A micronised, dispersible ferric pyrophosphate with high relative bioavailability in man

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Ferric pyrophosphate is a water-insoluble Fe compound used to fortify infant cereals and chocolate-drink powders as it causes no organoleptic changes to the food vehicle. However, it is only of low absorption in man. Recently, an innovative ferric pyrophosphate has been developed (Sunactive Fe™) based on small-particle-size ferric pyrophosphate (average size 0.3 μm) mixed with emulsifiers, so that it remains in suspension in liquid products. The aim of the present studies was to compare Fe absorption of micronised, dispersible ferric pyrophosphate (Sunactive Fe™) with that of ferrous sulfate in an infant cereal and a yoghurt drink. Two separate Fe absorption studies were made in adult women (ten women/study). Fe absorption was based on the erythrocyte incorporation of stable isotopes (57Fe and 58Fe) 14 d after the intake of labelled test meals of infant cereal (study 1) or yoghurt drink (study 2). Each test meal was fortified with 5 mg Fe as ferrous sulfate or micronised, dispersible ferric pyrophosphate. Results are presented as geometric means. There was no statistically significant difference between Fe absorption from micronised, dispersible ferric pyrophosphate- and ferrous sulfate-fortified infant cereal (3.4 and 4.1 % respectively; \( P = 0.24 \)) and yoghurt drink (3.9 and 4.2 % respectively; \( P = 0.72 \)). The results of the present studies show that micronised, dispersible ferric pyrophosphate is as well absorbed as ferrous sulfate in adults. The high relative Fe bioavailability of micronised, dispersible ferric pyrophosphate indicates the potential usefulness of this compound for food fortification.

Iron absorption: Iron fortification: Ferric pyrophosphate: Sunactive Fe™

Food fortification programmes are usually considered the most cost-effective and sustainable approach to combat Fe deficiency. However, the success of an Fe fortification programme depends largely on the careful choice of the Fe compound (Hurrell, 1997, 1998). A cheap and highly bioavailable Fe compound that causes no organoleptic changes would be the ideal fortification compound. Unfortunately, the water-soluble compounds, which are the most bioavailable, for example, ferrous sulfate, often cause unacceptable colour or flavour changes in the food vehicle (Hurrell & Cook, 1990). Ferric pyrophosphate is a water-insoluble Fe compound often used by European food companies to fortify infant cereals and chocolate-drink powders. Its main advantage is that it causes no adverse colour and flavour changes to food vehicles. However, it is only poorly soluble in dilute acid, such as the gastric juice, and is thus only of mediocre absorption in man. Human studies have reported absorption values between 15 and 75 % relative to ferrous sulfate, depending on batch and processing (Hurrell et al. 1989, 1991, 2000). A further disadvantage of ferric pyrophosphate is that it cannot be used to fortify liquid products due to its water insolubility.

Recently, a micronised, dispersible ferric pyrophosphate has been developed for food fortification. This innovative compound (Sunactive Fe™; Taiyo Kagaku (Yokkaichi, Japan)) is produced from ferric chloride and sodium pyrophosphate using a dispersion technique resulting in ferric pyrophosphate particles of very small average size (approximately 0.3 μm). Further, the formation of agglomerates is avoided by adding emulsifiers. This has the additional advantage that the micronised ferric pyrophosphate is dispersible in aqueous solutions and can be used to fortify liquid foods or drinks such as milk. Micronised, dispersible ferric pyrophosphate has been reported to have a similar bioavailability as ferrous sulfate in rat Hb repletion studies (Juneja et al. 2003).

The aim of the present study was to compare Fe absorption from micronised, dispersible ferric pyrophosphate (Sunactive Fe™) with ferrous sulfate. Fe absorption was measured in healthy women from a wheat-based infant cereal and a yoghurt drink by using a stable-isotope

Abbreviation: RBV, relative bioavailability.

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technique based on the incorporation of Fe stable isotopes into erythrocytes 14 d after administration.

**Subjects and methods**

**Subjects**

Twenty apparently healthy adult women (20–30 years; maximum body weight 60 kg) were recruited from the student and staff population at the Swiss Federal Institute of Technology Zurich and the University of Zurich. The subjects were randomly allocated into two separate studies (ten subjects/study). Exclusion criteria included pregnancy or lactation and known gastrointestinal or metabolic disorders. No medication (except oral contraceptives) or vitamin and mineral supplements were allowed during the study. Women regularly taking vitamin and mineral supplements discontinued the supplementation 2 weeks before the start of the study.

The study protocol was reviewed and approved by the ethical committee at the Swiss Federal Institute of Technology Zurich, Switzerland. Subjects were informed orally and in writing about the aims and procedures of the study. Written informed consent was obtained from all study subjects.

**Study design**

Fe absorption was based on erythrocyte incorporation of Fe stable-isotope labels 14 d after the intake of labelled test meals. The Fe compounds were labelled with $^{57}$Fe or $^{58}$Fe and added to the different test meals as described later. All test meals were fed, after an overnight fast, on two consecutive days under strictly standardised conditions and close supervision. A crossover study design was used with each woman acting as her own control. On the day before the intake of the first test meal (day 0), a venous blood sample was drawn after an overnight fast for the determination of Fe status parameters (Hb, and plasma ferritin) and body weight and height were measured. The two test meals were fed on the following days (days 1 and 2) between 07.00 and 09.00 hours. No intake of food or fluids was allowed for 3 h after the test-meal intake. A second venous blood sample was drawn 14 d after the intake of the second test meal (day 16).

**Test meals**

The test meals in study 1 consisted of 50 g roller-dried wheat-based infant cereal (Nestlé PTC, Orbe, Switzerland) fed with reconstituted milk (8 g Sano Lait milk powder; Coop Schweiz, Basel, Switzerland, and 75 ml deionised water). The infant cereal was made from 79.7 % partially hydrolysed wheat flour, 10 % sucrose, 4 % honey, 3 % palm oil, 0.3 % calcium carbonate and 3 % water. Except for Ca, no minerals or vitamins were added. The test meals in study 2 consisted of a yoghurt drink made from 170 g unskimmed yoghurt (Joghurt Nature 3.5 % fat; Migros Bio, Zurich, Switzerland) and 100 g unskimmed milk (Vallflora 3.8 % fat; Migros, Zurich, Switzerland). Each test meal contained 5 mg added Fe, 4 mg Fe as $^{58}$FeSO$_4$ plus 1 mg Fe as FeSO$_4$ of natural isotopic composition or 5 mg Fe as micronised, dispersible $^{57}$Fe ferric pyrophosphate. Deionised water (200 g) was served as a drink in study 1.

**Stable isotope labels**

$^{58}$FeFerrous sulfate was prepared from isotopically enriched elemental Fe (Chemgas, Boulogne, France) by dissolution in sulfuric acid and dilution to the appropriate concentration. Micronised, dispersible $^{57}$Fe ferric pyrophosphate was prepared from isotopically enriched elemental Fe (Chemgas, Boulogne, France) by firstly dissolving the elemental Fe in concentrated HCl. Formed $^{57}$FeCl$_2$ was oxidised to $^{57}$FeCl$_3$ by the addition of H$_2$O$_2$ (30 %, v/v). To remove impurities (iron oxides), the soluble $^{57}$FeCl$_3$ was extracted into diethyl ether, followed by re-extraction into deionised water. This FeCl$_3$ solution was evaporated under vacuum at 80°C using a rotovap (Rotavapor; Buechi, Flawil, Switzerland). Thereafter, the resulting dark red paste was crystallised to bright yellow $^{57}$FeCl$_3$·6H$_2$O. From this base compound, micronised, dispersible $^{57}$Fe ferric pyrophosphate was produced by Taiyo Kagaku (Yokkaichi, Japan) by mixing $^{57}$FeCl$_3$·6H$_2$O, emulsifiers (enzymically hydrolysed soya lecithin and polyglycerol fatty acid ester) and sodium pyrophosphate (Nanbu et al. 1998). Particle size was measured using a submicron particle sizer (NicoComp 370; Particle Sizing Systems, Santa Barbara, CA, USA) and the labelled compound was found to be equivalent to commercial Sunactive Fe$^{57}$ with respect to particle-size distribution (average particle size 0.24 μm; Fig. 1) and visual appearance. As a comparison, the particle-size distribution of a commercial food-grade ferric pyrophosphate (Dr Paul Lohmann Ltd, Emmerthal, Germany) was measured by laser light diffraction (Masterizer X; Malvern Instruments Ltd, Malvern, UK; Fig. 1).

**Fig. 1.** Particle-size distribution shown as relative volume percentage frequency curve of $^{57}$Fe-labelled micronised, dispersible ferric pyrophosphate ($^{57}$Fe Sunactive Fe$^{57}$; Taiyo Kagaku, Yokkaichi, Japan) (−○−). For comparison, the particle-size distribution of commercial micronised, dispersible ferric pyrophosphate (Sunactive Fe$^{57}$; Taiyo Kagaku, Yokkaichi, Japan) (−●−) and commercial ferric pyrophosphate (Dr Paul Lohmann Ltd, Emmerthal, Germany) (−▲−) are shown. Particle-size distribution was measured by laser diffraction (NicoComp 370; Particle Sizing Systems, Santa Barbara, CA, USA and Mastersizer X; Malvern Instruments Ltd, Malvern, UK).
Quantification of iron isotopes in labelled iron fortificants

Isotope-dilution MS was used to determine the concentration of $^{57}$Fe and $^{58}$Fe stable isotopes in the micronised, dispersible ferric pyrophosphate and ferrous sulfate solutions. An accurately measured amount of Fe of natural isotopic composition was added to samples taken from the prepared solutions of labelled Fe fortificants. The Fe standard was prepared gravimetricically from an isotopic reference material (IRM-014; EU Institute of Reference Materials, Geel, Belgium). Isotopic analysis was performed using negative thermal ionisation MS (Walczyk, 1997). Fe concentrations in each labelled Fe fortificant solution were calculated based on the shift in Fe isotopic abundances, the determined isotopic abundances of the pure isotopic labels and the natural Fe isotopic abundances (Walczyk et al. 1997).

Iron status measurements

Venous blood samples (7 ml) were drawn in EDTA-treated tubes at each sampling. Samples were analysed for Fe status indices (Hb, plasma ferritin) and for the incorporation of $^{57}$Fe and $^{58}$Fe into erythrocytes (day 16). Whole blood samples were portioned for the analysis of Hb and isotopic composition and plasma was separated, sampled and frozen for the later analysis of plasma ferritin. Hb was measured by the cyanmethaemoglobin method (Sigma kit; Sigma, St Louis, MO, USA) and plasma ferritin by ELISA (Ramco Laboratories, Houston, TX, USA). Commercial quality-control materials (DiaMed, Cressier sur Morat, Switzerland and Ramco Laboratories, Houston, TX, USA) were analysed together with the samples analysed for Hb and plasma ferritin respectively.

Quantification of iron isotope in blood

Each isotopically enriched blood sample was analysed in duplicate for its Fe isotopic composition as previously described by Walczyk et al. (1997). The blood samples were mineralised by microwave digestion using a mixture of HNO$_3$ and H$_2$O$_2$. Fe was separated from the matrix by anion-exchange chromatography and a solvent–solvent extraction step into diethyl ether. Isotopic analyses were performed by negative thermal ionisation MS (Walczyk, 1997).

Calculation of iron absorption

The amounts of $^{57}$Fe and $^{58}$Fe isotopic labels in blood 14 d after the test-meal administrations were calculated based on the shift in Fe isotope ratios and on the amount of Fe circulating in the body. The calculations were based on the principles of isotope dilution and took into account that the Fe isotopic labels were not monoisotopic (Walczyk et al. 1997). Circulating Fe was calculated based on blood volume and Hb concentration (Kastenmayer et al. 1994). Blood volume calculations were based on height and weight according to Brown et al. (1962). For calculations of fractional Fe absorption, 80 % incorporation of the absorbed Fe into erythrocytes was assumed (Hosein et al. 1967).

Food analysis

All test-meal components (infant cereal and milk powder, milk and yoghurt) were analysed for Fe and Ca by electrothermal–flame atomic absorption spectroscopy (SpectrAA 400; Varian, Mulgrave, Australia) after mineralisation by microwave digestion (MLS-Ethos plus; Mikrowellen-Labor-Systeme, Leutkirch, Switzerland) in a HNO$_3$–H$_2$O$_2$ mixture, using a standard addition technique to minimise matrix effects. Phytic acid in the infant cereal was determined by a modification of the Makower method (Makower, 1970) in which Ce replaced Fe in the precipitation step.

Statistics

Fractional Fe absorption values are presented as geometric means and standard deviations (−1 SD, +1 SD). Student’s paired t test was used to evaluate absorption data within each study. Absorption values were logarithmically transformed before statistical analysis (Excel 2002; Microsoft Corporation, Redmond, WA, USA).

Results

None of the subjects were found to be anaemic (Hb<120 g/l). However, nine women had no Fe stores indicated by low plasma ferritin values (<12 µg/l).

The test meals in study 1 (infant cereal) contained 0·6 mg Fe (1·1 mg Fe/100 infant cereal, 0·15 mg Fe/100 g milk powder), 167 mg Ca (148 mg Ca/100 g infant cereal, 115 mg Ca/100 g milk powder) and 84 mg phytic acid (168 mg phytic acid/100 g infant cereal). The yoghurt drink served in study 2 contained 0·06 mg Fe (23 µg Fe/100 g unskimmed milk, 22 µg Fe/100 g unskimmed yoghurt), and 340 mg Ca (109 mg Ca/100 g unskimmed milk, 137 mg Ca/100 g unskimmed yoghurt). The ascorbic acid content was not measured as it was assumed to be negligible in both test meals.

There was no statistically significant difference between Fe absorption from the micronised, dispersible ferric pyrophosphate- and the ferrous sulfate-fortified infant cereal (mean difference 5·4 and 4·1 % respectively; P=0·24) (Table 1). There was also no statistically significant difference between Fe absorption from the micronised, dispersible ferric pyrophosphate- and the ferrous sulfate-fortified yoghurt drink (mean difference 3·9 and 4·2 % respectively; P=0·72) (Table 2).

Discussion

When measuring Fe absorption from Fe fortification compounds using stable or radioisotope techniques it is extremely important that the physical and chemical properties of the labelled compounds are comparable with those of their commercial counterpart. In the case of ferrous sulfate, it is relatively easy to prepare a labelled compound with physical and chemical properties similar to commercially available ferrous sulfate. The production of labelled micronised, dispersible ferric pyrophosphate was however more complex. This was mainly due to the necessity to synthesise...
ferric trichloride in the hexahydrate form from isotopically enriched metal, free of acid residues and iron oxides. The labelled micronised, dispersible ferric pyrophosphate was made using a down-scaled manufacturing procedure similar to the commercial production procedure and the resulting compound was found to have a similar particle-size distribution as the commercial compound (Fig. 1).

The results of the present studies showed that micronised, dispersible ferric pyrophosphate is as well absorbed as ferrous sulfate from a wheat-based infant cereal as well as from a yoghurt drink. In previous studies with adult subjects, ferric pyrophosphate has been reported to have a relative bioavailability (RBV) compared with ferrous sulfate (RBV 100 %) varying from 15 to 75 %. In infant cereals, the values reported were between 15 and 39 % (Hurrell et al. 1989, 1991, 2000). The high RBV of Fe from micronised, dispersible ferric pyrophosphate, as demonstrated in the present study, is probably related to the extremely small particle size of the Fe compound which is approximately twenty times smaller than regular ferric pyrophosphate (average particle size 7·5 μm; Fig. 1). In rat studies, decreasing the particle size of water-insoluble Fe compounds has previously been shown to have a positive influence on Fe absorption. Shah & Belonje (1973), for example, showed that the RBV of electrolytic Fe powder increased from 12 to 32 % when the proportion of particles below 10 μm was increased from 62 to 99 %. Further, Motzok et al. (1975) demonstrated that decreasing particle size of CO-reduced Fe powders from 24–40 μm to 7–10 μm increased RBV from 11 to 31 %. Fe absorption from ferric orthophosphate has also been shown to be dependent on particle size as

<table>
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<th>Subject no.</th>
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<th>Ferrous sulfate</th>
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* Taiyo Kagaku, Yokkaichi, Japan.
† Fe absorption from ferrous sulfate = 100 %.

**Table 1.** Iron absorption by ten healthy adult women from infant cereal (study 1) fortified with ferrous sulfate or micronised, dispersible ferric pyrophosphate (Sunactive Fe'e*) (5 mg iron/meal)

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<tr>
<th>Subject no.</th>
<th>Plasma ferritin (μg/l)</th>
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<td>1·7</td>
<td>157</td>
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</table>

* Taiyo Kagaku, Yokkaichi, Japan.
† Fe absorption from ferrous sulfate = 100 %.

**Table 2.** Iron absorption by ten healthy adult women from yoghurt drink (study 2) fortified with ferrous sulfate or micronised, dispersible ferric pyrophosphate (Sunactive Fe'e*) (5 mg iron/meal)
RBV increased nearly 8-fold (from 6 to 46%) when particle size was decreased from approximately 15 μm to below 1 μm (Harrison et al. 1976). In human subjects, Björn-Rasmussen et al. (1977) reported that Fe absorption from hydrogen-reduced elemental Fe powders was dependent on their solubility in dilute acid, which in turn was partly dependent on particle size and active surface area. In the present study, it was not technically feasible to produce labelled ferric pyrophosphate with the same particle size distribution as Sunactive Fe™ without the addition of emulsifiers. Therefore, we were not able to evaluate if the high RBV of micronised, dispersible ferric pyrophosphate was only due to the small particle size or whether the emulsifiers influenced Fe absorption significantly.

Based on the results from the present studies, micronised, dispersible ferric pyrophosphate could be a very useful Fe fortificant, especially since it can be expected to cause fewer organoleptic problems than water-soluble Fe compounds. Extensive organoleptic studies, however, still remain to be carried out. Presently, Sunactive Fe™ is being used in Japan to fortify milk and milk products. Milk products have previously been shown to be difficult to fortify with readily absorbable Fe due to organoleptic problems (Demott, 1971; Edmondson et al. 1971; Kurtz et al. 1973; Wang & King, 1973). Fe fortificants that have been shown to be suitable for fluid milk fortification include ferric ammonium citrate, ferrous bisglycinate and encapsulated ferrous sulfate (Edmondson et al. 1971; Wang & King, 1973; Boccio et al. 1997; Olivares et al. 1997). While ferrous bisglycinate would be expected to be at least as well absorbed as ferrous sulfate (Fox et al. 1998), if not better (Bovell-Benjamin et al. 2000; Layrisse et al. 2000), ferric ammonium citrate has been reported to be less well absorbed than ferrous sulfate (Grebe et al. 1975; Layrisse et al. 1976; Gonzalez et al. 2001).

In addition to milk products, micronised, dispersible ferric pyrophosphate is potentially a suitable Fe fortificant for food vehicles that are difficult to fortify with readily available Fe such as chocolate-drink powders, cereal products, iodised salt, and bouillon cubes. Further, the overall acceptability of simulated rice grains (Kapanidis & Lee, 1996) may be improved by using micronised, dispersible ferric pyrophosphate instead of ferrous sulfate as less discoloration of fortified rice grains can be expected.

Although not statistically different, absorption from micronised, dispersible ferric pyrophosphate relative to ferrous sulfate was somewhat lower from the infant cereal than from the yoghurt drink in the present study (Tables 1 and 2). The differences in relative Fe absorption from different meals could be related to the differences in the dissolution of micronised, dispersible ferric pyrophosphate in the gastric juice as well as gastric emptying rate which both depend on meal composition (Hallberg et al., 1986). Further studies are needed to evaluate the RBV of micronised, dispersible ferric pyrophosphate added to different food vehicles.

In conclusion, the results of the present studies show that Fe absorption from micronised, dispersible ferric pyrophosphate (Sunactive Fe™) is similar to that of ferrous sulfate from a fortified infant cereal as well as from a fortified yoghurt drink. The high RBV is presumably due to the very small particle size. Micronised, dispersible ferric pyrophosphate can be expected to provoke fewer unacceptable sensory changes than water-soluble Fe compounds in different food vehicles; however, comprehensive sensory studies are now needed to fully evaluate the usefulness of this compound.

Acknowledgement

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References


