IRON SUPPLEMENTATION DOES NOT WORSEN RESPIRATORY HEALTH IN CYSTIC FIBROSIS

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Background

An estimated 10-29% of adults with cystic fibrosis (CF) are anemic, and 23-100% of these patients also have low blood iron concentrations (hypoferremia) (1-3). Biomarkers of iron homeostasis are affected by inflammation in CF (4), which could lead to inappropriate treatment of hypoferremic anemia with supplemental iron. *In-vitro*, CFTR dysfunction increases the iron content of airway surface liquid and augments biofilm growth of *Pseudomonas aeruginosa* (5). These observations suggest that taking an iron supplement could potentiate lung infection by increasing sputum iron. We questioned whether ferrous sulfate: 1) increased serum iron, transferrin saturation (TSAT), and hemoglobin, 2) increased sputum iron, 3) altered the CF lung microbiome, and 4) was associated with onset of CF pulmonary exacerbation (CFPE).

1) Pond MN et al. *Respir Med* 1996; 90: 409-13.
2) von Drygalski A, Biller J. *Nutr Clin Pract* 2008; 23: 557-63.
3) Gifford AH et al. *Pediatr Pulmonol* 2011; 46: 160-5.
4) Gifford AH et al. *Clin Trans Sci* 2012; 5: 368-73.
5) Moreau-Marquis S et al. *Am J Physiol Lung Cell Mol Physiol* 2008; 295: L25-37.

Study Design

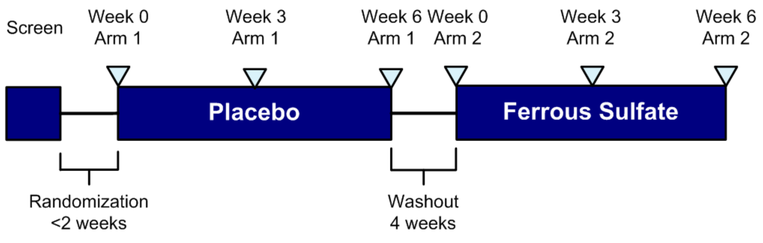

Figure 1. This was a randomized, double-blind, placebo-controlled, crossover trial of ferrous sulfate 325 mg taken by mouth daily for 6 weeks in adults with CF. At screening, subjects were required to have hemoglobin <15.5 gm/dl (♂) or <13.6 gm/dl (♀) and TSAT ≤21% (all subjects). Hemoglobin cutoffs are below gender-specific means for 20-29 year old Caucasians in NHANES III. TSAT ≤21% is below the mean for 20-39 year old Caucasian women in NHANES III.

Methods

- CBC, serum iron, TSAT, reticulocyte count: autoanalyzer
- Serum hepcidin-25 and erythropoietin (EPO): ELISA
- Sputum iron: ICP-MS
- CFPE: Akron Pulmonary Exacerbation Score (PES) ≥5
- Sputum microbiome: 454 pyrosequencing of 16S rRNA
- Sputum bacterial diversity: Simpson Diversity Index (SDI)
- Fixed-effect models describing changes from baseline

Baseline Characteristics

Number of patients (*N*)	22
Age (years)	32.1 (13.6)
Gender (M/F)	14/8
dF508 homozygote (%)	77
CF-related diabetes (%)	68
Body weight (kg)	63.9 (11.9)
FEV_1 (% predicted)	56 (21)
Hemoglobin (gm/dl)*	13.6 (0.9) (♂), 12.6 (0.7) (♀)
TSAT (%)*	13 (5) (♂), 10 (4) (♀)
Serum hepcidin-25 (ng/ml)**	48.6 (41.9)
Sputum iron (ng/mg sample)†	1.44 (1.0)

Table 1. Data are presented as mean and (SD); * = measured at screening; ** = measured at randomization; † = samples collected from 21 of 22 subjects

Effect on Serum Iron & TSAT

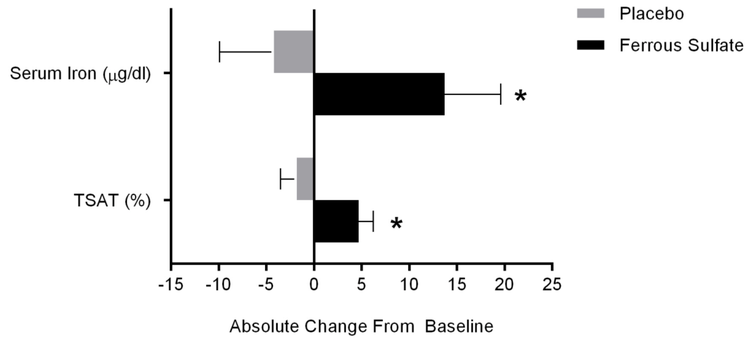

Figure 2. Bars denote mean differences, and whiskers signify SD. After 6 weeks of ferrous sulfate, serum iron improved by 13.7 (5.9) μg/dl, and TSAT improved by 4.7 (1.5) %. Hemoglobin was not significantly affected by treatment (data not shown). * = p <0.05 compared to placebo.

Model for Sputum Iron

Parameter	Estimate	S.E.	*t*-value	*p*-value
Ferrous sulfate	-0.281	0.196	-1.43	0.16
Serum hepcidin-25	0.011	0.004	2.51	0.02
Serum erythropoietin	0.052	0.020	2.66	0.01

Table 2. Ferrous sulfate use did not significantly predict sputum iron levels. Each ng/ml increase in serum hepcidin-25 was associated with a 1.1% increase in sputum iron. Each mU/ml increase in serum erythropoietin was associated with a 5.2% increase in sputum iron.

Effect on Sputum Microbiome

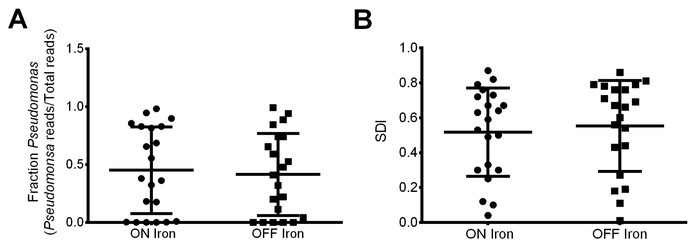

Figure 3. The CF sputum microbiome is unaltered by iron supplementation. Panel A shows that relative abundance of *Pseudomonas aeruginosa* in sputum, as calculated by deep sequencing, does not change within-subjects at week 6 of each arm. Panel B shows that overall bacterial diversity expressed as SDI is also unaffected by ferrous sulfate. Horizontal lines denote mean values, and the error bars denote SD. Paired Student's *t*-tests were used to make the comparisons.

Model for Akron PES

Parameter	Estimate	S.E.	*t*-value	*p*-value
Ferrous sulfate	-0.738	0.684	-1.08	0.29
Serum hepcidin-25	0.042	0.014	2.98	0.006
Antibiotic use	1.585	0.731	2.17	0.04
Body weight	-0.424	0.206	-2.06	0.047

Table 3. Ferrous sulfate use was unrelated to PES. Adjusting for baseline levels, each ng/ml increase in serum hepcidin-25 was associated with a PES increase of 0.04 points. Gaining 1 kg of body weight was associated with a reduction in PES of 0.4 points. Antibiotic use predicted a 1.6 point increase in PES from baseline.

Conclusions

- Ferrous sulfate improved iron status but did not increase hemoglobin in CF patients with hypoferremic anemia.
- Iron supplementation did not influence sputum iron content, the sputum microbiome, and exacerbation status.
- Serum hepcidin-25 is a biomarker associated with sputum iron variation and incremental changes in Akron PES.

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