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ORIGINAL CONTRIBUTION

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Relative bioavailability of micronized, dispersible ferric pyrophosphate added to an apple juice drink

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Abstract *Background* Food iron fortification is a sustainable and relatively simple strategy to reduce/prevent iron deficiency but is a challenge for the food industry because of possible adverse organoleptic changes caused by the added iron. A micronized dispersible ferric pyrophosphate, trademarked as SunActive Fe®, has recently been developed. SunActive Fe® has a small particle size, is water soluble and may be suitable for fortifying liquid products. *Aim of the study* To determine the relative bioavailability of SunActive Fe® and its suitability for addition to pure apple juice. *Methods* Iron absorption from SunActive Fe® added to pure apple juice (Minute Maid®) was compared with absorption from ferrous sulphate, a highly bioavailable form of iron, in 15 women with relatively low iron stores. Both forms of iron were enriched with an iron stable isotope and iron absorption from the apple juice drinks was calculated from the isotopic enrichment of red blood cells 14 days after the last test meal. *Results* Although mean absorption of iron from SunActive Fe® was significantly lower than from ferrous sulphate (5.5% compared with 9.1%), the mean bioavailability of SunActive Fe® iron relative to ferrous sulphate was 0.6, indicating that it is a good source of bioavailable iron. Iron Absorption from SunActive Fe® was positively correlated ($r = 0.97$, $P = 0.01$) with absorption from ferrous sulphate, and negatively correlated with serum ferritin concentration (ferrous sulphate $r = -0.81$, $P < 0.001$; SunActive Fe® $r = -0.76$, $P = 0.01$). *Conclusions* SunActive Fe® was well absorbed from apple juice and is a potentially useful fortificant for liquid food products.

Key words iron absorption – fortification – SunActive Fe® – ferric pyrophosphate – iron bioavailability

Introduction

Iron deficiency anaemia is the most common nutritional deficiency disorder, affecting approximately 500 million people worldwide [1] with infants, children and pre-menopausal women most at risk. Since only a fraction of iron ingested in the diet is actually absorbed (on average 15% from Western type diets), the cause of nutritional deficiency is often related to low bioavailability rather than inadequate intake of iron. Strategies for combating iron deficiency include iron supplementation, food diversification and food fortification. The latter is recognised as a sustainable,

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relatively simple and realistic way to reduce and prevent iron deficiency but is a challenge for the food industry because of adverse organoleptic changes caused by the added iron during preparation or storage [8, 9]. Forms of iron which are water soluble and highly bioavailable, e.g. ferrous sulphate, often cause sensory problems when added to foods while forms which are less soluble in water cause fewer problems but are not generally well absorbed and are therefore not effective fortificants. One strategy for overcoming this problem is to reduce the particle size of poorly water-soluble iron compounds to increase their dissolution rate and thereby improve bioavailability.

A micronized dispersible ferric pyrophospate (MDFP), trademarked as SunActive Fe®, with a very small particle size, coated in monoglycerides and diglycerides to minimize particle aggregation, has recently been developed [14] and was reported to have high relative bioavailability in humans when added to a wheat-milk infant drink and a yogurt drink [3]. MDFP was developed for addition to liquid products but its high bioavailability makes it potentially useful in any food that may be subject to sensory change when fortified with water-soluble iron compounds. Although MDFP is a promising fortificant, the effect of food matrix or food processing on bioavailability is unpredictable [5] and bioavailability cannot necessarily be extrapolated from previously studied foods.

Intestinal iron absorption by human volunteers can be measured using stable isotope techniques in order to study different aspects of iron bioavailability. Erythrocyte incorporation of the absorbed isotopic label 14–18 days after oral administration of a test dose or meal is a direct measure of iron bioavailability [6, 11] and this technique has been successfully used in many studies [2].

MDFP has been considered as a possible fortificant for drinks and the aim of this study was to determine the bioavailability, relative to ferrous sulphate, of MDFP and its suitability for addition to pure apple juice. Absorption of stable isotope enriched SunActive Fe® added to a pure apple juice drink was compared with stable isotope enriched ferrous sulphate using a randomized cross-over study design.

Methods

Subjects

A total of 15 women aged between 18 and 65 years, with iron stores at the lower end of the normal range, were recruited for the study. Subjects with chronic or acute illness, BMI > 35, or those taking regular medication that could affect iron absorption were excluded. Women with low iron stores were chosen to reflect a target population that could benefit from iron fortified drinks and also to maximise iron absorption and isotopic enrichment of the blood. The study was approved by the Norfolk1 Research Ethics Committee and written informed consent was obtained from all subjects. A 10 ml fasting blood sample was taken from the subjects to exclude those whose biochemical and haematological indices fell outside the normal range. Subjects with serum ferritin concentration less than 12 μg/l (indicative of iron deficiency) or more than 50 μg/l were also excluded.

Test meals

Subjects attended the Human Nutrition Unit (HNU) at the Institute of Food Research for test meals and blood sampling. Test drinks containing added iron, labelled with stable isotopes of iron (Fe-58 enriched ferrous sulphate or Fe-57 enriched SunActive Fe®), were consumed on two consecutive days.

On day 1, a 15 ml venous blood was taken from fasting subjects and iron-isotope and iron-status indices were measured (haemoglobin, serum ferritin, serum iron, total iron binding capacity, and C-reactive protein). Subjects were given 500 ml pure apple juice, with no added ascorbic acid, (Minute Maid®, Coca-Cola, Brussels, Belgium), containing approximately 4.2 mg of stable isotope enriched iron that was added to the apple juice immediately before consumption. A standard serving of Minute Maid® apple juice was 250 ml and if fortified would contain 2.1 mg of added iron (15% of recommended daily intake). Volunteers were given 2 × 250 ml servings in order to ensure sufficient isotope enrichment in the blood. 1.5 h after consuming the apple juice drink, subjects were given a standard breakfast containing low quantities of inhibitors or enhancers of iron absorption [white toast with butter and jam and the choice of additional fruit (bananas and melons)]. 1.5 h after consuming breakfast, subjects were given another 500 ml of pure apple juice drink, containing 4.2 mg added stable isotope enriched iron. A lunch consisting of foods containing low levels of inhibitors or enhancers of iron absorption (white bread sandwiches with cheese or egg filling, fruit cake and apple) was provided 1.5 h after consuming the apple juice drinks. Drinking water was freely available throughout the day. The 1.5 h intervals between test drinks and meals were to minimize any possible effect of enhancers or inhibitors of iron absorption contained in the meals.

On day 2, following an overnight fast, subjects attended the HNU and consumed apple juice containing added iron, breakfast and lunch as for day 1. Subjects

given iron as ferrous sulphate on day 1 were given iron as SunActive Fe® on day 2 and vice versa. Volunteers reported no sensory problems or adverse events following consumption of the apple juice drinks containing added iron. Fourteen days after the last test drink was consumed (day 15), a 15 ml venous blood sample was taken from fasting subjects for determination of iron-status indices (haemoglobin, serum ferritin, serum iron, total iron binding capacity, and C-reactive protein) and iron-isotope enrichment of red blood cells.

Iron absorption from the apple juice drinks was calculated from the isotopic enrichment of red blood cells 14 days after the last test meal, the total iron concentration in whole blood and an estimate of blood volume, as previously described by Roe et al. [16]. The utilization of absorbed iron into red blood cells was assumed to be 80% [12].

Preparation of stable isotope enriched iron

Ferrous sulphate solutions were prepared from isotopically enriched elemental Fe (Fe-58 93.18 atom%; Chemgas, Boulogne, France) dissolved in acid and diluted to the appropriate concentration according to a previously developed method [4]. Micronized dispersible ferric pyrophosphate was prepared using a method modified from Fidler et al. [3]. Isotopically enriched elemental Fe (Fe-57, 95.99 atom%; Chemgas, Boulogne, France), was dissolved in concentrated HCl and then oxidized from ferrous to ferric iron by addition of hydrogen peroxide. Ferric chloride was purified by extraction into diethyl ether, followed by re-extraction into water. Aqueous ferric chloride was evaporated to near dryness under nitrogen with gentle heat and then crystallized to produce hydrated ferric chloride ($FeCl_3 \cdot 6H_2O$). The hydrated ferric chloride was then shipped to Taiyo Kagaku Ltd, Yokkaichi, Japan, for preparation of SunActive Fe® by mixing with emulsifiers (enzymatically hydrolyzed lecithin and polyglycerol fatty acid ester) and sodium pyrophosphate [14]. Physical properties of the labelled SunActive Fe® were very similar to the commercial product (Table 1).

Table 1 Characteristics of commercially available and iron-isotope enriched SunActive Fe® (mean of 2 batches)

	Commercial SunActive Fe®	SunActive Fe® Fe-57 enriched
Concentration of iron (mg/g)	11.3	11.8
Average diameter (median diameter) (nm)	159 ± 147[a]	218 ± 148[a]
Zeta potential (mV)	−40.8	−41.7

[a]SD

Analytical methods

Haemoglobin, serum iron, total iron binding capacity, transferrin saturation, serum ferritin and C-reactive protein (normal range 0–10 mg/l) were measured by the Chemical Pathology Department of the BUPA hospital (Norwich, UK).

The iron content of whole blood was calculated from the haemoglobin concentration [16] and the iron-isotope enrichment of red blood cells was measured as previously reported [16].

Statistics

To compare iron absorption from SunActive Fe® with iron absorption from ferrous sulphate, Wilcoxon matched pairs signed ranks test, STATS package R [15] was used. The level of significance was set at P values < 0.05.

Results

Subject characteristics, measured on study day 1, are shown in Table 2. All subjects had a serum ferritin concentration between 16 and 48 μg/l. The addition of SunActive Fe® or ferrous sulphate to pure apple juice in this study caused no sensory problems and there were no reported adverse events following consumption of the test meals. Volunteers did not report any differences between the two test drinks. Longer term stability of SunActive Fe® added to apple juice was not tested during this study but SunActive Fe® is reported to be stable [3].

Iron-status indices and iron absorbed from SunActive Fe® and ferrous sulphate are shown in Table 3. Mean absorption of a 9.2 mg dose of iron as SunActive Fe® was 5.5% (0.51 mg iron) compared with 9.1% (0.82 mg iron) from a 9.0 mg dose of iron as ferrous sulphate. Absorption from SunActive Fe® was significantly lower than absorption from ferrous sulphate (Wilcoxon matched pairs signed ranks test, $P < 0.001$). However, the mean bioavailability of SunActive Fe® iron relative to ferrous sulphate was

Table 2 Subject characteristics ($n = 15$)

Age (years)	47 ± 10
Body mass index (kg/m^2)	24.3 ± 4.0
Haemoglobin (g/dl)	13.7 ± 0.8
Serum ferritin (μg/l)	33 ± 11
Serum iron (μmol/l)	14.8 ± 6.5
Total iron binding capacity (μmol/l)	61.5 ± 13.5
Transferrin saturation (%)	23.4 ± 10.0

All values are mean ± SD

Table 3 Iron absorption from 9.0 mg iron as ferrous sulphate or 9.2 mg iron as SunActive Fe® added to pure apple juice

Subject	Age (years)	BMI[a] (kg/m^2)	Hb[a] (g/dl)	Ferritin[a] (μg/l)	Trans Sat[a] (%)	Absorption from ferrous sulphate		Absorption from SunActive Fe®		Relative bioavailability
						mg	%	mg	%	
1	60	22.0	12.8	40	19.7	0.50	5.5	0.34	3.7	0.67
2	51	21.0	13.1	40	13.5	0.95	10.6	0.41	4.5	0.42
3	54	33.0	13.8	39	22.7	0.35	3.8	0.16	1.8	0.46
4	58	30.5	13.5	20	27.2	1.40	15.5	0.86	9.3	0.60
5	51	24.0	13.2	48	13.6	0.35	3.8	0.21	2.3	0.59
6	36	26.5	14.2	16	36.2	1.72	19.1	0.90	9.7	0.51
7	45	21.0	13.0	24	11.4	0.87	9.7	0.59	6.4	0.66
8	36	22.0	14.9	48	20.6	0.33	3.7	0.33	3.6	0.96
9	62	20.7	13.8	47	27.6	0.41	4.5	0.12	1.3	0.29
10	38	29.0	13.1	27	21.7	0.40	4.5	0.28	3.0	0.68
11	43	21.0	13.4	26	26.4	0.09	1.0	0.04	0.4	0.43
12	62	26.0	14.6	21	21.1	1.18	13.1	0.59	6.4	0.49
13	43	26.0	13.2	39	12.9	0.62	6.9	0.52	5.6	0.82
14	42	22.1	13.9	25	25.6	2.75	30.5	2.01	21.8	0.72
15	30	20.0	15.7	31	50.3	0.34	3.8	0.24	2.6	0.68
Mean	47	24.3	13.7	33	23.4	0.82	9.1	0.51	5.5	0.60
SD	10	4.0	0.8	11	10.0	0.70	7.8	0.49	5.3	0.17

[a]Study day 1

0.6, indicating that SunActive Fe® is a good source of bioavailable iron.

There was a significant correlation between iron absorption from the two iron sources ($r = 0.97$, $P = 0.01$; Fig. 1). Log transformed serum ferritin concentration was negatively correlated with iron absorption (ferrous sulphate $r = -0.81$, $P < 0.001$; SunActive Fe® $r = -0.76$, $P = 0.01$). Total iron binding capacity was also significantly correlated with iron absorption from both ferrous sulphate ($r = -0.657$, $P = 0.008$) and SunActive Fe® ($r = -0.672$, $P = 0.006$).

Discussion

Previous studies have consistently shown iron absorption to be negatively related to serum ferritin concentration, particularly when serum ferritin is below 70 μg/l, at which point iron absorption is similar to basal iron requirements [6, 7, 16]. These relationships were upheld for both ferrous sulphate and SunActive Fe® in the present study.

SunActive Fe® has previously been reported to have a similar bioavailability to ferrous sulphate in rat haemoglobin regeneration studies [17]. However, Moretti et al. [13] concluded that the relative bioavailability of micronized dispersible ferric pyrophosphate varies according to the food vehicle (0.62 in a wheat-milk infant cereal compared with 0.15–0.25 in a rice meal). Fidler et al. [3] reported that iron absorption by adult women from SunActive Fe® added to an infant cereal and a yoghurt drink was similar to absorption of iron from ferrous sulphate.

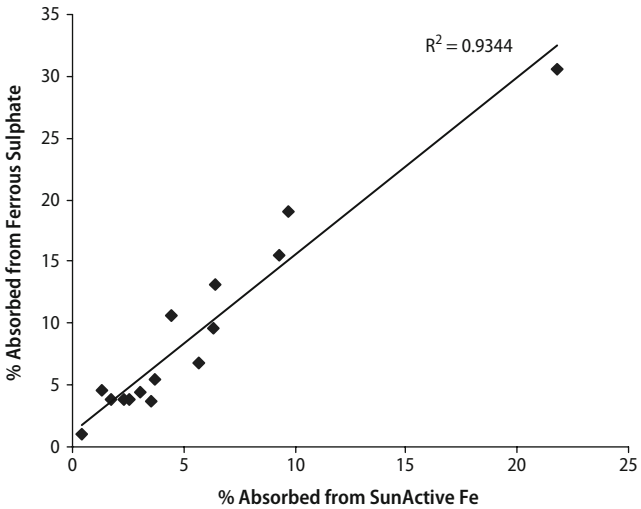

Fig. 1 Iron absorption (%) from 8.4 mg iron as ferrous sulphate and SunActive Fe® given on two different occasions to 15 women ($r = 0.97$, $P = 0.01$)

Mean iron absorption in the present study (5.5%) was similar to the mean values observed in the Fidler et al. study (3.4% from infant cereal and 3.9% from yoghurt drink). However, relative bioavailability was higher in the Fidler study [3] compared with our study (0.82 from infant cereal and 0.93 from yoghurt drink vs. 0.6 from apple juice). Absorption from SunActive Fe® was similar in both studies, but absorption of ferrous sulphate in our study was higher (9.1% from apple juice) compared with the Fidler study [3] (4.1% from infant cereal and 4.2% from yoghurt drink). The higher absorption of ferrous sulphate from apple juice

may be explained by the difference in food matrices, with wheat based infant cereal and yoghurt drink both likely to contain more inhibitors of iron absorption than apple juice, and also being associated with different rates of gastric emptying. The absence of inhibitors or enhancers of iron absorption in pure apple juice is likely to have maximised the difference in absorption between ferrous sulphate and SunActive Fe®, particularly if iron is not able to be completely solubilised from the SunActive Fe® complex with rapid gastric emptying.

The results from this study reinforce the results from the Fidler study [3] and support their conclusion that SunActive Fe® is potentially a very useful fortificant. The relatively high bioavailability of SunActive Fe® is thought to be due to the very small particle size, although it is possible that emulsifiers used in the product also facilitate iron absorption [3]. SunActive Fe® may be especially useful since it can be added to liquids with minimal organoleptic problems compared with other water-soluble iron compounds.

The relative bioavailability of iron compounds is useful for ranking the potential of iron compounds for food fortification purposes, but the efficacy of a fortified food depends on the absolute iron absorption rather than the relative bioavailability [10]. In the present study, subjects consumed 1,000 ml apple juice, containing 8.4 mg iron, each day and absorbed an average of 0.51 mg of iron from SunActive Fe®. The apple juice is commercially available in 250 ml portions and if four drinks, each containing 2.1 mg added iron (15% recommended daily intake), were consumed daily, then iron absorption of the magnitude reported here would make a significant contribution towards iron requirements and would therefore help to maintain or improve iron status.

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