Recovery from dietary iron deficiency anaemia in rats by the intake of microencapsulated ferric saccharate

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Abstract This study examined the bioavailability of iron contained in microencapsulated ferric saccharate in a rat model of iron deficiency anaemia. Three groups of male Sprague–Dawley rats with induced iron deficiency anaemia were subsequently treated with a control Fe-deficient diet (2–6 mg Fe/Kg of diet) with or without the addition of 10 mg Fe/Kg of diet (in form of ferrous sulphate or microencapsulated ferric saccharate) for 2 weeks. The bioavailability of microencapsulated ferric saccharate was examined by measuring body weight gain, feed efficiency and reticulocyte parameters, and compared with the bioavailability of ferrous sulphate. Final body weight, feed efficiency, mean corpuscular volume of reticulocytes and average haemoglobin content in reticulocytes were significantly higher in anaemic rats supplemented with either microencapsulated ferric saccharate or ferrous sulphate, compared to anaemic controls. No significant differences were found between the two iron-supplemented groups. The total number of reticulocytes showed a similar trend. The results demonstrated that ingestion of microencapsulated ferric saccharate is as effective as ferrous sulphate in recovery from iron deficiency anaemia.

Keywords Iron absorption · Iron deficiency anaemia · Microencapsulation · Ferric saccharate · Rats · Reticulocytes

Introduction

Iron deficiency anaemia is one of the most prevalent nutritional problems in the world (De Benoist et al. 2008). Its prevalence is particularly high among young children and women of reproductive age. It can cause developmental delays and behavioural disturbances in children (Lozoff et al. 2006). In women of reproductive age, it reduces physical performance (Pasricha et al. 2014) and increases the risk of preterm delivery and of having babies with a low birth weight (Leung and Chan 2001).

Iron supplementation or fortification is commonly recommended to prevent iron deficiency anaemia, but current solutions have drawbacks such as dose limitation, food oxidation and short shelf life. Moreover, soluble iron salts produce several side effects (i.e. unpleasant taste, vomiting, pyrosis, altered transit and/or stool darkening) (Hyder et al. 2002; Waldvogel et al. 2012; Pasricha et al. 2013). Microencapsulation of iron sources can reduce these problems, avoiding the interaction of iron with the environment until reaching the intestine, increasing its compatibility with other ingredients and reducing side effects. Iron usually exists in the ferrous (Fe^{2+}) or ferric (Fe^{3+}) state. The ferrous form is directly absorbed in the duodenum by a divalent metal transporter (DMT1) (Chua et al. 2007), while ferric iron is reduced to the ferrous form by a ferrireductase at the apical surface of the epithelial brush border.

In the present study, we compared the bioavailability of a microencapsulated ferric saccharate form (MFS), trademarked as AB-Fortis[®] (AB-Biotics S.A, Spain), to that of ferrous sulphate. Ferrous sulphate was chosen as a positive control because it is commonly used as a standard in iron bioavailability studies due to its good bioavailability (Jeppsen and Borzelleca 1999; Lysionek et al. 2003;

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Sakaguchi et al. 2004). MFS is a patented micro-encapsulated preparation consisting of a calcium alginate matrix that holds and protects a salt of Fe^{3+} inside. MFS is produced by ionotropic gelation of alginate with calcium, entrapping the iron salt inside. Calcium displays a stronger interaction with alginate, stabilizing the microcapsules and avoiding the release of iron. Ideal iron fortifiers should permit supplementing high doses of iron in food (50% of recommended daily intake and above) without changing their physical, chemical or sensory properties (Hurrell 2002).

Because of the ubiquity of iron, its compartmentalized sites of action, and its complex metabolism, usual pharmacokinetic measurements such as serum concentration are largely irrelevant when evaluating the bioavailability and efficacy of iron preparations (Schümann et al. 1997). As such, pharmacokinetic and pharmacodynamic assessments of iron supplements cannot be based on the standard principles that apply to non-endogenous drugs. In a previous study (Contreras et al. 2014) the iron absorption of MFS was tested in healthy non-iron deficient volunteers in a cross-over, single-dose study. This study demonstrated that iron absorption in MFS was equivalent to ferrous sulphate as denoted by a similar increase of the saturation of transferrin (iron-binding blood plasma glycoprotein) for a period of 6 h. The present study aimed to demonstrate not only that iron was absorbed, but also whether it was incorporated into haemoglobin by studying MFS bioavailability in a rat model of iron deficiency anaemia.

Materials and methods

This study was approved by the Animal Care and Use Committee of the University of Barcelona. The study design was based on the official AOAC 974.31 (Association of Official Analytical Chemists) method (AOAC 1974), but modified by specifically measuring haemoglobin content and corpuscular volume in reticulocytes.

Animals and diets

Twenty male Sprague–Dawley weanling rats (aged 21 days, weighing 35–40 g) from Harlan Interfauna Ibérica (Barcelona, Spain) were randomly assigned to one of three dietary groups ($n = 6$ /negative control group, $n = 6$ /positive control group, and $n = 8$ /test group) as illustrated in Fig. 1. Animals were housed in stainless steel cages (two animals per cage) in a controlled environment (20–22 °C, 30–50% of relative humidity and 12 h light cycles). Anaemic animals were prepared by feeding them AIN-93G Fe-deficient diets (2–6 mg Fe/kg diet) for 23 days. Next,

Fig. 1 Design of rat feeding experiments

the negative control group was fed the same Fe-deficient diet for an additional 14 days, while the other two groups were fed one of the following diets for the same period: (1) positive control group, AIN-93G Fe-deficient diet plus FeSO_4 (10 mg Fe/kg diet); and (2) test group, AIN-93G Fe-deficient diet plus MFS (10 mg Fe/kg diet). Rats were allowed free access to their diet and to deionised water (Millipore Milli-Q System) during the experiment.

Sampling procedure

Body weight and food consumption were periodically measured throughout the experimental period. After 23 days on Fe-deficient diet, animals were anesthetized with inhaled isoflurane and a volume of 0.1–0.3 ml of blood was extracted from the saphenous vein and collected in microtubes containing dipotasic EDTA as anti-coagulant. The total haemoglobin content of blood was measured with the haematology autoanalyzer ADVIA 120 (Bayer, Spain) in order to verify that animals had become anaemic. All rats had haemoglobin <6 g/dL, and thus were satisfactorily depleted of iron. Next, rats received either one of the two Fe-fortified diets or the negative control diet. After an additional 14 days, the animals were anesthetized in the same conditions stated before and blood (0.3 mL) was extracted by intracardiac puncture and collected in tubes containing dipotasic EDTA as anticoagulant. Full haemograms were measured with the haematology autoanalyzer ADVIA 120 (Bayer, Spain). Number of reticulocytes, mean corpuscular haemoglobin concentration in reticulocytes (MCHC_R) and mean corpuscular volume of reticulocytes (MCV_R) were measured for each animal at the end of the study. The average haemoglobin content of reticulocytes was calculated by multiplying $\text{MCHC}_R \times \text{MCV}_R$.

Release of iron in simulated gastrointestinal conditions

Ferrous sulphate, ferric saccharate and MFS were solved in distilled water at 35 mg/L of elemental iron each. Subsequently, pH was adjusted to 2.0 with HCl and solutions were incubated for 2 h. Then, pH was adjusted to alkaline conditions with NaOH and bile salts (Oxgall) were added, and the solutions were further incubated for 4 h. Several aliquots were taken at different time points to determine free iron content.

Statistical analysis

Data were analysed by IBM® SPSS Statistics v.20 for Windows and expressed as mean $\pm$ standard error of the mean (SEM). Two-way analysis of variance (ANOVA) was used to determine the effect of diet, time and their interaction on body weight. One-way ANOVA was used to assess the effect of diet on the remaining variables at the beginning and at the end of the treatment period. The exact nature of the differences between treatments was determined by Tukey's post hoc test. A confidence coefficient of 95% was used.

Results and discussion

Effect of iron supplementation on body weight

Animals had similar initial weight, and no significant differences were noted among groups at the beginning of the anaemia recovery period (see Table 1). Evolution of body weight during the study is shown in Fig. 2. All animals gained body weight through the study, although data show a clear difference among the three study groups. Body weight was increased in both fortified groups to a similar extent, whereas body weight gain was markedly lower in the group fed the negative control diet. A two-way

Fig. 2 Body weight increase during all the study. The anaemia induction period starts on day 0, and the recovery period starts on day 23. Data represents averages and SEM. $n = 6$ for negative control and ferrous sulphate groups, and $n = 8$ for MFS group

ANOVA analysis of body weight versus time shows that time ($P < 0.001$), treatment ($P = 0.005$) and interaction ($P < 0.001$) effects are clearly significant. From the sixth day of the recovery period (29th day) onwards, rats treated with either ferrous sulphate or MFS had statistically higher body weights than controls ($P < 0.001$ for both groups). No statistically significant differences between MFS and the positive control (ferrous sulphate) were found. Feed efficiency was significantly higher in both supplemented groups than in negative controls, again without significant differences between iron supplemented groups.

Effect of iron supplementation on reticulocytes

The average haemoglobin content of reticulocytes was determined to evaluate iron status. As can be seen in Table 2, MCV_R values were similarly increased in both the positive control (ferrous sulphate) and MFS group, compared to negative controls ($P < 0.01$), while there were no differences between both fortified groups. The total number

Table 1 Weight gain, food consumption and feed efficiency during the anaemia recovery period (last 14 days)

	Negative control	Positive control (FeSO_4)	Test group (MFS)
Initial weight (g) ^a	121.8 $\pm$ 4.6	122.2 $\pm$ 4.3	132.7 $\pm$ 3.5
Final weight (g) ^a	147.9 $\pm$ 6.3*	208.5 $\pm$ 9.6**	195.8 $\pm$ 5.0**
Food consumption (g animal ⁻¹ day ⁻¹) ^b	9.6 $\pm$ 0.5*	14.4 $\pm$ 1.0**	12.6 $\pm$ 0.4**
Feed efficiency (g of animal 100 g consumed ⁻¹) ^a	19.3 $\pm$ 1.2*	42.5 $\pm$ 3.2**	35.8 $\pm$ 1.3**

^a $n = 6$ for negative control and positive control groups, and $n = 8$ for MFS group

^b $n = 3$ for negative control and positive control groups, and $n = 4$ for MFS group

Results are expressed as mean $\pm$ SEM. Results in the same row not sharing a common superscript are significantly different based on Tukey post hoc test at $P < 0.05$

Table 2 Blood parameters related to reticulocytes

	Negative control	Positive control (FeSO ₄)	Test group (MFS)
MCV _R (fL)	43.48 ± 0.87*	51.73 ± 1.45**	51.50 ± 1.49**
Reticulocytes (10 ⁶ cells/mL)	335.96 ± 74.14	772.35 ± 107.77	697.53 ± 143.62
MCHC _R (g/dL)	24.94 ± 0.17	24.60 ± 0.43	24.25 ± 0.19

MCV_R mean corpuscular volume of reticulocytes and MCHC_R mean corpuscular haemoglobin concentration in reticulocytes. Results are expressed as mean ± SEM. Results in the same row not sharing a common superscript are significantly different based on Tukey post hoc test at $P < 0.01$. $n = 6$ for negative and positive control groups, and $n = 8$ for MFS group

of reticulocytes also showed a similar trend, although the difference between the fortified groups and the negative controls did not reach statistical significance ($P = 0.083$) owing to the larger standard deviation. Conversely, there were no differences in MCHC_R between groups. The average haemoglobin content in reticulocytes is shown in Fig. 3, both the positive controls and MFS showing a highly significant increase compared to negative controls ($P < 0.01$). However, no differences were found between positive control and MFS group. Supplementation with ferrous sulphate and MSF increased average haemoglobin content in reticulocytes by 1.86 ± 0.28 pg and by 1.64 ± 0.46 pg, respectively. Thus, based on the increase in average haemoglobin content of reticulocytes, relative bioavailability of MFS compared to FeSO₄ would be estimated to be 0.88.

Compared to the official AOAC 974.31 method, we sought to determine iron bioavailability by specifically measuring haemoglobin content in reticulocytes. Reticulocytes are immature red blood cells that are released into the blood stream to become mature red blood cells. When the iron status is low, reticulocytes are released into the bloodstream with an insufficient amount of haemoglobin (Fukui

et al. 2002; Handelsman and Levin 2008). Taken together with their short lifespan of 60 days (Derelanko 1987), this makes reticulocytes a suitable marker of the iron stores right before the analysis. Therefore, we hypothesized that their haemoglobin content could be more sensitive to short-term iron bioavailability than total blood haemoglobin in erythrocytes. Although the official AOAC 974.31 method requires the testing of doses of up to 24 ppm of iron and groups of at least 8 animals, by focusing the analysis on reticulocytes it was possible to observe a statistically significant effect on blood parameters in both iron-supplemented groups with doses of 10 ppm and using 6 animals per group only. Therefore, although further validation is required, these results suggest that measuring specifically haemoglobin content in reticulocytes could be a good alternative to refine the official AOAC method, as there is a growing interest in performing studies with fewer animals, following the principles of the 3Rs (Replacement, Reduction and Refinement).

Iron replacement therapy is necessary to correct haemoglobin levels and replenish iron stores in iron deficiency anaemia. Oral treatment is usually preferred being an inexpensive and effective treatment. Moreover, it can reduce the risk of adverse effects such as iron overload usually associated to other forms of administration (i.e. intravenous iron administration). Many iron-containing preparations are available on the market, but they vary widely in dosage, chemical state of iron (ferrous or ferric form), bioavailability and cost. The bioavailability of trivalent versus bivalent preparations was studied by several authors (Kaltwasser et al. 1987; Mehta 2003; Ruiz-Argüelles et al. 2007), concluding that anaemic patients treated with ferric preparations failed to respond in several cases, while the same patients responded to the administration of a ferrous form.

Ferrous salts are cheap and present good bioavailability (between 10 and 15%), but their main drawback is a high incidence of side effects, such as nausea, heartburn, pain, diarrhoea or constipation, that increase the rejection of iron-fortified products by the consumer (Mannar 2006). In a systematic review about tolerability of different oral iron supplements, it was found that the incidence of gastrointestinal events with ferrous sulphate preparations was

Fig. 3 Average haemoglobin contained in reticulocytes calculated by multiplying MCHC_R × MCV_R. Data represents averages and SEM. $n = 6$ for negative control and ferrous sulphate groups, and $n = 8$ for MFS group

markedly higher than with ferric iron such as iron protein succinylate (31.6 vs. 7.0%) (Cancelo-Hidalgo et al. 2013). Microencapsulation is a good technique to avoid interaction of the encapsulated ingredient with the environment and reduce side effects. Iron supplements typically produce pyrosis and nausea when free iron reaches the stomach. MFS minimizes these adverse effects, since the microcapsules are designed to be stable at acidic pH. This was confirmed using an in vitro experiment simulating gastrointestinal conditions. Results are presented in Fig. 4, showing that iron was well protected in the microcapsules under acidic conditions simulating the stomach (first 2 h).

Alterations in gut transit are a common side effect of iron supplementations. Iron is a key growth factor for many bacterial species (Andrews et al. 2003), promoting the overgrowth of opportunistic iron-dependent bacterial genera (e.g. *Enterobacteria*), but not of other bacteria such as *Bifidobacteria* or *Lactobacilli*, thus leading to microbiota imbalance (dysbiosis) in some individuals. Bacteria of different genera have been shown to induce different contraction patterns in the gut (Massi et al. 2006). To reduce the risk of dysbiosis, oral iron supplements should release iron in a controlled manner, in order to avoid high concentrations of free iron in the gastrointestinal tract. In this regard, MFS acts as an extended release formulation, keeping the iron encapsulated in the stomach, and then releasing iron gradually in the intestine, thus reducing the amount of free iron and the risk of dysbiosis.

It is important not only to achieve protection and stability, but also to have good bioavailability. In a previous study (Contreras et al. 2014) it was shown that iron absorption from MFS was equivalent to that from ferrous sulphate in healthy, non-iron deficient volunteers. The present study in a murine model of anaemia has demonstrated not only that MSF iron is absorbed but also

incorporated into haemoglobin, allowing the animals to recover from anaemia.

Since iron is well absorbed, the microcapsules must be able to completely release their content in the intestine. Calcium displays a strong interaction with alginate, thus stabilizing the microcapsules and avoiding the release of iron. However, when MFS reaches the intestine, the calcium layer is dissolved and iron is gradually released due to the ionic interaction of iron with alginate being weaker. This assumption was confirmed in an in vitro experiment (see Fig. 4), most iron becoming soluble after 1 h under simulated gut conditions.

In terms of cost, although microencapsulated products are more expensive than soluble iron salts, the impact on the cost of the fortified food product is almost negligible (i.e. approximately 1–2 USD cents for daily dose), as the amount of MFS required to achieve the desired recommended daily intake is very small.

Conclusion

MFS displayed similar bioavailability to ferrous sulphate at the dose tested (10 mg Fe/Kg diet). This conclusion is supported by the analysis of the average amount of haemoglobin in reticulocytes (reflecting bioavailable iron in the short term), the total number of reticulocytes, their mean corpuscular volume, body weight gain and feed efficiency. Both MFS and ferrous sulphate resulted in statistically significant differences compared to negative control ($P < 0.01$), but no differences could be found between them ($P > 0.05$ in all comparisons).

References

- Andrews SC, Robinson AK, Rodríguez-Quinones F (2003) Bacterial iron homeostasis. *FEMS Microbiol Rev* 27:215–237. doi:10.1016/S0168-6445(03)00055-X
- AOAC (1974) Bioavailability of iron. Rat hemoglobin repletion bioassay. Method 974.31. In: Official methods of analysis of the AOAC, 16th edn. Gaithersburg
- Cancelo-Hidalgo MJ, Castelo-Branco C, Palacios S et al (2013) Tolerability of different oral iron supplements: a systematic review. *Curr Med Res Opin* 29:291–303. doi:10.1185/03007995.2012.761599
- Chua ACG, Graham RM, Trinder D, Olynyk JK (2007) The regulation of cellular iron metabolism. *Crit Rev Clin Lab Sci* 44:413–459. doi:10.1080/10408360701428257
- Contreras C, Barnuevo MD, Guillén I et al (2014) Comparative study of the oral absorption of microencapsulated ferric saccharate and ferrous sulfate in humans. *Eur J Nutr* 53:567–574. doi:10.1007/s00394-013-0565-z
- De Benoist B, Mclean E, Egli I CM (2008) Worldwide prevalence of anemia 1993–2005: WHO global database on anemia

Fig. 4 Iron release under simulated gastrointestinal conditions. Filled square Blank, open square ferrous sulphate, x MFS and open triangle ferric saccharate

- Derelanko MJ (1987) Determination of erythrocyte life span in F-344, Wistar, and Sprague-Dawley rats using a modification of the [³H] diisopropylfluorophosphate ([³H] DFP) method. *Toxicol Sci* 9:271–276. doi:[10.1093/toxsci/9.2.271](https://doi.org/10.1093/toxsci/9.2.271)
- Fukui Y, Samma S, Fujimoto K et al (2002) Reticulocyte hemoglobin content as a marker of iron status in patients receiving maintenance hemodialysis. *Clin Exp Nephrol* 6:147–153. doi:[10.1007/s101570200025](https://doi.org/10.1007/s101570200025)
- Handelman GJ, Levin NW (2008) Iron and anemia in human biology: a review of mechanisms. *Heart Fail Rev* 13:393–404. doi:[10.1007/s10741-008-9086-x](https://doi.org/10.1007/s10741-008-9086-x)
- Hurrell RF (2002) Fortification: overcoming technical and practical barriers. *J Nutr* 132:806S–812S
- Hyder SMZ, Persson LÅ, Chowdhury AMR, Ekström E-C (2002) Do side-effects reduce compliance to iron supplementation? A study of daily- and weekly-dose regimens in pregnancy. *J Health Popul Nutr* 20:175–179. doi:[10.3329/jhpn.v20i2.142](https://doi.org/10.3329/jhpn.v20i2.142)
- Jeppsen RB, Borzelleca JF (1999) Safety evaluation of ferrous bisglycinate chelate. *Food Chem Toxicol* 37:723–731. doi:[10.1016/S0278-6915\(99\)00052-6](https://doi.org/10.1016/S0278-6915(99)00052-6)
- Kaltwasser JP, Werner E, Niechzial M (1987) Bioavailability and therapeutic efficacy of bivalent and trivalent iron preparations. *Arzneimittelforschung* 37:122–129
- Leung AK, Chan KW (2001) Iron deficiency anemia. *Adv Pediatr* 48:385–408
- Lozoff B, Beard J, Connor J et al (2006) Long-lasting neural and behavioral effects of iron deficiency in infancy. *Nutr Rev* 64:S34–S43. doi:[10.1301/nr.2006.may.S34](https://doi.org/10.1301/nr.2006.may.S34)
- Lysionek AE, Zubillaga MB, Salgueiro MJ et al (2003) Stabilized ferrous gluconate as iron source for food fortification: bioavailability and toxicity studies in rats. *Biol Trace Elem Res* 94:73–78. doi:[10.1385/BTER:94:1:73](https://doi.org/10.1385/BTER:94:1:73)
- Mannar MG (2006) Successful food-based programmes, supplementation and fortification. *J Pediatr Gastroenterol Nutr* 43(Suppl 3):S47–S53. doi:[10.1097/01.mpg.0000255850.30400.ae](https://doi.org/10.1097/01.mpg.0000255850.30400.ae)
- Massi M, Ioan P, Budriesi R et al (2006) Effects of probiotic bacteria on gastrointestinal motility in guinea-pig isolated tissue. *World J Gastroenterol* 12:5987–5994
- Mehta BC (2003) Ineffectiveness of iron polymaltose in treatment of iron deficiency anemia. *J Assoc Physicians India* 51:419–421
- Pasricha SR, Hayes E, Kalumba K, Biggs BA (2013) Effect of daily iron supplementation on health in children aged 4–23 months: a systematic review and meta-analysis of randomised controlled trials. *Lancet Glob Health* 1:e77–e86. doi:[10.1016/S2214-109X\(13\)70046-9](https://doi.org/10.1016/S2214-109X(13)70046-9)
- Pasricha S, Low M, Thompson J, Farrell A (2014) Iron Supplementation benefits physical performance in women of reproductive age: a systematic review and meta-analysis 1–3. *J Nut* 6:906–914. doi:[10.3945/jn.113.189589.economic](https://doi.org/10.3945/jn.113.189589.economic)
- Ruiz-Argüelles GJ, Díaz-Hernández A, Manzano C, Ruiz-Delgado GJ (2007) Ineffectiveness of oral iron hydroxide polymaltose in iron-deficiency anemia. *Hematology* 12:255–256. doi:[10.1080/10245330701214160](https://doi.org/10.1080/10245330701214160)
- Sakaguchi N, Rao TP, Nakata K et al (2004) Iron absorption and bioavailability in rats of micronized dispersible ferric pyrophosphate. *Int J Vitam Nutr Res Int Zeitschrift für Vitamin- und Ernährungsforschung J Int Vitaminol Nutr* 74:3–9. doi:[10.1024/0300-9831.74.1.3](https://doi.org/10.1024/0300-9831.74.1.3)
- Schümann K, Classen HG, Hages M et al (1997) Bioavailability of oral vitamins, minerals, and trace elements in perspective. *Arzneimittelforschung* 47:369–380
- Waldvogel S, Pedrazzini B, Vaucher P et al (2012) Clinical evaluation of iron treatment efficiency among non-anemic but iron-deficient female blood donors: a randomized controlled trial. *BMC Med* 10:8. doi:[10.1186/1741-7015-10-8](https://doi.org/10.1186/1741-7015-10-8)