European Journal of Clinical Investigation (1997) 27, 228–233

47, 59,

Bovine ferritin iron bioavailability in man

B. SKIKNE, D. FONZO*, S. R. LYNCH & J. D. COOK Department of Medicine, Division of Hematology, Kansas University Medical Center, USA, and *Ospedale Mauriziano Umberto 10, Turin, Italy

Received 20 March 1996 and in revised form 17 September 1996; accepted 30 September 1996

Abstract. The bioavailability of ferritin iron was evaluated in human subjects using radiolabelled [^{55}Fe]ferritin isolated from bovine spleen and liver. Preliminary studies with bovine spleen ferritin labelled *in vitro* demonstrated an inappropriately high absorption compared with ferritin labelled *in vivo*, and the latter was therefore used in all subsequent absorption studies. In 10 subjects, geometric mean absorption from 5 mg of ferritin iron was 3·8% when taken without and 3·2% when taken with food ($P > 0{\cdot}05$). These values were significantly lower than absorption from the same dose of iron given as ferrous sulphate, which averaged 24·1% without and 8·2% with food. When the iron dose was increased 10-fold, absorption of ferritin iron averaged only 0·6–0·7% with or without food as compared with 7·9% without and 2·6% with food when the iron was given as ferrous sulphate. In a further study, mean absorption from bovine spleen ferritin of 4·0% did not differ significantly from the mean of 2·7% observed with bovine liver ferritin. These findings confirm previous studies indicating that ferritin iron is poorly absorbed. Furthermore, its use as a pharmaceutical iron preparation cannot be advocated.

Keywords. Absorption, bioavailability, ferritin, iron, man.

Introduction

The iron storage protein ferritin is ubiquitous in nature [1] and widely present in most foods of both animal [2] and vegetable origin [3,4]. In general, iron bioavailability is high from animal sources. Absorption from haem iron is significantly elevated, especially when compared with that from ferritin iron [5]. However, the previous studies examining ferritin iron absorption employed radiolabelled ferritin purified from either rats or rabbits. Ferritin thus labelled has been mixed with unlabelled ferritin derived from large animal species for studying ferritin iron absorption [5,6]. There is no information on whether the absorption of ferritin iron differs when isolated from different animal species or from different organs in the same species.

Correspondence: B. Skikne, Division of Hematology, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160–7402, USA.

© 1997 Blackwell Science Ltd

The present study was undertaken to evaluate iron absorption in human subjects from purified bovine ferritin, the form of animal ferritin that is most commonly consumed in the diet. Because of the technical difficulties in preparing radiolabelled bovine ferritin *in vivo*, the feasibility of using *in vitro* radiolabelled bovine ferritin was first evaluated. Further studies were then carried out to compare ferritin iron absorption with ferrous sulphate.

Method

In vitro *radiolabelling of commercial bovine spleen ferritin*

An aliquot of 2 mg of commercial bovine spleen ferritin was placed in a 50-mL conical flask containing 0·3 mL of 0·1 mol L^{-1} phosphate buffer, pH 7·5. To promote oxidation of added ferrous iron to the ferric hydrolysis polymer [7], the mixture was rapidly vortexed while 0·035 mL of $^{59}FeSO_4$ containing 20 μCi and 1·6 μg of iron was added dropwise over 10 min with a Hamilton syringe. The ferritin solution was then incubated at 37°C for 16 h, after which the reaction mixture was transferred to an Eppendorf microtube and centrifuged at $10\,000 \times g$ for 30 min. The supernatant was discarded and the labelled ferritin in the precipitate was resuspended in a buffer and purified by passage through a Sepharose 6B column (100 × 2·5 cm) in the same buffer.

No differences were observed between *in vitro* labelled ferritin and unlabelled ferritin when examined by polyacrylamide gel electrophoresis and isoelectric focusing. With autoradiography, ^{59}Fe was shown to be present in the full range of isoferritins, with slightly higher amounts in the more acidic forms. Anti-ferritin immune serum to commercial bovine ferritin produced in rabbits was added in varying dilutions. After overnight incubation, the samples were centrifuged for 20 min at 2500 × g, the supernatant discarded and centrifugation repeated after resuspension of the ferritin–antiferritin pellet in a phosphate buffer [8]. Recovery of the radioiron-labelled ferritin was then measured.

Production of in vivo *radiolabelled ^{55}Fe ferritin*

Radiolabelled ferritin was produced *in vivo* by injecting

30 mCi of $^{55}FeCl_3$ in 4% sodium citrate into 6-month-old Holstein calves with an estimated blood volume of 10 L in which erythropoiesis had been stimulated by removing 400 mL of blood over the preceding 3 days to enhance incorporation of the injected radioiron into circulating red blood cells. As most of the latter would remain in the circulation rather than becoming deposited in iron stores, the uptake of radioactive iron by the reticuloendothelial system in the liver and spleen was enhanced by coating the circulating red cells with antibovine antiserum as follows. Two weeks after the initial injection of radioiron, 380 mL of whole blood was removed through the jugular vein into a sterile blood collection bag containing citrate, phosphate, dextrose, adenine solution (CPDA-1 Whole Blood; Fenwal Laboratories, IL, USA). After centrifuging the bag for 5 min at 4000 rpm at 4°C, the serum and white-cell layer were expelled and replaced with an equal volume of normal saline. After gentle mixing, the red blood cells were washed again in saline and the supernatant replaced with an equal volume of saline containing 0·3–0·6 mL L^{-1} rabbit antibovine red blood cell IgG antibody with an agglutination titre of 1 : 1280 (Cooper Biomedical, Malvern, PA, USA). The red blood cells were mixed gently and incubated at 37°C for 1 h. The supernatant containing unbound red blood cell antibodies was removed by centrifugation and, following a further saline wash, the coated red blood cells were reinfused into the animal. This procedure was repeated 10 times over the next 2 weeks. The estimated iron contained in the reinfused, treated red blood cells was approximately 1400 mg. Sixteen hours after the final antibody-coated red blood cell infusion, the animal was sacrificed and the liver and spleen were removed.

Purification of radiolabelled liver and spleen ferritin

Radiolabelled ferritin was isolated by a modification of the method of Cetinkaya *et al.* [9] as follows. Spleen or liver tissue was homogenized in water, heat treated for 10 min at 70–75°C and incubated for 2 h on ice after adjusting the pH to 4·8 with HCl. The homogenate was then centrifuged at 10 000 × *g* for 30 min at 4°C, the sediment was discarded and the pH of the supernatant was adjusted to 6·5 with 1 mol L^{-1} sodium hydroxide. Solid ammonium sulphate was added to 50% saturation and after incubating overnight at 4°C, the preparation was centrifuged at 1600 × *g* for 30 min at 4°C, the supernatant discarded and the pellet dissolved in deionized water. The precipitation with ammonium sulphate was repeated and the recovered pellet was dissolved in 0·05 mol L^{-1} phosphate buffer at pH 7·5. After dialysing for 72 h, the material was chromatographed on Sepharose 6B in the same buffer. Iron-containing protein was identified in the eluate using a Gilford Response spectrophotometer set at wavelengths of 280 and 420 nm [10]. After concentrating the ferritin peak by ultrafiltration (PM10 membrane Amicon), the preparation was ultracentrifuged at 100 000 × *g* for 2 h at 4°C and the ferritin pellet dissolved in 0·05 mol L^{-1} phosphate buffer at pH 7·5. Solid sodium acetate was added to bring the concentration to 0·05 mol L^{-1}, the pH was adjusted to 5·5 and ammonium sulphate precipitation at 50% saturation was performed as described previously. After centrifugation at 1600 × g for 30 min at 4°C, the precipitate was dissolved in distilled water, dialysed in a 0·05 mol L^{-1} phosphate buffer and stored at 4°C.

The protein content of the ferritin was measured by the method of Lowry *et al.* [11] using bovine serum albumin as the standard. The non-haem iron content was measured by the method of Torrance & Bothwell [12]. Precipitation of haem iron was carried out in 10% trichloroacetic acid–3 mol L^{-1} HCl solution with incubation for 20 h at 65°C. Radiolabelled ferritin was prepared for liquid scintillation counting by a modification of the method of Eakins & Brown [13]. The protein content of commercial ferritin was 57·8 mg mL^{-1} and the iron content was 0·3 mg mg^{-1} protein (30%) compared with 7·7 mg mL^{-1} and 0·1 mg mg^{-1} protein (10%), respectively, for the bovine ferritin prepared for the present investigation.

Bioavailability studies

Three studies assessing ferritin iron bioavailability were performed in 27 healthy normal volunteer subjects between the ages of 23 and 41 years. Three of the subjects had iron deficiency as defined by a serum ferritin $\leq 12\ \mu g\ L^{-1}$ and one of these female subjects was also anaemic as defined by a haematocrit <36%. A fourth subject had a haematocrit <36% with a normal ferritin. All of the subjects were healthy and denied a history of disorders known to influence the gastrointestinal absorption of iron. Written, informed consent was obtained from all subjects and all protocols were approved by the Human Subjects Committee of the University of Kansas Medical Center.

Using dual ^{59}Fe and ^{55}Fe tags sequentially, three separate absorption measurements were performed in each subject in study 1 and four in the remaining two studies [14]. All test preparations were administered in the morning between 07.00 and 09.00 hours after an overnight fast and no food or liquid was allowed for 4 h after ingesting the test meal. Before giving the first test dose, 30 mL of blood was obtained for measurement of background blood radioactivity, packed cell volume and serum ferritin concentration. Serum ferritin concentration was measured using an enzyme-linked immunosorbent assay employing monoclonal antibody [15]. On the following two mornings, test meals tagged with either 90 kBq of ^{55}Fe or 74 kBq of ^{59}Fe were fed. Blood was obtained 14 days later for determination of incorporated red cell radioactivity and the third and fourth test meals tagged with separate radioiron labels were again fed on 2 successive days. The subjects returned 14 days after the final test dose when blood was again drawn to measure the increase in radioactivity in circulating red blood cells. All measurements of blood radioactivity were performed on duplicate 10-mL samples of whole blood by a modification of the method of Eakins & Brown [13].

Absorption was estimated from the blood volume estimated from the height and weight of each subject [16]. Red cell incorporation of absorbed radioactivity was assumed to be 80% in all subjects [17].

Study 1 was designed to assess the suitability of *in vitro* labelled ferritin for iron absorption measurements. In four male and four female subjects, the absorption of bovine spleen ferritin labelled by the two methods was compared, and a third absorption test using *in vivo* tagged bovine liver ferritin was also measured to evaluate for possible absorption differences in the tissue source of ferritin. Each of the labelled preparations was added to unlabelled spleen or liver ferritin to bring the iron dose to 2·7 mg for the spleen preparation and 1·1 mg for the liver ferritin. The total ferritin protein content was 40 mg for the two spleen preparations and 34 mg for the liver preparations.

Study 2 was performed in three men and seven women. The purpose was to compare absorption of 5 mg of ferritin iron with the same iron dose given as ferrous sulphate directly. In the first pair of absorption studies, the test doses were given with food, whereas in the second pair they were given with water only. The food was a wheat-based meal containing Farina, a white wheat flour commonly used for porridge. The flour (250 g) was fed with 120 mL of milk, 14 g of butter and 24 g of sugar. The nutrient composition of the meal was 407 kcal, 8·7 g of protein, 14·8 g of fat, 60 g of carbohydrates and 3·14 mg of iron.

Study 3, performed in nine volunteer subjects, was identical to the second study except that 50 mg of iron as ferritin or ferrous sulphate was used as the test dose.

Statistical analysis

Analysis of absorption data was performed after converting to logarithms. A paired *t*-test was used to examine whether the mean log absorption ratios for each study differed significantly from 0 [18].

Results

There were significant differences in the organ distribution of radioiron after the injection of *in vivo* radiolabelled antibody-coated bovine red blood cells. It was presumed that the non-haem iron fraction was present predominantly as ferritin. Approximately one-third of the radioiron was in the non-haem iron fraction in the spleen, whereas two-thirds was in the non-haem iron fraction in the liver. As a reference, less than 5% of the radioiron was non-haem in whole blood obtained from the animals at the time of sacrifice. It is noteworthy that only 20% of the non-haem radioiron was recovered as ferritin. The remainder was either not incorporated into ferritin or lost in the ferritin isolation procedure.

With *in vitro* radiolabelled ferritin, 90% of radioiron added to cold ferritin was bound to the protein, with an estimated incorporation of 28 radioiron atoms per molecule of ferritin. The immunoreactivity of the *in vitro* labelled ferritin as measured with rabbit antibovine ferritin did not change significantly following the labelling procedure. Thus, after 16 days, 90% of the iron radiolabel was precipitated compared with 92% on day 2 and 93% on day 8. Both *in vivo* labelled and *in vitro* labelled ferritins had similar elution patterns on the Sepharose 6B. However, in addition to the major ferritin peak, a minor small molecular weight fraction was noted on the initial elution pattern of the *in vitro* labelled ferritin, which probably represented denatured ferritin. This peak disappeared after repeat chromatography on Sepharose 6B. The elution pattern of the *in vivo* labelled ferritin preparation did not demonstrate the smaller molecular weight peak.

In study 1, a marked difference in the absorption of the *in vivo* and *in vitro* labelled ferritin was observed in eight volunteer subjects (Table 1). In comparing the two spleen ferritin preparations, absorption of the *in vivo* labelled material ranged from 0·4 to 16·9% with a geometric mean of 4·0%. A fourfold higher mean absorption of 16·7% was observed with *in vitro* labelled ferritin and the difference was highly significant ($P<0·001$). On the

Table 1. Absorption from *in vivo* and *in vitro* radiolabelled bovine spleen and liver ferritin

				Spleen ferritin		Liver ferritin
Subject	Sex/age	Packed cell volume (%)	Serum ferritin* ($ng\,mL^{-1}$)	*In vivo* label (%) (A)	*In vitro* label (%) (B)	*In vivo* label(%) (C)
1	F/32	38	119	6·8	18·7	4·7
2	M/23	45	118	0·4	2·6	0.4
3	F/26	39	102	1·7	13·7	1·4
4	F/38	36	59	1·9	6·2	0·5
5	F/32	39	22	4·6	36·9	9·3
6	M/23	44	16	9·9	19·0	5·9
7	M/41	46	14	9·0	58·7	16·3
8	M/25	42	11	16·9	34·8	2·4
Mean	30	41	40	4·0	16·7	2·7
−SE				2·6	11·6	1·7
+SE				6·1	23·9	4·3

* Geometric mean.
A vs. B: $P=0·0003$; A vs. C: $P=0·2428$; B vs. C: $P=0·0001$.

Table 2. Iron absorption from 5 mg of ferritin iron and from 5 mg of ferrous sulphate in the presence or absence of food (Farina meal)

				Iron absorption*			
				+Food		−Food	
Subject	Sex/age	Packed cell volume (%)	Serum ferritin* (ng L^{-1})	Ferrous sulphate (% of dose)	Ferritin (% of dose)	Ferrous sulphate (% of dose)	Ferritin (% of dose)
1	M/33	49	261	3·5	2·2	15·0	2·2
2	M/34	49	139	23·4	13·9	6·7	3·8
3	F/38	36	86	18·2	3·6	49·9	8·1
4	M/25	45	81	5·0	2·6	15·0	2·1
5	F/32	41	76	5·4	2·0	30·2	1·3
6	F/27	43	53	15·4	6·4	22·2	6·4
7	F/35	47	42	1·0	0·9	25·0	8·3
8	F/29	38	39	9·7	3·1	10·7	2·2
9	F/32	39	8	12·9	4·3	90·7	12·1
10	F/32	35	4	16·6	3·1	54·2	2·4
Mean	32	42	38	8·2	3·2	24·1	3·8
+1 SE			57	6·0	4·1	18·7	4·8
−1 SE			24	11·1	2·6	31·0	3·0

* Geometric mean.

other hand, the mean absorption of 2·7% observed with *in vivo* labelled liver ferritin did not differ significantly from *in vivo* labelled spleen ferritin ($P > 0·05$). Because of the significantly higher absorption observed with ferritin tagged *in vitro*, all subsequent absorption measurements were performed with ferritin labelled *in vivo*.

In study 2, absorption of 5 mg of ferritin iron was compared directly with the same dose as ferrous sulphate first with and then without food (Table 2). The 10 subjects had varied iron status with serum ferritins ranging from 4 μg L^{-1} to 261 μg L^{-1}. When taken with food, the mean absorption of ferrous sulphate iron was 8·2% as compared with a significantly lower mean of 3·2% with ferritin iron (absorption ratio = 2·5, $P < 0·05$). An even greater relative difference was observed when the two forms of iron were taken with water. Mean absorption with ferrous sulphate was 24·1% as compared with 3·8% with ferritin, which gave a mean absorption ratio of 6·4 ($P < 0·01$). Interestingly, there was a threefold reduction in the absorption of ferrous sulphate when taken with food whereas the absorption of ferritin iron was unaffected.

Similar findings were obtained in study 3 when the iron dose was increased to 50 mg (Table 3). Absorption of ferrous iron averaged 2·6% with food and was threefold higher when taken with water. The absorption of ferritin iron was again much lower than ferrous sulphate and was unaffected by food administration as reflected in mean absorption values of 0·6% and 0·7% with and without food respectively. The differences in absorption

Table 3. Iron absorption from 50 mg of ferritin iron and from 50 mg of ferrous sulphate in the presence or absence of food (Farina meal)

				Iron absorption*			
				+Food		−Food	
Subject	Sex/age	Packed cell volume (%)	Serum ferritin* (ng L^{-1})	Ferrous sulphate (% of dose)	Ferritin (% of dose)	Ferrous sulphate (% of dose)	Ferritin (% of dose)
1	M/23	45	133	2·3	0·3	3·9	0·6
2	F/36	35	115	1·1	0·4	7·0	0·2
3	F/37	38	87	1·3	0·3	10·9	1·2
4	F/31	37	52	2·5	0·2	6·9	0·4
5	F/26	40	52	1·3	0·3	6·4	0·5
6	M/31	46	43	5·7	2·2	18·9	1·2
7	M/33	44	38	7·6	3·2	3·4	4·5
8	F/33	37	34	2·5	0·5	5·7	0·2
9	F/22	39	14	5·5	2·1	25·1	2·0
Mean	30	38	52	2·6	0·6	7·9	0·7
+1 SE		27	41	2·1	0·4	6·3	0·5
−1 SE		55	66	3·3	0·9	9·9	1·0

* Geometric mean.

rates between ferrous sulphate and ferritin iron were not significant when the iron dose was increased 10 times. Thus, the mean absorption ratio when given with food of 4·4 for 50 mg of ferrous sulphate compared with 50 mg of ferritin iron did not differ significantly from the ratio of 2·5 with the 5-mg dose. Similarly, ratios of 10·7 and 6·4 were observed without food with the 50- and 5-mg iron dose respectively. It should be noted that a more direct comparison of absorption data in the two studies is not possible because of the moderate differences in iron status as reflected by mean serum ferritin values of 38 and 52 $\mu g\,L^{-1}$ in studies 2 and 3 respectively.

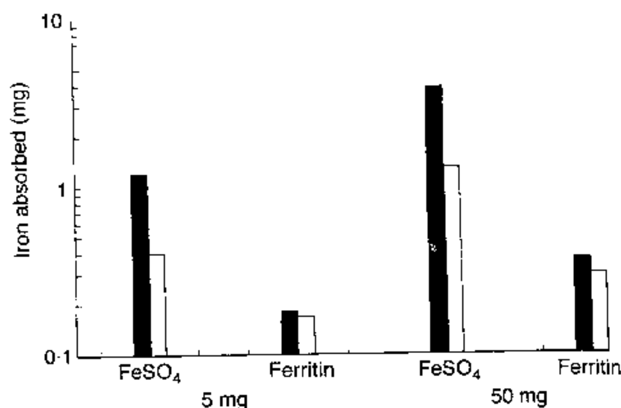

Figure 1. Absolute iron absorbed in mg from 5 mg of iron and 50 mg of iron taken as ferrous sulphate or ferritin iron without (■) and with food (□).

Discussion

The most convenient way of preparing radioactive ferritin to determine its bioavailability in human subjects would be to label *in vitro*, because producing *in vivo* radiolabelled ferritin in large domestic animals is expensive and technically burdensome. In the present study, despite apparent similarity between *in vivo* and *in vitro* labelled material when tested by various biochemical methods, a fourfold difference in the absorption of ferritin iron was noted when evaluated in humans. This significant difference presumably reflects the fact that the radioiron added to ferritin *in vitro* becomes loosely associated with the ferritin molecule, either stratified in the outer region of the protein core or perhaps situated within the channels between the subunits of the molecule. It is unlikely that the major portion of the added radioiron was incorporated into the highly insoluble iron complex within the inner core of the protein. Our findings indicate that ferritin labelled *in vitro* under the conditions that we used is not equivalent to *in vivo* labelling and is not a suitable method for bioavailability studies. Thus the remaining studies employed *in vivo* labelled ferritin.

Although sufficient *in vivo* radiolabelled spleen and liver ferritin were obtained in the present investigation to perform the required absorption measurements, the overall yield of radiolabelled ferritin was low. This may have been due to the young age of the animals and perhaps also to their relative iron-deficient status. The latter would presumably result in a rapid egress of radioactivity from the spleen following ingestion of the antibody-coated labelled red blood cells. An alternative explanation is that a significant destruction of antibody-coated red blood cells occurred within the circulation, leading to a preferential uptake of haemoglobin–haptoglobin complexes by the liver. These possibilities were not examined further in the present investigation.

Ferritin iron is widely present in food of both animal and vegetable origin, and consequently forms a substantial component of the dietary iron intake. Ferritin iron absorption is minimally influenced by the presence of food in contrast to other forms of iron. In the present study, ferritin iron absorption was not inhibited significantly when fed with a wheat-based meal, whereas ferrous sulphate absorption declined approximately threefold. This held true with doses of both 5 mg and 50 mg of administered iron. These results are similar to data presented previously by Layrisse *et al.* [19] and Derman *et al.* [6]. Another feature of ferritin iron absorption is that the absolute amount of iron absorbed increases less when higher amounts of iron are given compared with ferrous sulphate. Thus, when given with food, the absorption of ferrous sulphate increased from 409 μg with a 5-mg dose to 1315 μg with a 50-mg dose, a threefold difference. On the other hand, ferritin iron absorption increased from 162 to 300 μg with a tenfold increase in iron dose, only a twofold increase. Similar relationships held when the two forms of iron were given without food (Fig. 1). These observations suggest that ferritin iron is a relatively insoluble iron pool that equilibrates only partially with the common pool of dietary non-haem iron.

The mechanisms for the absorption of ferritin iron have not been fully elucidated, and many aspects remain controversial. Early studies indicated a lower absorption of ferritin iron compared with the common pool of non-haem iron, leading to the proposal of a third luminal iron pool [19,20]. Other workers have suggested that ferritin molecules are taken up into the mucosal cell in an intact form [21]. It is more likely that ferritin iron must first be released from the protein within the lumen of the gastrointestinal tract, and it is likely that both an acidic and a reducing environment promote this release [22,23]. Thus, Derman *et al.* [6] have suggested that the nature of the diet and its concentration of enhancing ligands, such as ascorbic acid, determines the extent of ferritin iron release within the gastrointestinal tract. The mean absorption of 2·7% from liver ferritin and 4·0% from spleen ferritin observed in the present study was similar to that reported previously by Layrisse *et al.* [19] but somewhat higher than reported by Derman *et al.* [6]. In our present study, we did not find a relationship between percentage absorption of ferritin iron and iron stores as measured by the serum ferritin concentration in individual studies, but when the results from study 2 examining absorption of 5 mg ferritin iron were pooled with those of the first study examining absorption from 2·7 mg iron

a significant although weak negative relationship between iron stores and ferritin absorption was seen ($r = 0{\cdot}479$, $P < 0{\cdot}05$). This relationship was documented in a previous study using a different animal species as a source of labelled ferritin [19], and provides support for the contention that ferritin iron is, in part, absorbed by the same mechanism that is responsible for iron uptake from the common non-haem iron pool. A specific receptor-mediated uptake of ferritin with release of iron within the mucosal cell may also be operational. This may be the reason why the inhibitory effect of food on absorption occurring with ferrous sulphate was not seen with ferritin iron. This, however, could not be confirmed in our studies.

From a historical perspective, ferritin iron has been used for iron supplementation in certain European countries because it is not associated with the gastrointestinal side-effects of inorganic iron, such as nausea and epigastric discomfort. This lack of side-effects may be because relatively large amounts of endogenous ferritin are normally present in the gastrointestinal tract [24]. However, ferritin iron is poorly absorbed. Based on the findings in our second study, 162 μg of iron was retained from a 5-mg dose in the iron-replete subjects, which would provide 10–15% of the daily iron requirement. This is far lower than the 409 μg achieved with 5 mg of ferrous sulphate. Indeed, absorption from 50 mg ferritin iron is 300 μg, lower than that from 5 mg ferrous sulphate. However, efficacy of ferritin iron has been shown in haemoglobin repletion studies [25–27]. This disparity between low intrinsic ferritin iron absorption shown by us and others and efficacy in haemoglobin repletion may be due to the ferritin protein having an enhancing effect on food iron absorption similar to that observed with other animal proteins [28]. This possibility has not been investigated. From a practical standpoint however, because of the costs involved in the purification of ferritin iron, its significantly lower intrinsic bioavailability and the recent problem with BSE, ferritin iron is not recommended for treating iron deficiency.

References

1 Harrison PM, Treffry A, Lilley TH. Ferritin as an iron-storage protein: mechanisms of iron uptake. J Inorg Biochem 1986;27:287–93.
2 Clement N, Torrance JD, Bothwell TH, Charlton RW. Iron compounds in muscle. S Afr J Med Sci 1972;37:7–14.
3 Crichton RR, Ponce-Ortiz Y, Koch MHJ, Parfait R, Stuhrmann HB. Isolation and characterisation of phytoferritin from pea (*Pisum sativum*) and lentil (*Lens esculenta*). Biochem J 1978;171:349–56.
4 Sczekan SR, Joshi JG. Isolation and characterization of ferritin from soyabeans (*Glycine max*). J Biol Chem 1987;262:13780–8.
5 Martinez-Torres C, Leets I, Taylor P, Ramirez J, del Valle Camacho M, Layrisse M. Haem, ferritin and vegetable iron absorption in humans from meals denatured of haem iron during the cooking of beef. J Nutr 1986;116:1720–5.
6 Derman DP, Bothwell TH, Torrance JD *et al.* Iron absorption from ferritin and ferric hydroxide. Scand J Haematol 1982;29:18–24.
7 Treffry A, Sowerby JM, Harrison PM. Oxidant specificity in ferritin formation. FEBS Lett 1979;100:33–6.
8 Covell AM, Worwood M. Turnover and tissue uptake of rabbit ferritin from rabbit plasma. Comp Biochem Physiol [B] 1984;77:829–34.
9 Cetinkaya N, Lengemann FW, Kogan P. Isolation, purification and characterization of bovine spleen ferritin. Comp Biochem Physiol [B] 1985;80:773–8.
10 Treffry A, Harrison PM. The binding of ferric iron by ferritin. Biochem J 1979;181:709–16.
11 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
12 Torrance JD, Bothwell TH. A simple technique for measuring iron concentrations in formalinised liver samples. S Afr J Med Sci 1968;33:9–11.
13 Eakins JD, Brown DA. An improved method for the simultaneous determination of iron-55 and iron-59 in blood by liquid scintillation counting. Int J Appl Radiat Isot 1966;17:391–7.
14 Hurrell RF, Lynch SR, Trinidad TP, Dassenko SA, Cook JD. Iron absorption in humans as influenced by bovine milk proteins. Am J Clin Nutr 1989;49:546–52.
15 Flowers CA, Kuizon M, Beard JL, Skikne BS, Covell AM, Cook JD. A serum ferritin assay for prevalence studies of iron deficiency. Am J Hematol 1986;23:141–51.
16 Brown E, Hopper J Jr, Hodges JL *et al.* Red cell, plasma, and blood volume in healthy women measured by radiochromium cell-labeling and hematocrit. J Clin Invest 1962;41:2182–90.
17 Hosain F, Marsaglia G, Finch CA. Blood ferrokinetics in normal man. J Clin Invest 19167;46:1–9.
18 Cook JD, Layrisse M, Finch CA. The measurement of iron absorption. Blood 1969;33:421–9.
19 Layrisse M, Martinez-Torres C, Renzy M, Leets I. Ferritin iron absorption in man. Blood 1975;45:689–98.
20 Martinez-Torres C, Renzi M, Layrisse M. Iron absorption by humans from hemosiderin and ferritin, further studies. J Nutr 1976;106:128–35.
21 Modesti A, Ceccarelli G, De Masi E, Caramia FG. Investigation on intestinal absorption of ferritin using electron microscopic procedures. Curr Ther Res 1985;37:1081–90.
22 Theil EC. The ferritin family of iron storage proteins. Adv Enzymol Relat Areas Mol Biol 1990;63:421–49.
23 Watt GD, Jacobs D, Frankel RB. Redox reactivity of bacterial and mammalian ferritin: is reductant entry into the ferritin interior a necessary step for iron release. Proc Natl Acad Sci USA 1988;85:7457–61.
24 Skikne BS, Whittaker P, Cooke A, Cook JD. Ferritin excretion and iron balance in humans. Br J Haematol 1995;90:681–7.
25 Del Signore R, Baroni MC, Mineo F *et al.* Clinical efficacy of ferritin as a well tolerated iron donor in sideropenic anemias. Acta Therapeut 1987;13:185–92.
26 Layrisse M, Martinez-Torres C, Ceccarelli G *et al.* Iron absorption from ferritin: Its possible utilization in the treatment of iron deficiency anemia. Curr Ther Res 1986;40:248–54.
27 Fochi F, Ciampini M, Ceccarelli G. Efficacy of iron therapy: A comparative evaluation of four iron preparations administered to anemic pregnant women. J Intern Med 1995;13:1–11.
28 Lynch SR, Hurrell RF, Dassenko SA, Cook JD. In: Mineral Absorption in the Monogastric GI tract, Dintzis FR, Laszlo JA, eds. New York: Plenum Press, 1989: 117–32.