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ORIGINAL ARTICLE

# Erythropoiesis stimulating agents are associated with serum fibroblast growth factor 23 metabolism in patients on hemodialysis

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## ABSTRACT

**Background.** This study aimed to determine associations among short- and long-acting erythropoiesis stimulating agents (ESAs), changes in serum fibroblast growth factor 23 (FGF23) and biomarkers of iron metabolism.

**Methods.** Among 108 patients on hemodialysis (HD), 44 received every 2 weeks or monthly doses of continuous erythropoiesis receptor activator (CERA), 31 received weekly doses of darbepoetin-$\alpha$, 24 received three doses per week of epoetin-$\beta$ and 9 were not treated with an ESA. Intact and C-terminal FGF23 and transferrin saturation (TSAT), ferritin, erythroferrone and hepcidin 25 were measured in blood samples collected before the HD session at the end of the dialysis week (baseline, Day 0) and on Days 3, 5, 7 and 14 thereafter.

**Results.** Levels of ferritin, hepcidin 25 and erythroferrone as well as TSAT were significantly decreased or elevated in patients treated with CERA compared with other types of ESAs. Levels of C-terminal FGF23 increased in all groups during the observation period. Levels of intact FGF23 and ratios of intact FGF23 to C-terminal FGF23 gradually decreased between Days 3 and 7 in the CERA but not in the other groups. Multivariate models associated changes in hepcidin 25 and phosphate with those of intact FGF23.

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**Conclusion.** The long-acting ESA CERA might influence levels of intact FGF23 by increasing FGF23 cleavage in patients on HD in association with prolonged hepcidin 25 suppression.

**Keywords:** erythroferrone, erythropoiesis, hepcidin 25, iron metabolism, phosphate

## INTRODUCTION

Fibroblast growth factor 23 (FGF23) is secreted by osteocytes and regulates serum phosphate levels and vitamin D homeostasis. Recent studies have shown that FGF23 metabolism is influenced by iron deficiency and inflammation [1, 2], which independently and/or synergistically increase FGF23 production and cleavage [2]. Accelerated FGF23 production and cleavage during the acute phase of inflammation and iron deficiency result in an increase in the amount of cleaved C-terminal FGF23 compared with bioactive intact FGF23. Increased furin activity due to an iron deficiency leads to increased FGF23 transcription and decreased polypeptide *N*-acetylgalactosaminyltransferase 3 activity that subsequently results in increased FGF23 degradation [3]. The production and cleavage of FGF23 are diminished and seem to gradually stabilize during the chronic inflammatory phase, whereas levels of intact FGF23 become relatively increased compared with those in the acute phase [2]. High FGF23 values, iron deficiency and inflammation are independently associated with increased mortality among patients with chronic kidney disease (CKD) [4–6]. Thus, adequate management of these factors would help to improve the survival of such patients.

Notably, high FGF23 values are associated with anemia in patients with CKD [7, 8] and high C-terminal FGF23 values might be associated with erythropoietin (EPO) levels in patients after kidney transplantation [9]. Thus, many factors that are associated with anemia and erythropoiesis might influence FGF23 production and metabolism, indicating that managing these conditions might decrease and stabilize FGF23 levels, potentially reducing the risk of mortality for patients with CKD [4, 10,11]. The effects of the specific compounds and preparations used to treat iron deficiency on FGF23 differ in patients on hemodialysis (HD); some forms of intravenous iron increase [12–14] whereas oral ferric citrate or sucroferric oxohydroxide can decrease [15–18] FGF23 values in such patients.

Experimental (murine models) and clinical studies have confirmed the impact of epoetin, a short-acting erythropoiesis stimulating agent (ESA), on FGF23 production [19, 20]. Injected epoetin dose-dependently stimulates serum FGF23 and the expression of bone FGF23 messenger RNA in mice and one intravenous bolus of epoetin increases serum FGF23 values in patients with anemia [19]. Thus interventions to decrease FGF23 levels might not only target iron, but also ESAs. However, iron levels are dramatically decreased during erythropoiesis induced by long-acting, compared with short-acting, ESAs [21, 22] and this might exert different effects on FGF23 metabolism.

Therefore the present study aimed to determine whether the effects of the long-acting ESA darbepoetin-$\alpha$ (DA) and continuous erythropoiesis receptor activator (CERA) on serum FGF23 values differ among patients on HD.

## MATERIALS AND METHODS

### Patients

This study included 108 patients on maintenance HD at four outpatient dialysis clinics in Japan (Suiyukai Clinic, Shibagaki Dialysis Clinic Jiyugaoka, Shibagaki Dialysis Clinic Togoshi and Ebara Clinic). The exclusion criteria comprised malignant, chronic inflammatory or severe liver or lung disease and under medication with anti-inflammatory or immunosuppressive agents. Ninety-nine patients were treated with an ESA as follows: 44, 31 and 24 received biweekly CERA, weekly DA and three doses of epoetin-β per week, respectively. The Showa University Institutional Committee on Human Research approved the protocol of the study, which proceeded according to the Declaration of Helsinki (2008 revision). Details of the inclusion criteria are provided elsewhere [21, 22]. All patients provided written informed consent to participate in this study.

### Administration of ESAs

The included patients were treated with intravenous DA (Nesp; Kyowa Hakko Kirin, Tokyo, Japan) once per week, the CERA Mircera (Chugai Pharmaceutical, Tokyo, Japan) once every 2 or 4 weeks or epoetin-β (Eposin; Chugai Pharmaceutical) three times per week. The ESA dose was administered according to the Guidelines for Renal Anemia published by the Japanese Society for Dialysis Therapy in 2008 and 2015 [23, 24]. During the 14-day observation period, DA was injected at baseline (Day 0) and on Day 7 and the CERA was injected at baseline (Day 0). Epoetin was administered once to three times per week according to the treatment plan for each patient; that is, once at baseline (Day 0), twice (Days 0 and 5) and three times (Days 0, 3 and 5).

### Iron supplementation

Intravenous iron in the form of 40-mg doses of saccharated ferric oxide (Fesin; Nichiiko Pharmaceutical, Toyoma City, Japan) was administered at least 2 weeks before the study commenced. Intravenous iron was administered once weekly at baseline (Day 0) and on Day 7 if hemoglobin levels decreased to $<$10 g/dL and serum transferrin saturation (TSAT) and ferritin values reached $<$20% and $<$100 ng/mL, respectively [23, 24]. Phosphate binders of ferric citrate hydrate containing iron (Riona; Torii Pharmaceutical, Tokyo, Japan) or sucroferric oxyhydroxide (Petol; Kissei Pharmaceutical, Matsumoto, Japan) were administered daily for 14 days and the doses were not changed during the study period.

### Blood sampling

Venous blood was sampled from all groups before the HD session at the end of the dialysis week (baseline, Day 0) and on Days 3, 5, 7 and also on Day 14 for the group that received CERA.

### Measured parameters

Routine biochemical parameters and levels of albumin, calcium, phosphate, intact parathyroid hormone, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, high-sensitivity C-reactive protein, biomarkers of iron metabolism (serum iron, total iron-binding capacity, ferritin, hepcidin 25 and erythroferrone) and intact and C-terminal FGF23 were measured in venous blood samples

obtained at baseline. Phosphate, biomarkers of iron metabolism, intact FGF23 and C-terminal FGF23 were measured in venous blood samples at various time points thereafter. Serum samples were immediately frozen and stored at −80°C. Hepcidin 25 was measured using liquid chromatography–tandem mass spectrometry [25], erythroferrone was analyzed using an enzyme-linked immunosorbent assay (ELISA) with paired rabbit antihuman erythroferrone monoclonal antibodies [26, 27], intact FGF23 was assessed using ELISA kits (Kainos Laboratories, Tokyo, Japan) and C-terminal FGF23 was assessed with ELISA kits (Immutopics, San Clemente, CA, USA).

### Statistical analysis

Data are presented as mean (standard deviation) or as medians (range) unless otherwise stated, with statistical significance set at $P < 0.05$. Normally distributed variables were compared among three groups using analysis of variance and nonnormally distributed variables were analyzed using Wilcoxon rank-sum tests. Nominal variables were compared among three groups using chi-squared tests. Changes in parameters between Days 0 and 3, 5 or 7 were compared using paired t-tests. Associations among changes in intact FGF23, C-terminal FGF23 or the ratio of intact FGF23 to C-terminal FGF23 with independent variables were assessed using multivariate analyses. Data were statistically analyzed using JMP Pro 14.0 (SAS Institute, Cary, NC, USA) and Prism 8.1 (GraphPad Software, San Diego, CA, USA).

## RESULTS

The mean epoetin-β doses at each HD session on Days 0, 3 and 5 were 2093 ± 732, 1096 ± 1092 and 1093 ± 1148 units, respectively. The mean doses of DA and CERA on Day 0 were 22 ± 10 and 49 ± 30 μg, respectively (Table 1). Biomarker assessment in 108 patients (Supplementary data, Table S1) showed that intact FGF23 closely correlated with C-terminal FGF23, phosphate correlated with intact and C-terminal FGF23 and hepcidin 25 correlated with TSAT and ferritin.

### Baseline characteristics according to ESA therapy

Table 1 shows the characteristics of the patients. Mean age, sex and dialysis vintage did not differ among the control and ESA groups.

Fewer patients in the DA group received a phosphate binder containing iron compared with the others, whereas ~20% of patients in each ESA group received intravenous iron therapy. Baseline hemoglobin, erythroferrone and C-terminal FGF23 values were lower in the groups treated with than without ESA. Ferritin levels were higher in the epoetin-β group than in the other groups, whereas TSAT and hepcidin 25 values did not differ among the four groups.

### Changes in parameters of iron metabolism and phosphate, FGF23 after ESA treatment

TSAT as well as ferritin and hepcidin 25 levels were significantly decreased and erythroferrone levels were significantly elevated in the CERAgroup compared with the other groups (Figure 1).

Levels of phosphate were increased at Day 3 in the group without ESA and the epoetin-β group but decreased in the CERA group on Day 5 (Figure 2). C-terminal FGF23 increased in all groups during the observation period, whereas intact FGF23 decreased between Days 3 and 7 in the CERA group (Figure 2). The ratio of intact to C-terminal FGF23 decreased in the CERA group between Days 3 and 7, then returned to baseline on Day 14 (Figure 2).

To separate the influence of changes in serum phosphate on FGF23 metabolism, changes in FGF23 were assessed according to decreasing or increasing levels of phosphate (Supplementary data, Figures S1 and S2). Values for intact FGF23 decreased along with those of phosphate in the three groups treated with ESA (Supplementary data, Figure S1) and similarly increased with those of phosphate in the epoetin-β and DA groups (Supplementary data, Figure S2d and e). However, these values did not become elevated in the CERA group during the study period (Supplementary data, Figure S2f).

### Associations between changes in FGF23 and biomarkers that influence such changes

Because CERA significantly altered biomarkers of iron metabolism, we assessed associations between changes in intact FGF23 and biomarkers of iron metabolism using multivariate models. Changes in intact FGF23 were significantly influenced according to phosphate levels (Table 2) and those in erythroferrone were significantly associated with levels of intact FGF23 (Table 2, Model 3). However, these associations disappeared when the model included changes in hepcidin 25 that were independently associated with those of intact FGF23 (Table 2, Model 4). Multivariate analyses did not identify associations between C-terminal FGF23 or the ratio of intact to C-terminal FGF23 and biomarkers of iron metabolism (Supplementary data, Table S2).

## DISCUSSION

This observational study investigated changes in FGF23 and in biomarkers of iron metabolism associated with ESA treatment and found that CERA decreased intact FGF23 levels more effectively than epoetin and DA. Furthermore, decreases in FGF23 induced by CERA were independent of phosphate metabolism.

A previous study of the effects of the short-acting ESA epoetin-β on serum FGF23 levels confirmed that one dose of epoetin-β increases levels of both intact and C-terminal FGF23 within 12–18 h in patients with anemia [19]. The present study associated epoetin with increased levels of both intact and C-terminal FGF23 after 3 days. Our findings differed from those of the previous study [19] because of different blood sampling times. The half-life of epoetin-β is about 6 h [28], therefore epoetin-β became ineffective over time and became similar to those in the group without ESA treatment.

On the other hand, the long-acting ESA CERA was associated with decreased and increased levels of intact and C-terminal FGF23, respectively, and the ratio of intact to C-terminal FGF23 decreased according to the half-life of CERA (~6 days [28]). The long-acting ESA DA (half-life when administered intravenously, 25 h [28]) did not affect intact FGF23 values for 7 days. The discrepancy between short- and long-acting ESAs might depend on the duration of continuous stimulation by exogenous EPO. Continuous EPO stimulation might increase FGF23 degradation, thus intact FGF23 values decreased according to an increase in FGF23 cleavage that yielded C-terminal FGF23.

The present study found that CERA profoundly affected iron metabolism; TSAT, ferritin and hepcidin 25 were decreased and erythroferrone was increased after 7 days of CERA compared with epoetin-β and DA. Multivariate models did not associate changes in intact FGF23 with those of TSAT and ferritin, but

**Table 1. Patient characteristics**

| Characteristics | Non-ESA treatment ($n = 9$) | Epoetin-β ($n = 24$) | Darbepoetin-α ($n = 31$) | CERA ($n = 44$) | P-value |
|---|---|---|---|---|---|
| Age (years) | 64 ± 13 | 67 ± 11 | 67 ± 10 | 69 ± 9 | 0.63 |
| Sex (male), % | 70 | 38 | 58 | 54 | 0.28 |
| Diabetes mellitus, % | 30 | 38 | 19 | 48 | 0.08 |
| Body mass index (kg/m$^2$) | 22.8 ± 2.7 | 21.7 ± 5.1 | 20.7 ± 3.9 | 23.0 ± 4.5 | 0.08 |
| History of CVD (yes), % | 20 | 29 | 32 | 34 | 0.83 |
| Hemodialysis vintage (months) | 185 (32–414) | 140 (22–411) | 122 (17–356) | 82 (12–435) | 0.26 |
| $K_t/V$ | 1.2 ± 0.2 | 1.3 ± 0.3 | 1.4 ± 0.3 | 1.3 ± 0.2 | 0.21 |
| Active vitamin $D_3$, % | 89 | 95 | 65 | 76 | 0.07 |
| Calcimimetics, % | 67 | 43 | 41 | 39 | 0.63 |
| Phosphate binder, % | 89 | 78 | 100 | 91 | – |
| Calcium carbonate, % | 78 | 43 | 59 | 53 | 0.50 |
| Polymers, % | 44 | 10 | 32 | 27 | 0.17 |
| Lanthanum carbonate hydrate, % | 56 | 33 | 54 | 27 | 0.14 |
| Iron-containing phosphate binder, % | 56 | 42 | 6 | 20 | 0.007 |
| I.V. ferrotherapy, % | 0 | 17 | 23 | 16 | 0.22 |
| Amount of iron administration for 3 months prior to baseline | | | | | |
| I.V. iron, *n* (mg) | 0 | 8, 390.0 ± 136.0 | 8, 328 ± 132 | 10, 342.9 ± 186.0 | 0.58 |
| Ferric citrate hydrate, *n* (g) | – | 2, 102.4 ± 48.3 | 1, 136.5 ± 0 | 4, 75.8 ± 57.3 | 0.65 |
| Sucroferric oxyhydroxide, *n* (g) | 5, 97.6 ± 47.1 | 8, 125.2 ± 49.5 | 1, 23.3 ± 0 | 5, 68.5 ± 19.8 | 0.11 |
| Doses of ESA at baseline | – | 2093 ± 732, 1096 ± 1092, 1093 ± 1148[a] units | 22 ± 10 μg | 49 ± 30 μg | – |
| Mean doses of ESA for 3 months before baseline | – | 4319 ± 2483[b] units | 20 ± 11[c] μg | 47 ± 32[d] μg | – |
| Hemoglobin (g/dL) | 12.9 ± 1.0 | 10.9 ± 0.9 | 11.1 ± 1.0 | 10.9 ± 1.0 | <0.0001 |
| Albumin (g/dL) | 3.7 ± 0.3 | 3.7 ± 0.3 | 3.8 ± 0.3 | 3.8 ± 0.2 | 0.34 |
| Creatinine (mg/dL) | 12.8 ± 2.8 | 9.5 ± 2.6 | 11.7 ± 2.7 | 10.4 ± 2.3 | 0.0009 |
| Calcium (mg/dL)[e] | 9.7 ± 0.7 | 9.0 ± 0.7 | 9.0 ± 0.5 | 9.1 ± 0.6 | 0.02 |
| Phosphate (mg/dL) | 5.6 ± 1.5 | 5.2 ± 1.5 | 5.5 ± 1.2 | 5.1 ± 11 | 0.44 |
| Intact-PTH (pg/mL), median (range) | 152 (21–435) | 131 (30–745) | 253 (49–533) | 145 (55–876) | 0.003 |
| 25-hydroxyvitamin D (ng/mL) | 14.4 ± 5.9 | 12.6 ± 4.4 | 16.3 ± 5.3 | 15.5 ± 4.4 | 0.03 |
| 1,25-dihydroxyvitamin D (pg/mL) | 13.6 ± 5.5 | 15.4 ± 8.9 | 14.2 ± 8.8 | 13.1 ± 6.7 | 0.82 |
| TSAT, % | 26.8 ± 12.2 | 26.0 ± 8.5 | 23.8 ± 8.3 | 29.1 ± 10.9 | 0.20 |
| Ferritin (ng/mL), median (range) | 70.5 (10–244) | 177.0 (11.9–411) | 34.5 (5.9–394) | 76.5 (6.5–486) | 0.02 |
| Hepcidin 25 (ng/mL), median (range) | 24.4 (0.4–69.0) | 41.8 (4.0–125.7) | 19.2 (0.7–143.7) | 37.3 (0.2–226.6) | 0.08 |
| Erythroferrone (ng/mL), median (range) | 26.5 (10.75–451.1) | 12.1 (1.49–52.73) | 7.6 (0.41–49.39) | 6.73 (0.1–112.3) | <0.0001 |
| Intact FGF23 (pg/mL), median (range) | 9357.5 (618.5–66 869) | 3397.3 (410.6–20 430) | 3765.6 (195.5–19378) | 1884.4 (84.3–17714) | 0.12 |
| C-terminal FGF23 (RU/mL), median (range) | 5435.2 (635.7–6517.9) | 1919.3 (454.3–6955.1) | 1421.3 (157.4–5743.2) | 1076.0 (44.0–6288.7) | 0.03 |
| High-sensitivity C-reactive protein (mg/dL), median (range) | 0.13 (0.025–0.62) | 0.13 (0.007–1.01) | 0.08 (0.005–1.76) | 0.09 (0.01–2.4) | 0.60 |

Values are expressed as mean (standard deviation) unless stated otherwise. P-values were calculated for differences in variables according to ESA and non-ESA treatment. Polymers include sevelamer hydrochloride and bixalomer; iron-containing phosphate binders includes ferric citrate hydrate and sucroferric oxyhydroxide.
[a]Doses of epoetin-β at baseline are expressed as mean epoetin dose at each HD session at Days 0, 3 and 5, mean total dose (units/week).
[b]Mean weekly dose (units).
[c]Mean weekly dose (μg).
[d]Mean dose per 2 or 4 weeks (μg).
[e]Adjusted for albumin.
CVD, cardiovascular disease; I.V., intravenous.

decreases in hepcidin 25 were independently associated with those of intact FGF23. A low-iron diet has been associated with increased FGF23 production and FGF cleavage in model mice with CKD and hepcidin knockdown and with increased FGF23 production and diminished FGF23 cleavage in model mice with CKD and normal hepcidin [29]. Thus a greater decrease in hepcidin might be associated with increased FGF23 cleavage and subsequently increased C-terminal FGF23 and decreased intact FGF23 in patients with HD.

Although DA significantly decreased hepcidin 25 at Days 3 and 5 in the present study, the amount of the decreases were less than those in the previous study, even though the doses of DA were similar between them [22]. The discrepancy might be associated with baseline levels of hepcidin 25 and stored iron, because the baseline levels of hepcidin 25 and ferritin were obviously lower in the present than the previous study. Low iron status might have also affected FGF23 changes in the present study because low iron levels result in FGF23 metabolism, as

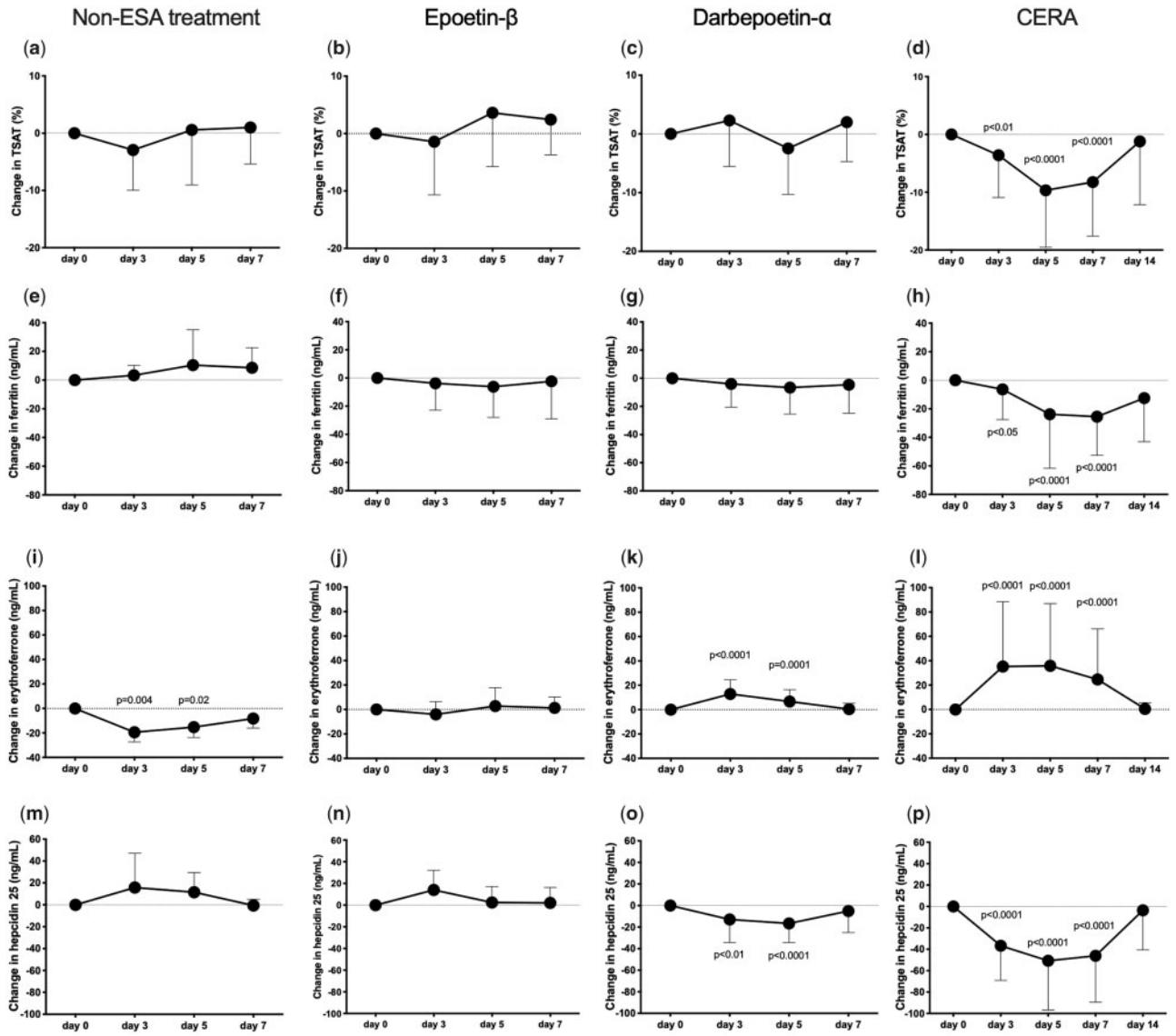


**FIGURE 1:** Changes in blood parameters with or without ESA.

Values for TSAT (**a**–**d**), ferritin (**e**–**h**), erythroferrone (**i**–**l**) and hepcidin 25 (**m**–**p**). No iron treatment (**a**, **e**, **i** and **m**), epoetin-β (**b**, **f**, **j** and **m**), DA (**c**, **g**, **k** and **o**) and CERA (**d**, **h**, **l** and **p**). Data are shown as mean (standard deviation).

noted above. Moreover, the rate at which phosphate binders containing iron were used was lower in DA than in other ESA groups in the present study (Table 1). The low rate of medicines containing iron in the present study might have influenced baseline ferritin values as well as changes in the biomarkers of iron and FGF23 after DA administration. With regard to these issues, intact FGF23 might decrease according to the half-life of DA.

All groups in the present study received intravenous iron as saccharated ferric oxide and oral phosphate binders containing iron. This intravenous iron form might increase [12–14], whereas oral phosphate binders can decrease [15–18] FGF23 levels. Thus iron therapy might have influenced our findings regarding FGF23.

The present findings should be interpreted with the following caveats. The number of patients was relatively small and this nonrandomized observational investigation could not determine whether the long-acting ESA actually caused changes FGF23 metabolism. An interventional study is warranted to conclusively determine this relationship.

In conclusion, long-acting ESAs might influence levels of intact FGF23 in patients on HD independent of phosphate and dependent on hepcidin 25 suppression.

## SUPPLEMENTARY DATA

Supplementary data are available at ckj online.

## CONFLICT OF INTEREST STATEMENT

None of the authors received any funding for this study. H.H. has received research funds from Torii Pharmaceutical, Chugai Pharmaceutical and Kyowa Hakko Kirin, as well as lecture fees from Kissei Pharmaceutical, Torii Pharmaceutical, Chugai Pharmaceutical and Kyowa Hakko Kirin. N.T. is the president of

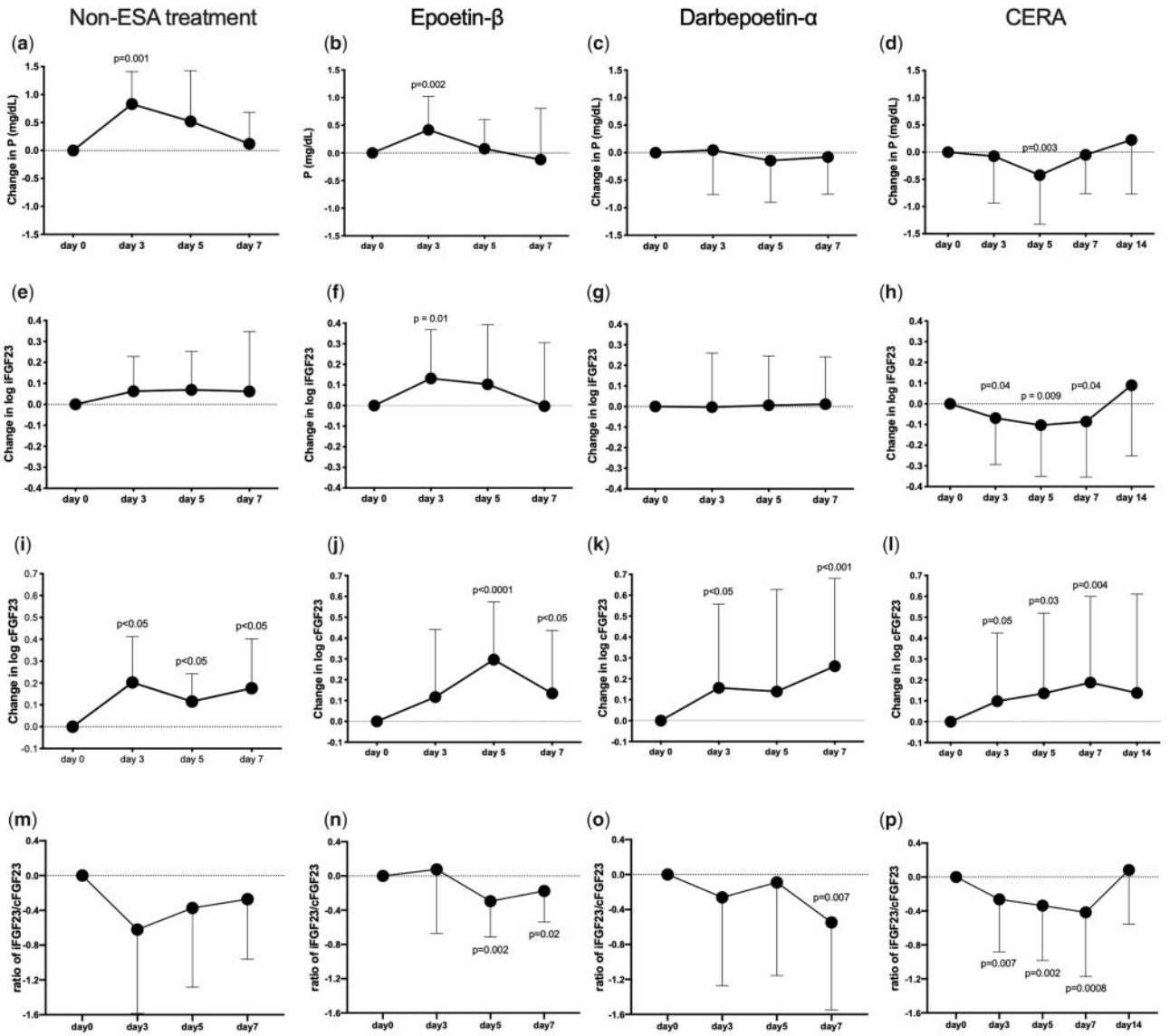


**FIGURE 2:** Changes in parameters associated with FGF23 with or without ESA.

Changes in levels of phosphate (**a**–**d**), log iFGF23 (**e**–**h**), log cFGF23 (**i**–**l**) and (iFGF23: cFGF23; **m**–**p**), among noniron treatment (**a**, **e**, **i** and **m**), epoetin-β (**b**, **f**, **j** and **m**), DA (**c**, **g**, **k** and **o**) and CERA (**d**, **h**, **l** and **p**) groups. Data are shown as mean (standard deviation). iFGF23:cFGF23, ratio of intact FGF23/C-terminal FGF23; log cFGF23, log-transformed C-terminal FGF23; log iFGF23, log-transformed intact FGF23.

**Table 2. Associations between changes in intact FGF23 and biomarkers that influence such changes**

| Changes in biomarkers | Model 1; P-value | Model 2; P-value | Model 3; P-value | Model 4; P-value |
|---|---|---|---|---|
| Phosphate (mg/dL) | 0.14, 0.03; <0.0001 | 0.14, 0.03; <0.0001 | 0.13, 0.03; <0.0001 | 0.12, 0.03; <0.0001 |
| TSAT (%) | 0.0009, 0.004; 0.71 | – | 0.002, 0.002; 0.56 | 0.0006, 0.003; 0.83 |
| Ferritin (ng/mL) | – | 0.002, 0.001; 0.12 | 0.002, 0.001; 0.22 | 0.001, 0.001; 0.32 |
| Erythroferrone (ng/mL) | – | – | −0.001, 0.0005; 0.04 | −0.0003, 0.0006; 0.63 |
| Hepcidin 25 (ng/mL) | – | – | – | 0.002, 0.0008; 0.03 |

Changes in dependent and independent variables between Days 0 and 3. Age and gender are included as independent variables in all multivariate models.

Medical Care Proteomics Biotechnology, which does not affect the adherence of the authors to all policies regarding data sharing, and has received lecture fees from Torii Pharmaceutical. T.G. is a scientific founder and consultant for Intrinsic LifeSciences (La Jolla, CA, USA) and Silarus Pharma (La Jolla, CA, USA) and a consultant for Keryx Pharma (New York, NY, USA), Akebia Pharma (Cambridge, MA, USA), La Jolla Pharmaceutical Company (San Diego, CA, USA) and Gilead Sciences (San Dimas, CA, USA). None of the other authors have any conflicts of interest to disclose.

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