both groups (overall mean 807.8 ± 123.9 relative units [RU]/mL). Baseline cFGF23 levels were negatively correlated with baseline levels of iron ($r = -0.61$; $p < 0.001$), TSAT ($r = -0.67$; $p < 0.01$), ferritin ($r = -0.55$; $p < 0.001$), and hepcidin ($r = -0.32$; $p = 0.049$). In contrast, baseline iFGF23 levels correlated positively and less strongly with iron levels ($r = 0.41$; $p = 0.008$) and TSAT ($r = 0.39$; $p = 0.01$), and did not correlate with ferritin ($r = 0.21$; $p = 0.19$) or hepcidin levels ($r = 0.21$; $p = 0.21$).

Effects of iron treatment on anemia and iron indices

The mean total doses of elemental iron were 918 mg in the FCM group and 911 mg in the iron dextran group. Hemoglobin increased by ≥2.0 g/dL in 60% of the FCM and 53% of the iron dextran groups (absolute difference 6.7%; 95% CI, −23.2% to 36.6%; $p = 0.79$), with no difference in the temporal change between groups (Fig. 2*A*, Table 2). FCM induced a greater increase in serum ferritin by day 7 compared to iron dextran, but thereafter, the levels were similar (Fig. 2*B*, Table 2). Both FCM and iron dextran increased TSAT and serum hepcidin levels significantly ($p \leq 0.001$; Table 2).

Effects of iron treatment on serum and urinary phosphate

In both the FCM and iron dextran groups, serum phosphate increased modestly within 24 hours after iron administration (Fig. 3*A*). In the iron dextran group, serum phosphate returned to near baseline levels by day 7 and remained stable for the remainder of the study. In the FCM group, serum phosphate decreased significantly by 0.6 mg/dL by day 7, and by 0.7 mg/dL by day 14, before returning toward normal by day 35 (Fig. 3*A*). Serum phosphate decreased to <2.0 mg/dL in 10 participants in the FCM group and none in the iron dextran group. Among the 6 participants whose serum phosphate remained below the normal range at day 35, levels normalized in all by day 80.

The mean FEPi trended downward on day 1 in both the FCM and iron dextran groups, but by day 7, mean urinary FEPi increased significantly compared with baseline in the FCM group ($p = 0.025$), and remained elevated through day 35 (Fig. 3*B*). There was no significant change over time in FEPi in the iron dextran group. There was no evidence of glycosuria, amino aciduria, or albuminuria in either group to suggest

Table 1. Demographic and Baseline Clinical Characteristics of the Evaluable Population

	Ferric carboxymaltose ($n = 17$)	Iron dextran ($n = 22$)
Demographics		
Age, years	35.5 ± 2.5	34.3 ± 2.3
Race, *n* (%)		
African American	14 (82)	11 (50)
White	2 (12)	8 (36)
Hispanic	1 (6)	3 (14)
Body mass index, kg/m^2	32.0 ± 1.9	33.6 ± 2.0
Iron deficiency parameters		
Hemoglobin, g/dL	9.5 ± 0.3	9.8 ± 0.3
Iron, μg/dL	24.2 ± 4.2	34.0 ± 4.2
Transferrin saturation, %	6.5 ± 1.2	9.9 ± 2.4
Ferritin, ng/mL	4.4 ± 0.6	6.9 ± 1.7
Hepcidin, ng/mL	14.3 ± 0.5	16.7 ± 0.6
Previous iron therapy, *n* (%)	6 (35)	11 (50)
Mineral metabolism parameters		
Serum phosphate, mg/dL	3.3 ± 0.1	3.5 ± 0.1
Fractional excretion of phosphate, %	11.9 ± 0.9	11.7 ± 0.9
Serum calcium, mg/dL	9.4 ± 0.1	9.4 ± 0.1
Serum parathyroid hormone, pg/mL	56.2 ± 4.9	57.5 ± 5.4
Serum 25(OH) vitamin D, ng/mL	16.0 ± 1.2	17.7 ± 1.6
Serum 1,25(OH)$_2$ vitamin D, pg/mL	50.7 ± 3.2	49.5 ± 2.5
Serum intact FGF23, pg/mL	28.6 ± 1.4	28.4 ± 1.6
Plasma C-terminal FGF23, RU/mL	783 ± 168	827 ± 181

The evaluable population included participants with baseline and at least one postrandomization set of FGF23 and blood and urinary phosphate levels. Continuous variables are presented as mean ± SEM, and categorical variables as proportions.

FGF23 = fibroblast growth factor 23; RU = relative units.

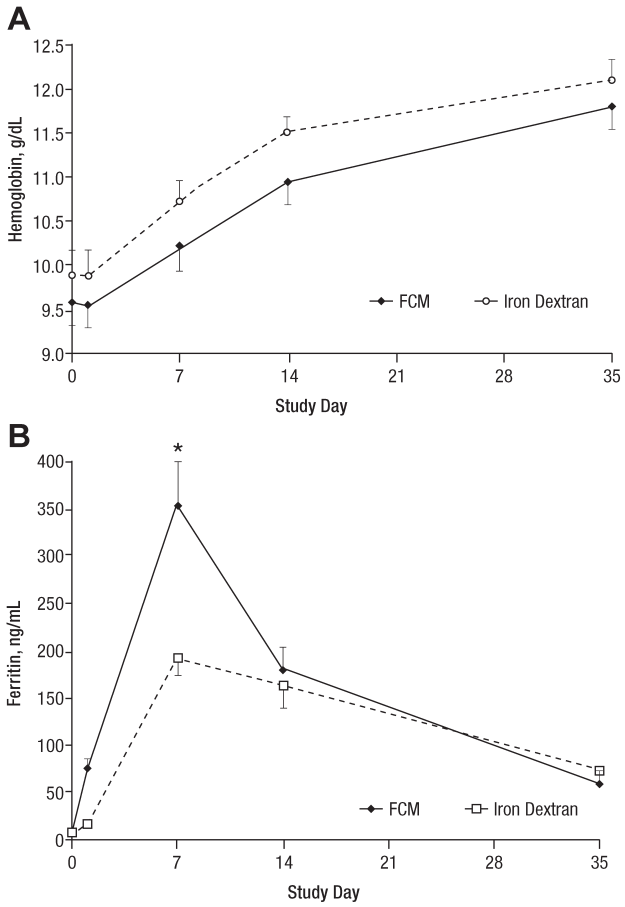

Fig. 2. Effect of FCM and iron dextran on anemia and iron status. (*A*) Hemoglobin; (*B*) serum ferritin. *Connotes $p < 0.05$ for between group differences in change from baseline values.

generalized proximal tubular dysfunction contributed to reduced serum phosphate, and there were no significant differences in bone turnover markers between the groups (data not shown).

Effects of iron treatment on cFGF23 and iFGF23 levels

In the overall study population, cFGF23 decreased by 81.4% ± 14.9% by day 1, and remained unchanged throughout the remainder of the study period without significant differences between the FCM and iron dextran groups (Fig. 3*C*, Table 3). The magnitude of reduction in cFGF23 from baseline to minimum value within individual participants was greatest among those with the most severe iron deficiency at baseline (correlation of baseline ferritin with maximal change in cFGF23; $r = 0.55$, $p < 0.001$).

Although there were no significant changes in iFGF23 levels in response to iron dextran, iFGF23 levels increased significantly by day 1 in the FCM group, remained elevated through days 7 and 14, before returning to baseline by day 35 (Fig. 3*D*, Table 3). The increase in iFGF23 from day 0 to day 1 in the FCM group correlated significantly with the concomitant increase in ferritin ($r = 0.59$; $p < 0.001$) and the magnitude of the subsequent decrease in serum phosphate at day 7 ($r = -0.50$; $p = 0.002$).

Effects of iron treatment on vitamin D, calcium, and parathyroid hormone levels

Neither FCM nor iron dextran induced a significant change in 25-hydroxyvitamin D levels (Fig. 4*A*). Iron dextran did not induce a significant change in 1,25-dihydroxyvitamin D levels. In the FCM group, 1,25-dihydroxyvitamin D levels fell significantly from baseline to day 1 and reached a nadir by day 7 before returning towards normal by day 35 (Fig. 4*B*). In the iron dextran group, serum calcium increased within 24 hours, returned toward baseline by day 7, and remained stable for the remainder of the study (Fig. 4*C*). In the FCM group, serum calcium increased within 24 hours, then decreased significantly below baseline on day 7 before returning toward normal by day 35 (Fig. 4*C*). Although the changes in parathyroid hormone (PTH) levels were not significant in either group, there was a trend toward an increase in PTH in the FCM group that peaked at day 14 ($p = 0.10$; Fig. 4*D*).

Stratified analyses by development of serum phosphate <2.0 mg/dL

To further characterize the effects of FCM on phosphate homeostasis, we compared laboratory results from the 10 participants who received FCM and developed serum phosphate levels <2.0 mg/dL with results from the remaining participants who

Table 2. Effects of Iron Therapies on Erythropoiesis and Iron Parameters

	Ferric carboxymaltose ($n = 25$)	Iron dextran ($n = 30$)	p[a]
Hemoglobin increase ≥2.0 g/dL, *n* (%)[b]	15 (60)	16 (53)	0.79
Mean increase in hemoglobin from baseline to highest value, g/dL	2.0 ± 0.3	2.2 ± 0.2	0.27
Mean increase in ferritin from baseline to highest value, ng/dL	306 ± 41	189 ± 21	0.02
Mean increase in transferrin saturation from baseline to highest value, %	68 ± 6.4	44 ± 3.0	0.002
Mean increase in hepcidin from baseline to highest value, ng/mL	33.4	77.8	0.04

Continuous variables are presented as mean ± standard error, and categorical variables as proportions.

[a] p for between group differences.

[b] This analysis was intention-to-treat in the safety population, which included all participants who received study drug regardless of whether they completed subsequent laboratory testing.

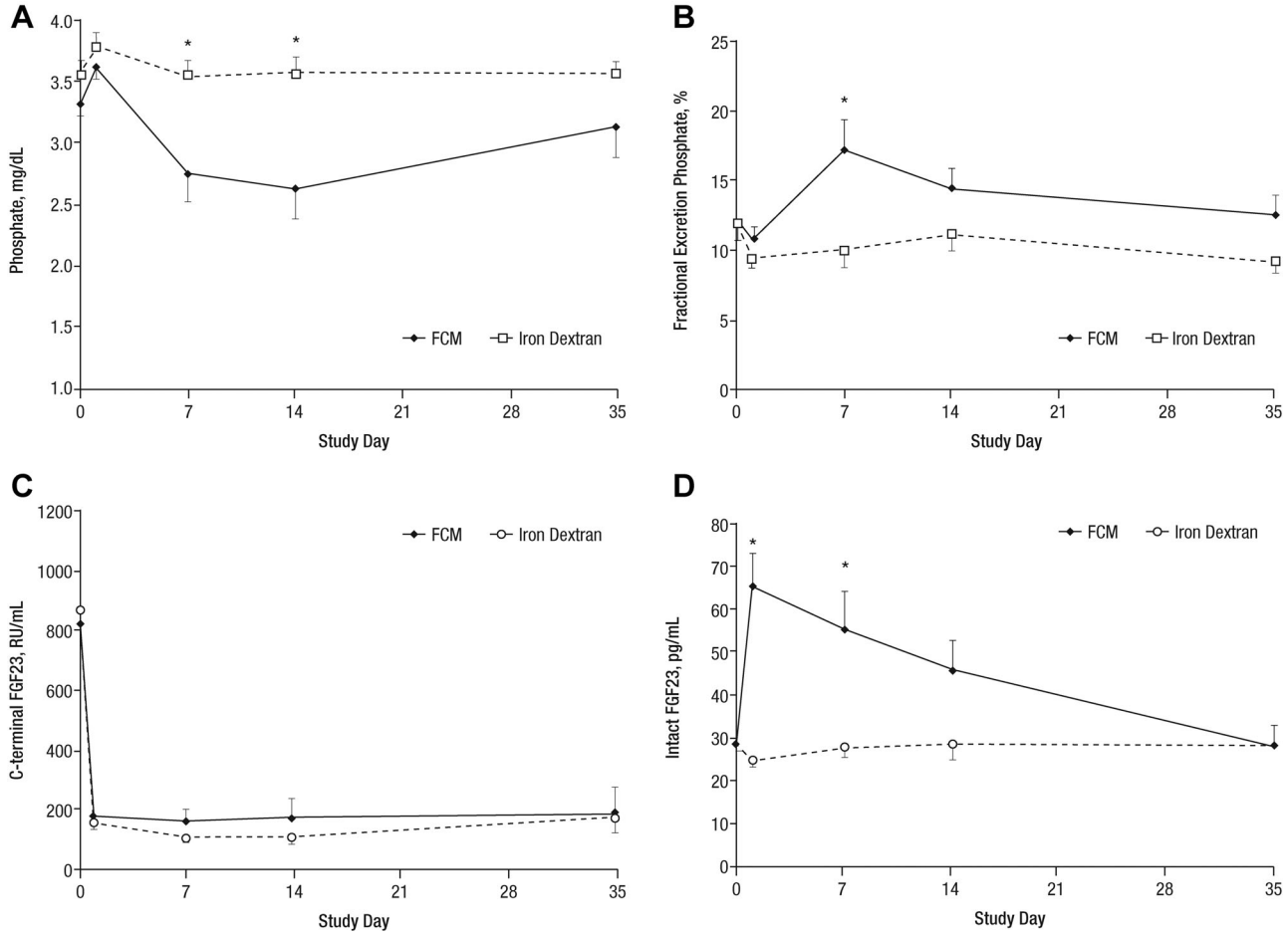

Fig. 3. Effect of FCM and iron dextran on phosphate and FGF23. (*A*) Serum phosphate; (*B*) urinary fractional excretion of phosphate; (*C*) plasma C-terminal FGF23; (*D*) serum intact FGF23. *Connotes $p < 0.05$ for between group differences in change from baseline values.

received FCM but maintained a serum phosphate $\geq$2.0 mg/dL. The nadir in serum phosphate occurred at day 14 (Fig. 5*A*). Although cFGF23 decreased similarly in both groups (Fig. 5*B*), those who developed serum phosphate <2.0 mg/dL manifested greater iFGF23 by day 1 that persisted through day 14 before normalizing by day 35 (Fig. 5*C*). FEPi increased to a significantly greater extent in the low-phosphate subgroup, peaked by day 14, and remained significantly elevated through day 35 (Fig. 5*D*). Levels of 25-hydroxyvitamin D did not change over time between groups (Fig. 6*A*). In contrast, levels of

Table 3. C-Terminal and Intact FGF23 Levels Throughout the Study Period

	C-terminal FGF23		Intact FGF23	
	FCM	Iron dextran	FCM	Iron dextran
Baseline	783 ± 168	827 ± 181	28.6 ± 1.4	28.4 ± 1.6
Day 1	177 ± 12.4	152 ± 17.5	65.2 ± 8.0	24.6 ± 1.9
Change from baseline, %	−78.6	−82.5	128.0	−13.4
Day 7	165 ± 34.9	114 ± 17.7	54.9 ± 18.0	27.5 ± 1.7
Change from baseline, %	−80.9	−85.6	91.8	−4.4
Day 14	170 ± 55.8	107 ± 16.0	45.8 ± 6.9	28.4 ± 2.1
Change from baseline, %	−77.5	−87.3	59.1	−1.3
Day 35	182 ± 73.7	170 ± 51.2	28.1 ± 2.9	28.3 ± 1.7
Change from baseline, %	−78.9	−80.0	2.0	−9.3

Results are presented as mean ± SEM.
FGF23 = fibroblast growth factor 23; FCM = ferric carboxymaltose.

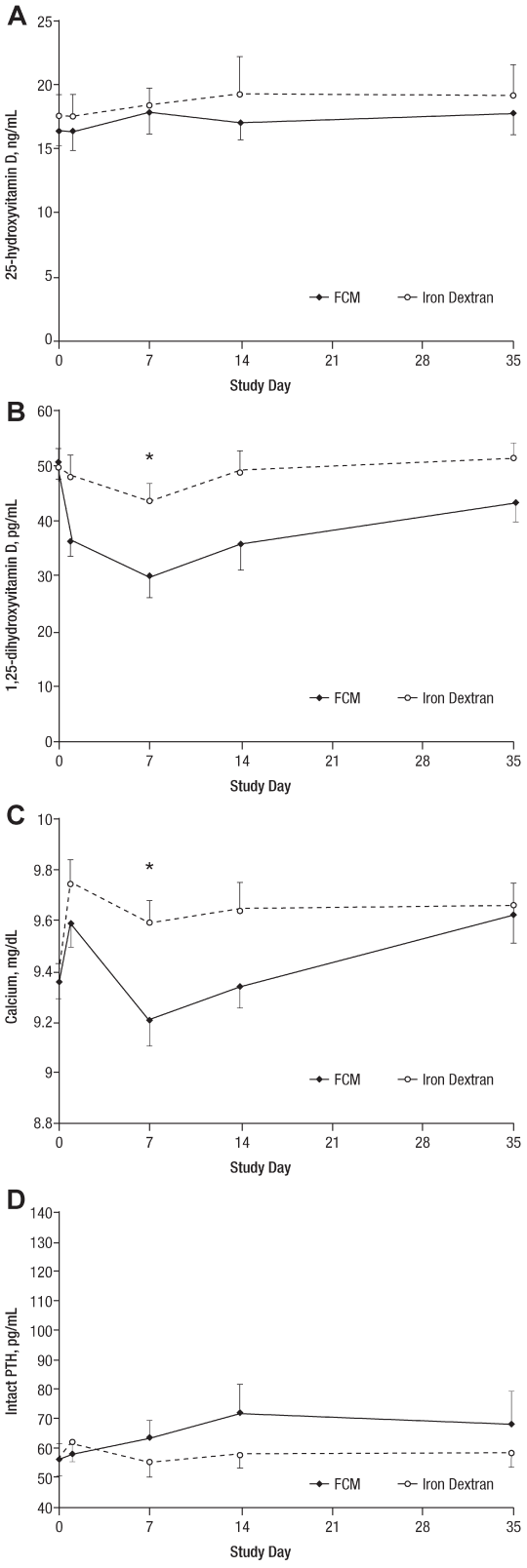

Fig. 4. Effect of FCM and iron dextran on calcium metabolism. (*A*) Serum 25-hydroxyvitamin D; (*B*) serum 1,25-dihydroxyvitamin D; (*C*) serum calcium; (*D*) plasma PTH. *Connotes $p < 0.05$ for between group differences in change from baseline values.

1,25-dihydroxyvitamin D and serum calcium decreased to a greater extent in the low-phosphate subgroup, reaching a nadir by day 7 before returning toward normal (Fig. 6*B*, *C*). Beginning at day 14, PTH levels were significantly higher in the low phosphate subgroup and decreased only partially by day 35 (Fig. 6*D*).

Safety

In the FCM group, 17 participants (68%) experienced one or more adverse events, eight (32%) of which were considered to be related to study drug (Table 4). In the iron dextran group, 18 participants (60%) experienced one or more adverse events, 11 (37%) of which were considered to be related to study drug. There were no significant differences between the groups. Reduced serum phosphate noted by participants' physicians was the most common adverse event reported in the FCM group (32%), and occurred significantly more frequently than in the iron dextran group (3%; $p = 0.008$).

Discussion

This randomized physiological study of intravenous iron therapy for iron deficiency anemia yields new insight into the relationship between iron and phosphate homeostasis. The first novel finding is that iron deficiency anemia is associated with normal iFGF23 but markedly elevated cFGF23 levels to an extent rarely seen except in renal failure or hereditary rachitic diseases.[23] Second, rapid correction of iron deficiency with different intravenous iron preparations reduced cFGF23 levels by approximately 80% within 24 hours. This provides the first direct evidence in humans that iron deficiency causes cFGF23 levels to rise, as was proposed in the seminal study of FGF23 in iron deficient mice.[21]

The third novel finding was that FCM but not iron dextran induced a significant *increase* in iFGF23 levels by 24 hours, which was associated with a subsequent increase in FEPi, and decreases in serum phosphate, 1,25-dihydroxyvitamin D, and calcium levels that were followed by an increase in PTH. This cascade was accentuated among participants who developed the greatest reductions in serum phosphate. The latter results recapitulate previously reported findings of acute, FGF23-mediated phosphate wasting in response to intravenous iron.[11–18] The results exclude the hypothesis that massive phosphate uptake by developing red blood cells lowered serum phosphate, because this would have caused urinary phosphate excretion to decrease. Interestingly, low serum phosphate persisted in some participants at day 35 when FEPi and PTH were persistently elevated but iFGF23 levels had already normalized. This suggests that transient secondary hyperparathyroidism due to FGF23-mediated reduction in 1,25-dihydroxyvitamin D and calcium levels also contributes to reduced serum phosphate in response to intravenous iron. Thus, in this single study, we reproduced the previously reported paradoxical findings that iron deficiency induces *fgf23* transcription, whereas its correction using certain forms of high-dose iron therapy

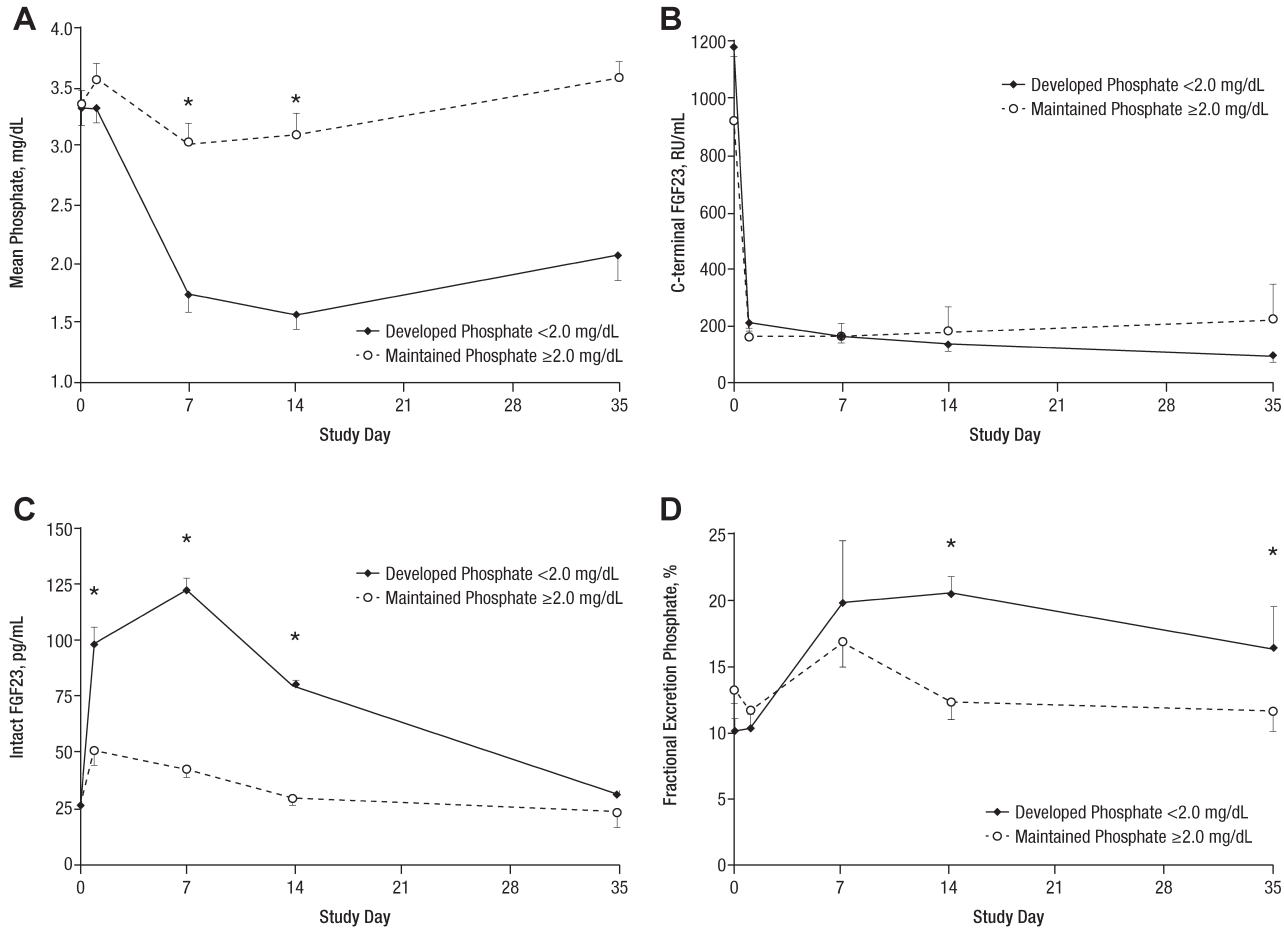

Fig. 5. Effect of FCM on phosphate and FGF23 among participants who did or did not develop serum phosphate <2.0 mg/dL. (*A*) Serum phosphate; (*B*) plasma C-terminal FGF23 (*C*) serum intact FGF23; (*D*) urinary fractional excretion of phosphate. *Connotes $p < 0.05$ for between group differences in change from baseline values.

can lower serum phosphate by raising circulating iFGF23 levels despite seemingly reducing transcription as suggested by rapidly reduced cFGF23 levels. Collectively, these findings suggest a complex relationship between iron status, intravenous iron preparations and regulation of FGF23 synthesis and degradation.

Iron deficiency stimulates *fgf23* transcription in osteocytes but does not cause hypophosphatemia in wild-type mice because of increased intracellular degradation of FGF23, which leaves a footprint of elevated circulating cFGF23 but normal iFGF23 levels (Fig. 7*A*).[21,22] The finding that both FCM and iron dextran rapidly lowered cFGF23 validates these aspects of FGF23 regulation in humans and suggests that the iron component these agents share in common likely drove the effect (Fig. 7*B*, *C*). In contrast, the acute increase in iFGF23 following FCM but not following iron dextran suggests that the main difference between the agents, namely their carbohydrate moieties, likely affect an additional aspect of FGF23 regulation. There are several possible hypotheses to explain these results. First, iron dextran and FCM could both reduce *fgf23* transcription, whereas FCM simultaneously inhibits FGF23 degradation in osteocytes (Fig. 7*B*, *C*). This could be achieved by enhancing posttranslational O-glycosylation of FGF23, which protects it from cleavage,[24] by inhibiting the currently unidentified system that degrades FGF23 in osteocytes, or accelerating iFGF23 secretion before it can be degraded. Second, different iron preparations may differentially reduce peripheral degradation or clearance of circulating iFGF23 after it is secreted by the osteocyte. Third, certain iron preparations could induce ectopic production of FGF23 by other organs that are involved in iron metabolism and have the capacity to express FGF23, including the liver and lymphatic system.[25] Additional mechanistic studies are needed to investigate these hypotheses.

This study has certain limitations. First, several participants who were initially randomized never returned to receive study drugs, and several more received study drugs but had incomplete laboratory testing. In the absence of complete laboratory data, these participants could not be included in the prespecified primary analyses of biochemical changes in mineral metabolism. However, since loss to follow-up appears to have been unrelated to randomized group, it is unlikely to have affected the primary results. Second, this was a small open-label trial of short duration from which we cannot extrapolate conclusions about the long-term safety of FCM.

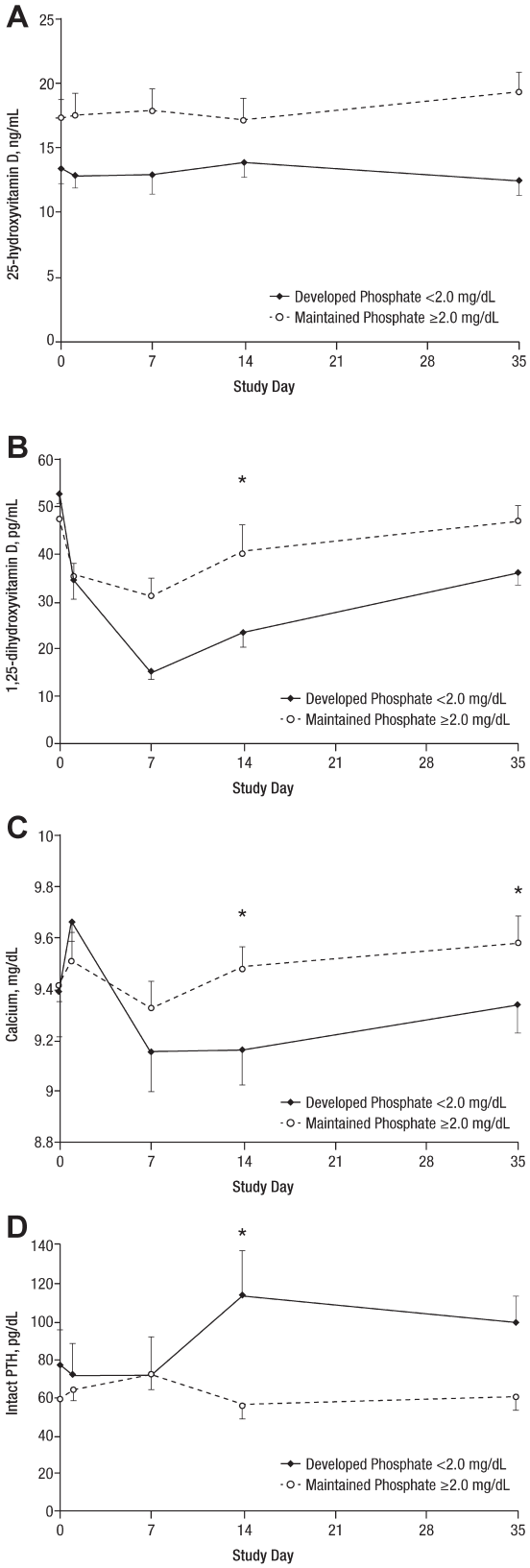

Fig. 6. Effect of FCM and iron dextran on calcium metabolism among participants who did or did not develop serum phosphate <2.0 mg/dL. (*A*) Serum 25-hydroxyvitamin D; (*B*) serum 1,25-dihydroxyvitamin D; (*C*) serum calcium; (*D*) plasma PTH. *Connotes $p < 0.05$ for between group differences in change from baseline values.

However, previous studies demonstrated an equivalent safety profile of FCM relative to other commercially available iron preparations, aside from transient reductions in serum phosphate levels that occurred in up to 60%.[2–10] A recent randomized trial of 2500 patients with chronic kidney disease and elevated cardiovascular risk that compared FCM to iron sucrose during 120 days of follow-up found no significant differences between groups in adverse events or the adjudicated composite end point of major cardiovascular events, despite a higher incidence of serum phosphate <2.0 mg/dL in the FCM arm (18.5% versus 0.8%).[26] These studies suggest that reduced serum phosphate following FCM is not associated with adverse clinical outcomes, but its long-term clinical significance requires further study. A third limitation is that this trial was conducted exclusively in premenopausal women, many of whom were African American. Although previous studies did not report racial or gender differences in serum phosphate levels following FCM, there are known differences in bone and mineral metabolism between African-Americans and whites that may include regulation of FGF23. Additional studies are needed to further investigate potential gender and racial differences in the interaction between iron and phosphate homeostasis.

In summary, a single dose of both FCM and iron dextran significantly increased hemoglobin and iron indices in this randomized study of women with iron deficiency anemia due to heavy uterine bleeding. Although cFGF23 levels fell in both groups, an increase in iFGF23 likely mediated transient and asymptomatic reductions in serum phosphate levels that were observed exclusively in the FCM-treated participants. These data should stimulate additional research into mechanisms of FGF23 synthesis and degradation, and the role of iron deficiency and its treatment.

Disclosures

As a consultant for Luitpold Pharmaceuticals, Inc., MW developed the study protocol, analyzed the data, and authored the manuscript. MW has also served as a consultant or received honoraria from Abbott, Amgen, Diasorin, Genzyme, Kai, Mitsubishi, Sanofi, Shire and Vifor. TK and DB are employees of Luitpold Pharmaceuticals, Inc.

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Authors' roles: Study design: MW, TAK, and DBB. Study conduct: TAK and DBB. Data collection: TAK and DBB. Data analysis: MW. Data interpretation: MW, DBB. Drafting manuscript: MW. Revising manuscript content: TAK and DBB. Approving final version of manuscript: MW, TAK, and DBB. MW takes responsibility for the integrity of the data analysis.

Table 4. Adverse Events

	Ferric carboxymaltose ($n = 25$)	Iron dextran ($n = 30$)	p
Patients experiencing one or more adverse events, *n* (%)	17 (68)	18 (60)	0.59
Patients experiencing one or more adverse events related to study drug, *n* (%)	8 (32)	11 (37)	0.78
Patients experiencing one or more serious adverse events, *n* (%)	0 (0)	0 (0)	1.00
Discontinuation from study due to adverse event, *n* (%)	2 (8)	2 (6)	1.00

Results are presented as proportions.

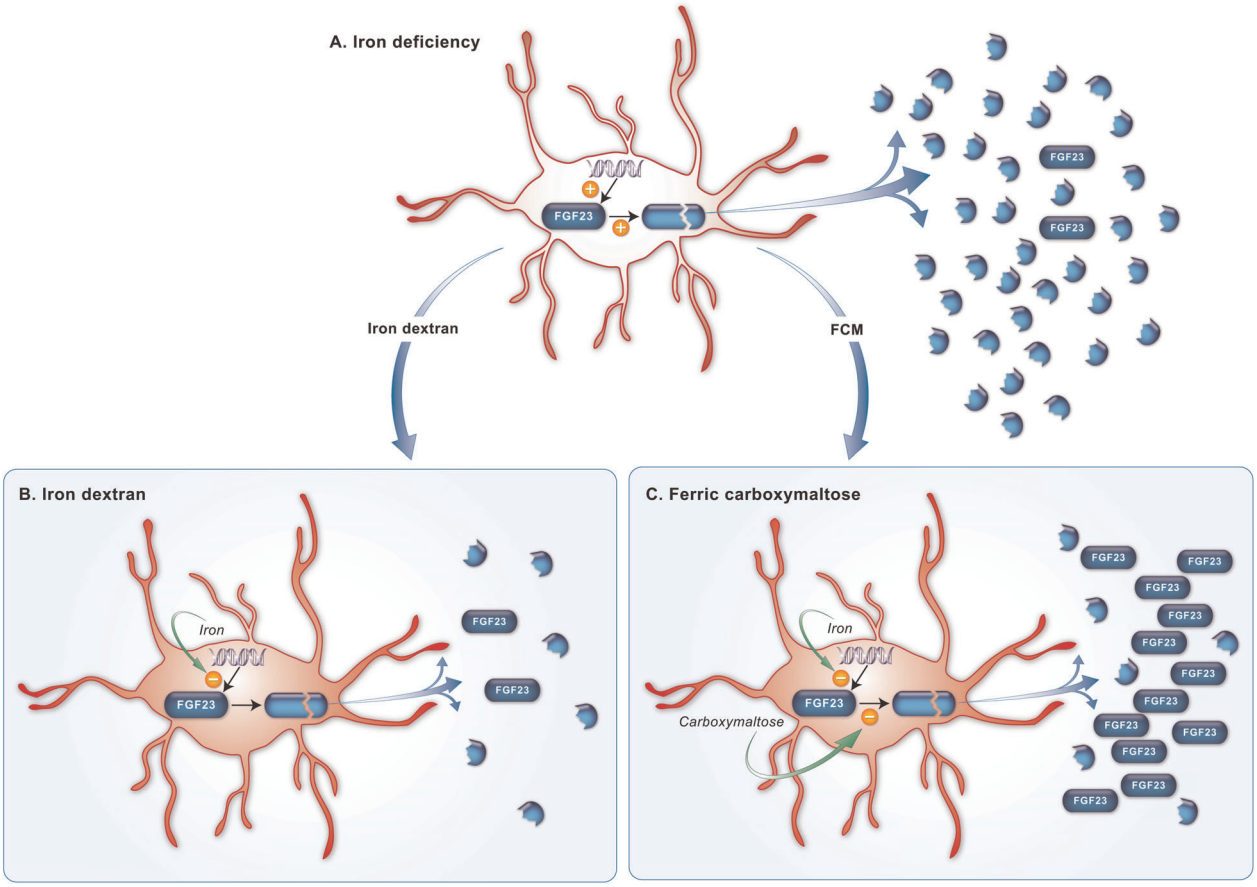

Fig. 7. Hypothesis for differential effects of iron deficiency and its correction with iron dextran or ferric carboxymaltose on regulation of iFGF23 and cFGF23 levels. (*A*) *fgf23* transcription in osteocytes is upregulated by iron deficiency, but a counterbalancing increase in posttranslational FGF23 cleavage maintains normal net production of intact protein. Increased *fgf23* transcription accompanied by increased intracellular FGF23 cleavage results in markedly elevated levels of FGF23 fragments that are detectable by the C-terminal assay. (*B*) Correction of iron deficiency with iron dextran restores normal *fgf23* transcription, thereby decreasing production of FGF23 fragments while maintaining normal production of intact protein. (*C*) Correction of iron deficiency with ferric carboxymaltose restores normal *fgf23* transcription, but production of intact FGF23 protein increases nevertheless, perhaps because of a greater magnitude of concomitant inhibition of FGF23 cleavage by carboxymaltose.

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