Hemoglobin regeneration efficiency and relative bioavailability of an electrolytic elemental iron powder to anemic rats.[1-3]

J. Swain[1, 2] and lab group[1] (D. Kim, C. Wong, and L. Shivers)

[1]Case Western Reserve University, Department of Nutrition, School of Medicine, 10900 Euclid Avenue, Cleveland, OH 44106.

[2]Correspondence to: James Swain, Case Western Reserve University, Department of Nutrition, School of Medicine, 10900 Euclid Avenue, Woods Bldg. WG48, Cleveland, OH 44106, Tel: 216-368-8554, Fax: 216-368-6644.

[3]Funding for this study was provided in part and provision of electrolytic iron powder by Industrial Metal Powders (IMP; India) Pvt. Ltd., 699/1, Koregaon Bhima, Behind Kalyani Forge, Nagar Road, Tal. Shirur, Dist. Pune - 412 216, Maharashtra, India.

Running Head: Bioavailability of electrolytic elemental iron powder.

ABSTRACT

Foods are fortified with various iron fortificants to reduce iron deficiency. Determining the nutritional efficacy of commercially produced products is an important endeavor. In this study, we determined the hemoglobin regeneration efficiency (HRE) ratio and relative bioavailability (RBV) of a electrolytic elemental iron powder (EIP; Industrial Metal Powders (IMP), India), treating iron deficient (anemic; hemoglobin 3-6 g/dL) rats with 14-d repletion diets fortified with graded (increasing) quantities (12, 24, 36, or 48 mg iron/kg diet) of EIP or bakery-grade ferrous sulfate (FS). A control diet (no added iron) was also used. The HRE ratio of diets containing EIP powder at 12, 24, 36, or 48 mg iron/kg diet were 0.35, 0.20, 0.19, and 0.16, respectively. The

RBV (%) of iron from EIP- relative to FS - at 12, 24, 36, or 48 mg iron/kg diet were 64.5, 59.1, 50.6, and 54.3, respectively. Food intake and thus, iron intake, weight gain, and hemoglobin generation were positively associated with dietary iron in both EIP and FS treatment groups.HRE ratio and thus, RBV calculations accounted for these factors. For EIP, the HRE ratio and RBV of iron from the 12 mg iron/kg diet were higher ($p<0.05$) as compared to the other diets containing greater quantities of EIP. The HRE ratio values for EIP, for the 24, 36, and 48 mg iron/kg diets, are similar - within the range - to other HRE ratio data values for electrolytic iron powders as noted previously in the literature. For treatments using FS, HRE ratio data varied among the diet treatments and was generally inversely associated with dietary iron content; though the overall range of HRE ratio values found for FS in this study, particularly of the 12 and 24 mg iron/kg diets, is similar to that found in the literature. Findings show that this electrolytic iron powder (Industrial Metal Powders, India) at each of the concentrations tested is an effective agent to replenish hemoglobin and correct anemia.

KEY WORDS: Electrolytic iron, iron absorption, food fortification, hemoglobin regeneration efficiency, relative bioavailability

INTRODUCTION

Iron deficiency anemia (IDA) is the most common nutrient deficiency in the world (1). IDA costs billions of dollars per year in medical care, lost productivity, and poor health (1, 2). To combat IDA, iron fortification of staple foods is used (3). Such fortification is considered to be a cost-effective, long-term strategy for reducing IDA. Iron powders - especially elemental forms of iron - are often used because they do not cause unacceptable sensory changes in the fortified food during storage and are relatively low cost (3). Elemental iron powders now account for most of the iron used for food fortification (3,4). Thus, understanding the bioavailability of these iron powders is very important to human health and to make specific qualitative and quantitative recommendations for their use in foods, as past studies indicate (5-7).

Electrolytic iron powders are often used to fortify foods with iron. Formation of these iron powders usually involves dissociation of sponge iron fines inside a porous diaphragm, and subsequent contact with a cathode of stainless steel onto which the EIP gets deposited during the electrolysis process. Thereafter, the iron is collected and processed based on sieve size requirements. The “flake-like” deposition of successive layers of iron bestows qualities upon the particle which favourably allow for gastric acid penetration and subsequent dissolution, which enhances bioavailability of this type of iron powder.

The objective of the present study was to determine the hemoglobin regeneration efficiency and bioavailability of a electrolytic elemental iron powder (EIP; Industrial Metal Powders (IMP), India),relative to bakery-grade ferrous sulfate (FS), assessed by determiningiron intakeand change in hemoglobin concentration in anemic rats as they consumed graded (increased) quantities of EIP and FS during a 14-d hemoglobin (Hb) repletion period diet.

METHODS

Iron powders. This study used the following two iron powders: 1) electrolytic elemental iron powder (EIP = electrolytic iron powder +325 mesh, Batch #05166 (99.6% iron);Industrial Metal Powders (IMP), India) and 2) a bakery-grade ferrous sulfate ($FeSO_4 \cdot H_2O$ (monohydrate); Crown Technologies, Inc., USA). Once received, EIP and FS were stored in a desiccatoruntil use.

Hemoglobin regeneration efficiency (HRE) and relative bioavailability (RBV) Determinations in anemic rats. The HRE and RBV of EIP were determined using an initial 220 weanling male Sprague-Dawley rats (Charles River/SASCO, Wilmington, MA), which were taken through a depletion, assessed for iron deficiency anemia, then anemic rats assigned to repletion period diets. Rats were housed individually in wire-bottom stainless steel mesh cages and given a 12-h light: dark cycle. After 24-d depletion with an iron deficient diet (1.6 mg iron/kg AIN-93G diet; 1.4 mg iron/kg diet - analyzed iron content), 162 rats - with hemoglobin values between 3 and 6 g/dL (mean ± SD of 4.1 ± 0.4 g/dL; range 3.1 to 5.9 g/dL) - were randomized into one of 9 different repletion period diet groups - rats consumed a repletion diet (modified AIN-93G diet) for 14 d, fortified with either EIP or FS (each at 12, 24, 36, or 48 mg iron/kg diet), or no added iron (analyzed 1.4 mg iron/kg diet); n=18/diet group at beginning of repletion period (n=15-18/diet group at end of repletion period). Mortality rates of rats among the diet treatment groups did not differ. For relative bioavailability determinations, FS was selected as the reference standard because of its known and higher bioavailability and the prevalence of data in the literature (5-6). HRE and RBV were calculated based on the following formulas:

1. HRE ratio = [Final Hb Fe (mg) - Initial Hb Fe (mg)]/Fe intake (mg total iron/14 d)
2. RBV = Percentage (%) HRE relative to ferrous sulfate monohydrate. (Diet matched.)

During the repletion period, daily and total food consumption (intake) measurements included adjustments for spilled food.All diets and deionized, distilled water were fed *ad libitum*.All experimental procedures conformed to U.S. National Institutes of Health, Public Health Service and Animal Welfare Act guidelines for the ethical treatment of laboratory animals and were approved by the Case Western Reserve University Institutional Animal Care and Use Committee.

Phlebotomy, hemoglobin, and hemoglobin iron determinations. At the beginning of the repletion period, rats were anesthetized with ethyl ether and blood was collected from the tail vein. Following repletion, the rats were anesthetized with ethyl ether, tail vein blood collected, then rats were killed by injection with ketamine/xylazineand subsequent exsanguinations. At both times, a modified met hemoglobin method was used for the colorimetric determination of hemoglobin (CELL-DYN® 4000 System; Abbott Diagnostics Division, Abbott Park, IL). The following calculation was used to determine hemoglobin (Hb) iron:

Hb Fe (mg) = BW X 0.067 X Grams Hb per mL X 3.35 mg Fe

Note: the calculation assumes 6.7% body weight (BW) is blood and the iron content of hemoglobin is 3.35 mg/g (8,9).

Dietary treatments.EIP orFS were incorporated into an AIN-93G (9) diet modified to have a very low base iron content, as conducted previously (5),by using vitamin-free casein(Harlan Teklad, Madison, WI), a reagent grade calcium carbonate (J.T. Baker, Phillipsburg, NJ), and a high-purity cellulose fiber source, Alphacel™ (ICN Biomedicals, Irvine, CA). Without added iron, the diet contained 1.4 mg iron/kg diet by analysis and contained (g/kg): 397.5 corn starch, 200 vitamin-free casein, 132 maltodextran, 100 sucrose, 70 soybean oil, 50 cellulose (Alphacel™), 35 iron-free mineral mix (AIN-93G mineral mix omitting ferric citrate, including reagent grade Calcium carbonate), 10 vitamin mix (AIN-93VX; Harlan Teklad, Madison, WI), 3 L-cystine, 2.5 choline bitartrate, and 0.014 TBHQ (an antioxidant) - **Table 1.**

Special care was taken when mixing the EIP and FS into the experimental diets to ensure that the iron powders were not subjected to excessive abrasive and/or frictional force to preserve the powder particle structure and the physicochemical characteristics of each powder per their original commercial forms. Before addition to the rest of the diet, each iron powder was combined with a 200 g portion of sucrose and mixed for 15 min using a non-chopping mixer (Model KSMC50 commercial mixer; Kitchen Aid, Inc., St. Joseph, MI) with an open-blade, plastic-covered stainless steel paddle (5). The sucrose/iron powder mixture was then added to the other dry diet ingredients and mixed (D330 mixer; Hobart Corp., Troy, OH) for 30 min. Oil was then added and the diet was mixed for an additional 30 min.

To determine iron content, each diet (n=4) was ashed by alternating dry heat (500°C) with reflux in concentrated nitric acid (J.T. Baker, Phillipsburg, NJ), then dissolving in 6 mol/L hydrochloric acid (J.T. Baker, Phillipsburg, NJ). Samples were analyzed by atomic absorption spectrophotometry (Optima 3100 XL; Perkin Elmer, Norwalk, CT).

Repletion period diets (base using modified AIN-93G low iron diet) were fortified with either EIP or FS (each at 12, 24, 36, or 48 mg iron/kg diet), or no added iron (calculated 1.6 (analyzed 1.4) mg iron/kg diet were then prepared; termed EIP and FS 1-4. **Table 2** shows calculated and analyzed iron content of each diet.

Statistical Analysis.HRE ratio and RBV were calculated as previously described(5, 8-10). Statistical analysis was performed using analysis of variance and the least significant difference (LSD) test was applied when treatment F was significant; expressed as $p < 0.05$. Results are expressed as mean values per animals within a diet treatment group.

SPECIFIC RESULTS

Food intake, iron intake, and growth. Food intake (and thus, iron intake), weight gain, and hemoglobin accumulation was positively associated with dietary iron in both EIP and FS treatment groups; generally there was a positive trend with increased food/iron intake - **Table 2.**

HRE and RBV.The HRE ratio of diets containing EIP powder at 12, 24, 36, or 48 mg iron/kg diet were 0.35, 0.20, 0.19, and 0.16, respectively. The RBV (%) of iron from EIP - relative to FS - at 12, 24, 36, or 48 mg iron/kg diet were 64.5, 59.1, 50.6, and 54.3, respectively. See**Table 3.**

FINDINGS & CONCLUSIONS

Findings demonstrate that this electrolytic iron powder was an effective agent to replenish hemoglobin. Data from this study also uniquely illustrate the HRE ratios and RBVs of this EIPwhen it is included at different concentrations in the diet.Food intake and thus, iron intake, weight gain, and hemoglobin generation were positively associated with dietary iron in both EIP and FS treatment groups. HRE ratio and thus, RBV calculations accounted for these factors.

Previously, rats have been used as a model for the assessment of iron absorption and to determine RBV and there exists a specific AOAC method for such studies (11). Forbes et. al explored use of different techniques to assess RBV and found good agreement between the rat hemoglobin repletion method and a human iron tracer technique (12).

For EIP, the HRE ratio and RBV of iron from the 12 mg iron/kg diet were higher ($p<0.05$) as compared to the other diets containing greater quantities of EIP. The HRE ratio values for EIP, for the 24, 36, and 48 mg iron/kg diets, are similar - within the range - to other

HRE ratio data values for electrolytic iron powders as noted previously in the literature (5, 13-15), though slightly higher in this study with a mean overall RBV of 57%. For treatments using FS, HRE ratio data varied among the diet treatments and was inversely associated with dietary iron content; though the overall range of HRE ratio values found for FS in this study, particularly of the 12 and 24 mg iron/kg diets, is similar to that found in the literature (5, 13-15).

Findings show that this electrolytic iron powder (Industrial Metal Powders, India) at each of the concentrations tested is an effective agent to replenish hemoglobin and correct anemia.Taking into consideration both EIP's favourable organoleptic profile and, as data from this study show- via HRE ratio and RBV - inclusion of this electrolytic iron powderas an iron fortificantin a variety of foods to reduce the incidence of iron deficiency anemia is advantageous.

REFERENCES

1. Lynch, S.R. Why nutritional iron deficiency persists as a worldwide problem. *J. Nutr.*2011, 141(4):763S-768S.

2. UNICEF/UNU/WHO/MI (1998) Technical workshop preventing iron deficiency in women and children: Background and consensus on key technical issues and resources for advocacy, planning, and implementing national programmes. UNICEF, New York.

3. Hurrell, R. F. Fortification: Overcoming Technical and Practical Barriers. *J. Nutr.*2002, 132: 806S-812S.

4. Hunt, J.R. How important is dietary iron bioavailability? *Am. J. Clin. Nutr*. 2001, 73(1):3-4.

5. Swain, J.H., Newman, S.M., Hunt, J.R. Bioavailability of elemental iron powders to rats is less than bakery-grade ferrous sulfate and predicted by iron solubility and particle surface area. *J. Nutr.*2003, 133:3546-3552.

6. Hallberg, L. and Hulthen, L. Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. *Am. J. Clin. Nutr*.2000, 71(5):1147-1160.

7. Beard, J.L., Murray-Kolb, L.E., Haas, J.D., Lawrence, F. Iron absorption prediction equations lack agreement and underestimate iron absorption. *J. Nutr.* 2007, 137(7):1741-1746.

8. Buchowski, M.S., Mahoney, A.W., Kalpalathika, M.P.V. Nonheme iron absorption, apparent iron absorption, and hemoglobin regeneration efficiency in anemic and normal rats fed with dietary heme and nonheme iron at various levels. *Nutr. Research* 1989, 9: 773-783.

9. Park, Y.W., Mahoney, A.W., Cornforth, D.P., Collinge, S.K., Hendricks, D.G. Bioavailability to anemic rats of iron from fresh, cooked or nitrosylated hemoglobin and myoglobin. *J. Nutr.*1983, 113:680-687.

10. Littell, R., Henry, P., Lewis, A., &Ammerman, C. (1997) Estimation of Relative Bioavailability of Nutrients Using SAS Procedures. J. Anim. Sci. 75: 2672-2683.

11. Reeves, P. G. (1996) AIN-93 purified diets for the study of trace element metabolism in rodents. In R.R. Watson, ed. Trace Elements in Laboratory Rodents CRC Press, New York.

12. Association of Official Analytical Chemists, A. (1984) Bioavailability of iron: Rat hemoglobin repletion bioassay (43.268-43.270). In S. Williams, ed. Official Methods of Analysis, 14 ed.

13. Forbes, A.L., Adams, C.E., Arnaud, M.J., and et. Al Comparison of in vitro, animal, and clinical determinations of iron bioavailability: International Nutritional Anemia Consultative Group Task Force report on iron bioavailability. *Am J ClinNutr,* 1989;49:225-38.

14. Hallberg, L., &Hulthen, L. (2000) Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron (See eratum Am. J. Clin. Nutr. 2000; 72:1242). Am. J. Clin. Nutr. 71: 1147-1160.

15. Hurrell, R.F. Preventing Iron Deficiency through Food Fortification. *Nutrition Reviews,* vol. 5(6), 1997, 210-222.

TABLE 1. Base Diet Composition.[1]

Component	**g/kg**
Corn starch	397.5
Casein (vitamin-free)	200.0
Maltodextran	132.0
Sucrose	100.0
Soybean oil	70.0
Cellulose, microcrystalline (Alphacel™)	50.0
Mineral mix, no iron added	35.0
Vitamin mix, AIN-93-VX	10.0
L-cystine	3.0
Choline bitartrate	2.5
TBHQ, antioxidant	0.014

[1]Modified AIN-93G without added iron. This is the base diet from which treatment diets were prepared.

Table 2: Food and iron intake, growth, and hemoglobin changein anemic rats fed graded quantities ofelectrolytic elemental iron powder (EIP; Industrial Metal Powders (IMP), India)orbakery-grade ferrous sulfate for a 14-day repletion period.[1]

	Control (No Added Iron)	Electrolytic Iron Powder (EIP)				Ferrous Sulfate (FS)				*p*
Diet Code	C	EIP-1	EIP-2	EIP-3	EIP-4	FS-1	FS-2	FS-3	FS-4	
Diet Fe (mg/kg) Calculated (Analyzed)	1.6 (1.4)	12 (11.7)	24 (24.8)	36 (35.6)	48 (48.1)	12 (11.8)	24 (24.2)	36 (36.3)	48 (46.9)	- (-)
Food intake (g/day)	11.7	13.4	13.8	15.0	16.2	12.9	14.2	14.9	16.8	0.04
Fe intake (mg/day)	0.016	0.157	0.342	0.534	0.779	0.152	0.344	0.541	0.788	0.03
Body weight (g) Initial (Gain)	83.9 (15.2)	84.4 (53.4)	83.2 (54.5)	83.1 (56.1)	84.2 (58.7)	84.7 (53.8)	84.2 (56.1)	83.1 (57.5)	85.2 (62.4)	NS (0.03)
Hemoglobin (g/dL) Initial (Gain)	4.63 (-0.42)	4.81 (0.66)	4.70 (1.32)	4.75 (2.79)	4.84 (3.56)	4.83 (1.9)	4.62 (4.11)	4.65 (6.42)	4.74 (8.23)	NS (0.02)
Hemoglobin Fe Gain (mg)	0.064	0.781	0.983	1.469	1.779	1.174	1.876	2.626	3.390	0.03

[1]Values are mean for diet group.
NS = not significant; - (dash) = not applicable.

Table 3: Hemoglobin regeneration efficiency (HRE) and relative bioavailability (RBV) of anemic rats fed graded quantities of electrolytic elemental iron powder (EIP; Industrial Metal Powders (IMP), India) or bakery-grade ferrous sulfate for a 14-day repletion period.

	Electrolytic Iron Powder (EIP)				Ferrous Sulfate (FS)			
Diet Code	EIP-1	EIP-2	EIP-3	EIP-4	FS-1	FS-2	FS-3	FS-4
Diet Fe (mg/kg) Calculated (Analyzed)	12 (11.7)	24 (24.8)	36 (35.6)	48 (48.1)	12 (11.8)	24 (24.2)	36 (36.3)	48 (46.9)
HRE ratio[1]	0.356	0.205	0.197	0.163	0.552	0.389	0.347	0.307
RBV[2]	64.5	59.1	50.6	54.3	-	-	-	-

[1]HRE ratio = [Final Hb Fe (mg) - Initial Hb Fe (mg)]/Fe intake (mg total/14 d)
[2]RBV = Mean percentage (%) HRE relative to ferrous sulfate (diet matched: i.e. 12 (EIP-1) vs. 12 (FS-1) mg Fe/kg diet)