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ORIGINAL CONTRIBUTION

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The relative bioavailability in humans of elemental iron powders for use in food fortification

Summary *Background* Bioavailability data in humans of elemental iron powders is limited although elemental iron is a common form of iron when used as a fortificant. *Aim of the study* The relative bioavailability (RBV) of seven elemental iron powders, five commercially available and two developmental are evaluated. In addition, one commercial electrolytic iron powder given with ascorbic acid (AA) was examined. *Methods* Based on a validated method this double-blinded randomized crossover study included three groups of male blood donors (n = 3*16) who were served rolls fortified with different elemental iron powders or ferrous sulfate ($FeSO_4$) nine weeks apart. Blood samples were drawn every hour for six hours. RBV was obtained by comparing the increase in serum iron concentration induced by the elemental iron with the increase induced by $FeSO_4$. *Results* All elemental iron powders studied were significantly less well absorbed compared to $FeSO_4$. The electrolytic iron given with 50-mg AA was as well absorbed as $FeSO_4$ (molar ratio = 1:6, AA:Fe). The mean RBVs of the iron powders were: electrolytic (A-131, RBV = 0.65); electrolytic (Electrolytic, RBV = 0.59); carbonyl (Ferronyl, RBV = 0.58); H-reduced (AC-325, RBV = 0.56); H-reduced (Hi-Sol, RBV = 0.50); carbonyl (CF, RBV = 0.37); reduced (Atomet 95SP, RBV = 0.36). The reduced iron was distinguished by having significantly lower RBV (0.36) although no significant overall ranking was possible. *Conclusions* Based on a validated method this double-blinded cross-over study in humans showed that the evaluated elemental iron powders currently available for commercial use are significantly less well absorbed compared to $FeSO_4$. The results indicate that the reduced iron powder was absorbed to a lower extent compared to the other iron powders and only 36 % compared to $FeSO_4$. Ascorbic acid seems to improve the bioavailability of elemental iron even though a rather low molar ratio is used. Thus, if confirmed, this enhancing effect of ascorbic acid on elemental iron when used as a fortificant could be used by co-fortifying them.

Key words serum iron – ferrous sulfate – elemental iron compound – iron absorption

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Introduction

A common method to manage risk for iron deficiency in a population is to add small amounts of iron to foods in the diet, i. e. fortification. The most commonly used commercial fortification iron powders are elemental iron since iron salts, such as ferrous sulphate ($FeSO_4$), can cause oxidation and undesirable organoleptic changes in the food stuffs when stored. When it comes to absorbability in human models there are very large differences between different elemental iron powders [1]. The bioavailability in humans of the elemental iron powders available on the market today is not known, which calls for studies on their nutritional value in humans. However, elemental iron can not be measured by using radioiron, which, when using whole body counting, is considered to be the present golden standard in

iron absorption methodology. A possible way of using radioiron isotopes in elemental iron absorption studies is to manufacture the elemental iron in such a way that it contains the iron isotopes. However, this can alter the physicochemical properties of the elemental iron powder so it differs from the original product. There are only a few such previous studies in human subjects using elemental iron containing radioiron. The elemental iron powders under investigation in these studies were electrolytic iron [2], carbonyl iron [3], and different kinds of reduced iron [4–7]. Even powders manufactured by the same method can vary considerably when it comes to solubility, particle size and reactive surface area [1, 8]. This suggests that physicochemical properties could be one possible factor that affects bioavailability.

Due to the lack of methods for use in humans the majority of studies on elemental iron bioavailability have been carried out in animal models. In many cases they have included studies of the relationship between metallurgical properties and absorbability. The ambition in these studies has been to find methods to estimate bioavailability in man [9]. But since there are always risks in misinterpretation when extrapolating data obtained in animals to humans this must be done with caution. Consequently it is uncertain what the observed correlation between solubility and RBV in rats [8, 10, 11] means in humans. Only a few studies have validated the results obtained through animal models with human data. A study by Reddy and Cook showed that dietary inhibitors and enhancers of non-heme iron absorption had different effects in humans and in rats [12]. When Forbes et al. used radioiron in both humans and in rats to assess the RBV of electrolytic iron, the results obtained in rats gave lower RBV values compared to the ones obtained in humans [2]. When the same group also used the rat depletion method by the Association of Official Analytical Chemists (AOAC) and compared it with the extrinsic method in humans, two different laboratories showed contradictory RBV results for the electrolytic iron powder, 0.66 and 0.77, compared to 0.75 in humans [2]. Thus, the best way to attain results useful for humans is preferably through human studies. And since the number of methods in humans to measure absorption from elemental iron powder are limited, it urged us to further develop the serum iron method used by Ekenved et al. [13]. This further developed method has been standardized and validated with radioisotope absorption measured with whole-body counting [14, 15]. It examines the increase in serum iron concentration (S-Fe) following an oral iron dose during a six hour time period and expresses it in the form of the area under curve (AUC_{0-6h}). The objective of this present study was to evaluate the bioavailability of seven elemental iron powders, relative to ferrous sulphate, by using this method. One of these elemental iron powders was also evaluated together with ascorbic acid.

Material and methods

Study design

Three groups of subjects (n = 3*16) were served bread rolls fortified with various elemental iron powders. The rolls were served approximately nine weeks apart. On the day of investigation blood samples were taken every hour during six hours, post test meal intake, in order to study the change in S-Fe. By comparing the induced change in S-Fe caused by the different elemental iron powders with the induced change in S-Fe caused by the control iron, ferrous sulphate monohydrate ($FeSO_4 \cdot H_2O$), a measure of the RBV was obtained in each subject. In this way each subject acted as his own control. In order to study the naturally occurring changes in the basal S-Fe under the conditions set up in this study, a wheat roll without iron added was also served.

Subjects

Forty-eight healthy males, all regular blood donors for several years (minimum 20 blood donations and mean 65) took part in this study. The age of the subjects ranged from 35 to 61 years (mean 50 years). As part of the exclusion criteria the subjects were asked about infections and iron supplement or medicine use. Informed consent was obtained from all subjects prior to the study. The Ethic Committee of the Medical Faculty of the University of Göteborg approved the study.

Meals and procedures

Eight weeks prior to each day of investigation the subjects had donated a ~ 450 ml blood sample at the blood donor centre at the Sahlgrenska University Hospital. This was done in order to keep the basic conditions the same for each test meal occasion. The subjects arrived at the laboratory between 08.00 and 09.00. They were not allowed to eat anything after 22.00 and not to drink anything after 24.00 the evening before. On arrival blood samples were drawn to determine initial blood parameters. The subjects were then served a test meal consisting of one wheat roll fortified with either no iron, 272 mg $FeSO_4 \cdot H_2O$ or 100 mg elemental iron. The weighed amount of iron was administered by an incision in the roll, which was baked from 40 g low-extraction wheat flour, 28 g water, 2.6 g yeast, 1.3 g sugar and 0.4 g salt in our laboratory. The native iron content in each roll was 0.15 mg. Together with the roll, 150 ml of tap water was served. After administration of the roll S-Fe blood samples were drawn every hour for six hours. Between the blood sampling the subjects remained in our laboratory

resting in a seated or supine position. During the first four hours no further food or beverage was allowed. After the fifth blood sample was drawn the subjects were served 150-ml coffee or tea and two unsweetened rusks. After each day of investigation the subjects donated blood after a minimum of two days and a maximum of seven days. Eight weeks later, counting from the day of blood donation, the next day of investigation was scheduled. In the first two groups, as a negative control, the S-Fe was also studied when the wheat roll was served without added iron. This was done in order to study the naturally occurring changes in the basal S-Fe during the time taken to carry out each day of investigation. This observed basal change in S-Fe during present standardized conditions is henceforth and throughout this paper referred to as diurnal variation. The third group was not served the negative control since the diurnal variation results obtained in the first two groups were considered constant enough to be applied in the third group as well.

Iron powders

The present study was part of an international project, commissioned by SUSTAIN[1] (Sharing United States Technology to Aid in the Improvement of Nutrition), to evaluate the bioavailability of elemental iron powders using many different methods and models [16, 17]. The elemental iron powders evaluated were chosen by SUSTAIN and were administered in a double-blind manner. A sample-coding key was submitted by SUSTAIN after the completion of the study. Seven different elemental iron powders were evaluated in this study; reduced (Atomet 95SP, Canada), H-reduced (Hi-Sol, USA), H-reduced (AC-325, USA), carbonyl (Ferronyl, USA), carbonyl (CF, Germany), electrolytic (A-131, USA) and electrolytic (Electrolytic, India). Except for elemental iron powders Hi-Sol and CF, which are developmental elemental iron powders, all are currently available commercial elemental iron powders. In an additional investigation, the electrolytic iron powder A-131 was administered with 50 mg AA. The subjects in the first group received a roll with no added iron, $FeSO_4 \cdot H_2O$, and elemental iron powders Atomet 95SP, AC-325 and Ferronyl on five different days of investigation. The subjects in the second group received a roll with no added iron, $FeSO_4 \cdot H_2O$, elemental iron powders A-131, Electrolytic and A-131 together with 50 mg AA. The subjects in the third group received a roll with $FeSO_4 \cdot H_2O$, and elemental iron powders Hi-Sol and CF on three different days of investigation.

1 Elemental iron powders and ferrous sulfate used in the present study were obtained from commercial suppliers in 2001 by the SUSTAIN (Sharing Science and Technology to Aid in the Improvement of Nutrition; Washington, DC) organization. This research was conducted collaboratively with SUSTAIN and its partners as part of its overall review of the bioavailability of elemental iron powders. Under our agreement with SUSTAIN, specific producer names have not been used, but this collection of samples reflects the products and production methods prominent on the market in 2001.

Blood samples

On arrival at the laboratory in the morning, a plastic catheter was inserted into an anticubital vein of each subject, where it remained for the study period of six hours. Initial blood samples were taken to determine erythrocyte sedimentation rate (ESR); initial S-Fe, haemoglobin concentration (Hb), and total iron binding capacity (TIBC). The methods used for analysis of TIBC and Hb were immunochemical turbometry and spectrophotometry, respectively, and were conducted at a reference laboratory (Clinical Chemistry Laboratory, Sahlgrenska University Hospital). Serum iron was determined according to a modified method described by the International Committee for Standardisation in Hematology [18]. All methods have previously been described [14].

Calculations and statistics

The iron added rolls were all designed to contain the same amount of elemental iron, i. e. 100 mg. Laboratory analysis of the control iron after the completion of the study found that it contained ferrous sulphate monohydrate ($FeSO_4 \cdot H_2O$) instead of anhydrous ferrous sulphate ($FeSO_4$) with which it was labelled. Thus, in order to compare the S-Fe response of the elemental iron powders with the S-Fe response from the same dose of iron as $FeSO_4 \cdot H_2O$ (272 mg which corresponded to 89.4 mg elemental iron), a correction was necessary. Based on the almost linear dose-response in this dosage range [13, 19–23], the individual increase in serum iron concentration following administration of 272 mg $FeSO_4 \cdot H_2O$ was divided by 0.894, giving a value corresponding to 100 mg elemental iron. After determining the change in S-Fe following each meal, the area under the curve during the six-hour time period (AUC_{0-6h}) was calculated using the trapezoidal rule. The AUC_{0-6h} for each individual's iron compound response was adjusted for diurnal variation by subtracting the AUC_{0-6h} following administration of a roll without added iron. Each elemental iron AUC_{0-6h} adjusted for diurnal variation was then compared to the corresponding $FeSO_4 \cdot H_2O$ AUC_{0-6h} adjusted for diurnal variation giving a ratio defined as RBV. One-way analysis of variance (ANOVA) with Tukey-Kramer as post-hoc test adjusting for multiple comparisons (least squares means) was used when comparing ratios within each of the three groups of subjects. ANOVA with Tukey as post hoc test was used when comparing ratios for all

different elemental iron powders. Variables used in these two analyses were subject and iron powder. ANOVA with Tukey test as post hoc was also used when comparing the difference between the AUC_{0-6h} induced by the elemental iron powders and the AUC_{0-6h} observed when no iron was added to the meal, i. e. diurnal variation. The Student's paired t-test was used when comparing dependent AUC_{0-6h} values and hour for hour increase in S-Fe after administration of a roll without added iron. All *p*-values are two-tailed and considered to be statistically significant if less than 0.05. The Statistical program used was SPSS for Windows version 10.0.5.

Results

Exclusions

Seven S-Fe curves were excluded from calculation due to infection (ESR cut-off > 15 mm). Another seven curves were excluded since the maximum S-Fe exceeded TIBC. If this happens, a part of the absorbed iron will be deposited in the liver during the first passage [24, 25]. One subject's total data were excluded from the study due to non-compliance. Another subject's total series of data was excluded due to a very marked change in iron status from one study occasion to the next. The $FeSO_4 \cdot H_2O$ response constitutes the control, which all elemental iron responses are compared against. Thus, if the $FeSO_4 \cdot H_2O$ response of an individual was excluded (n = 9), all the elemental iron responses obtained in that individual had to be excluded as well. This is the reason for such a large total number of exclusions.

The remaining subjects in the first group included in the analysis of Atomet 95SP, AC 325, and Ferronyl were 12, 14, and 14, respectively. In the second group 12, 11 and 11 subjects were included in the analysis of elemental iron powders A-131, Electrolytic and A-131 together with AA, respectively. In the third group, 14 and 9 subjects were included in the analysis of Hi-Sol and CF, respectively.

Diurnal variation

A certain proportion of the observed S-Fe increase following an administered iron powder dose is due to the basal occurring diurnal S-Fe increase. Thus, in order to determine the effect on S-Fe induced solely by an administered iron powder, the S-Fe must be corrected for basal diurnal variation. Therefore, this was done for each individual S-Fe response curve. The value used for this was the mean AUC_{0-6h} observed in 32 subjects when served a roll without any iron, which was 18.7 ± 1.7 μmol · h/L (mean ± SEM) [14]. The diurnal variation AUC_{0-6h} differed significantly from zero ($p < 0.001$).

Serum iron curves and RBV

All AUC_{0-6h} values are corrected for basal diurnal variation. In the first group the AUC_{0-6h} values of all three elemental iron powders (Atomet 95SP, AC-325 and Ferronyl) differed from $FeSO_4 \cdot H_2O$ AUC_{0-6h} ($p < 0.001$, $p < 0.001$ and $p < 0.001$, respectively). Also the RBV of Atomet 95SP (0.36 ± 0.04, mean ± SEM) was significantly lower than both AC-325 (0.56 ± 0.04) and Ferronyl (0.58 ± 0.08) (Fig. 1 and Table 1).

In the second group the AUC_{0-6h} values from $FeSO_4 \cdot H_2O$ and the two electrolytic iron powders (A-131 and Electrolytic) differed ($p < 0.023$ and $p < 0.007$). The mean RBV of the electrolytic iron powder A-131 given with AA was 99 % and significantly higher compared to the RBV of iron powder A-131 alone ($p < 0.05$) (Fig. 1 and Table 1).

In the third group the AUC_{0-6h} from $FeSO_4 \cdot H_2O$ and the elemental iron powders CF and Hi-Sol differed ($p < 0.001$ and $p < 0.001$, respectively). The RBV of iron powder CF did not differ from the RBV of Hi-Sol ($p = 0.067$) (Fig. 1 and Table 1). The AUC_{0-6h} induced by each of the evaluated iron compounds differed significantly from the diurnal variation AUC_{0-6h} ($p < 0.001$). Overall results are illustrated in Fig. 2.

Discussion

In an attempt to find an appropriate method for assessment of RBV of elemental iron powders in humans we have by standardization and validation re-established the Fe tolerance test [14, 15]. In a previous study we observed a strong correlation between the absorption measured with radioisotopes and the AUC_{0-6h} for S-Fe increase from 100 mg as $FeSO_4$ ($r^2 = 0.94$, $p < 0.001$) [15]. In this paper we have by using this method shown that the evaluated elemental iron powders currently available for commercial use are significantly less well absorbed compared to $FeSO_4 \cdot H_2O$.

This method demands administration of an iron dose that is much higher than what would be consumed in a common fortified meal. Nevertheless, recent results show that the induced serum iron increase following 100 mg Fe added to a food could predict the iron absorption of a small dose of iron added to the same meal [15].

When examining the plasma tolerance curves (Fig. 1) they show that only a fraction of the absorbed iron has been cleared within the observation period of six hours. For some of the elemental iron powders the nature of the curves differs from $FeSO_4 \cdot H_2O$ in that the uptake and the rate of decline are somewhat delayed. This could imply that in order to obtain a valid estimate of RBV an examination of total curve, i. e. back to baseline, is required. However the strong correlation between the ab-

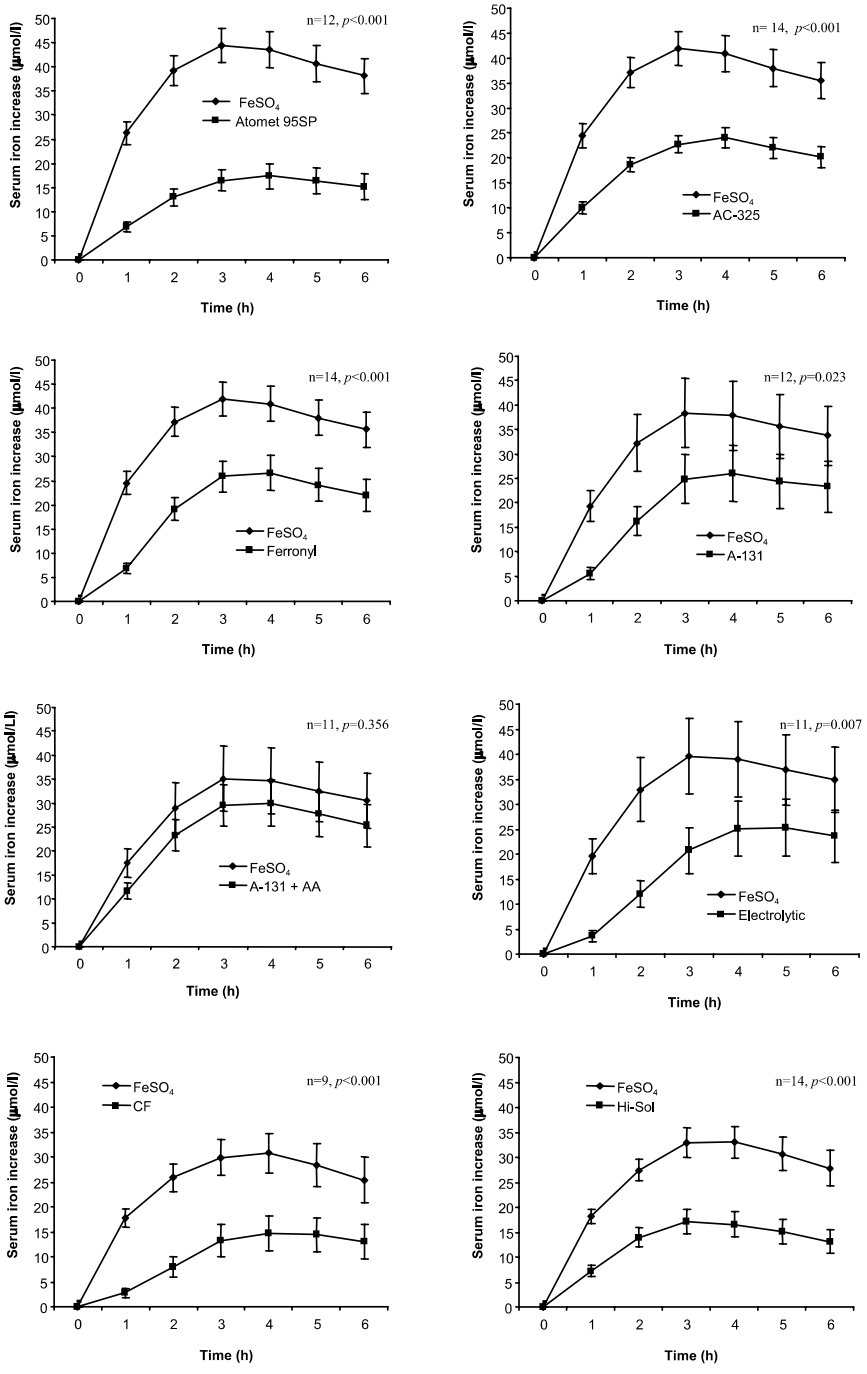

Fig. 1 Increase in serum iron concentration in healthy male blood donors after consuming 150 ml water and a wheat roll fortified with either ferrous sulphate or 100 mg elemental iron powder. Values are means ± SEM. All curves were adjusted for basal diurnal variation. The p-values relate to differences in AUC_{0-6h}

sorption measured with radioisotopes and the AUC_{0-6h} shows that this is not necessary. Furthermore, when studying the correlation we also found that the individual AUC_{0-6h} curves for some subjects were quick and steep, while for other subjects they were more delayed. Yet, there was a strong correlation [15]. A reasonable conclusion would therefore be that, as long as the S-Fe peak is passed the AUC_{0-6h} is a valid measure of iron absorption. Thus, since the correlation is valid even when the shapes of individual AUC_{0-6h} curves differ, this also applies when the shapes of mean curves differ. There were significant differences between Atomet 95SP (RBV = 0.36) versus Ferronyl (RBV = 0.58) and AC-325 (RBV = 0.56), which were evaluated in the same group of subjects.

The lack of further significant differences and RBV

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Table 1 Changes in serum iron concentration after consuming a wheat roll fortified with $FeSO_4$ or 100 mg elemental iron

Subjects			$FeSO_4$				Elemental iron powder					Ratio
n	Age (years) Mean (SEM)	Blood donations* Mean (SEM)	$S\text{-}Fe_{0h}$ (μmol/l) Mean (SEM)	$TIBC_{0h}$ (μmol/l) Mean (SEM)	Hb_{0h} (g/l) Mean (SEM)	AUC_{0-6h} [a, b] (μmol · h/l) Mean (SEM)	Product name	$S\text{-}Fe_{0h}$ (μmol/l) Mean (SEM)	$TIBC_{0h}$ (μmol/l) Mean (SEM)	Hb_{0h} (g/l) Mean (SEM)	AUC_{0-6h} [a] (μmol · h/l) Mean (SEM)	AUC_{0-6} Mean (SEM)
11	55 (2)	99 (15)	14.9 (1.7)	73 (3)	148 (3)	163.8 (30.6)	A-131 + AA	16.1 (2.4)	80 (4)	141 (2)	134.8 (20.2)	0.99 (0.18)
12	53 (2)	93 (15)	14.8 (1.6)	75 (4)	148 (3)	180.4 (32.5)	A-131	16.0 (1.4)	75 (4)	143 (2)	108.4[c] (22.5)	0.65 (0.12)
11	53 (2)	90 (16)	14.9 (1.7)	76 (4)	148 (3)	185.4 (35.2)	Electrolytic	17.7 (1.5)	74 (4)	147 (4)	98.5[c] (21.5)	0.59 (0.09)
14	48 (2)	56 (9)	14.3 (1.9)	83 (4)	145 (2)	200.6 (16.9)	Ferronyl	16.9 (2.1)	80 (2)	143 (3)	114.0[c] (14.9)	0.58 (0.08)
14	48 (2)	56 (9)	14.3 (1.9)	83 (4)	145 (2)	200.6 (16.9)	AC-325	16.5 (2.9)	83 (3)	144 (3)	107.7[c] (8.3)	0.56 (0.04)
14	43 (3)	50 (9)	18.4 (1.6)	75 (3)	152 (3)	157.6 (13.7)	Hi-Sol	14.0 (1.9)	75 (2)	147 (2)	76.7[c] (11.3)	0.50 (0.07)
9	42 (3)	45 (10)	18.9 (1.5)	76 (5)	156 (3)	146.6 (17.3)	CF	18.8 (2.3)	74 (3)	151 (3)	59.7[c] (14.2)	0.37 (0.07)
12	48 (2)	55 (11)	15.2 (2.1)	78 (4)	145 (3)	213.2 (17.0)	Atomet 95SP	17.6 (2.5)	81 (3)	146 (2)	77.7[c] (10.8)	0.36 (0.04)

* Number of blood donations before attending the study

[a] Total area under the curve for serum iron increase during six hours (AUC_{0-6h}). The AUC_{0-6h} values are corrected for basal diurnal variation. The value used was the AUC_{0-6h} obtained when the wheat roll was served without added iron

[b] The individual increase in serum iron concentration following administration of 272 mg ferrous sulphate monohydrate (corresponding to 89.4-mg elemental iron) was mathematically corrected in order to correspond to a 100 mg iron dose. See "Calculations and statistics"

[c] Significantly different from corresponding $FeSO_4$, $p < 0.05$

ranking could be explained by the possibility that the RBV of the other powders all are the same. Another explanation could be that the method, despite the Fe absorption validation, is too insensitive to detect differences in this interval.

As a consequence of the differences in RBV that were still observed, choosing the carbonyl iron, Ferronyl, for fortification purposes instead of the reduced iron, Atomet 95SP, would almost double the utilizable amount of iron. Together with economical aspects this finding can assist when considering type of fortificant to use in iron fortification programs. However, besides the importance of a fortificant iron powder's efficacy in improving iron status and economical aspects there are also other facts to consider. The possibility of negative effects of an excess of iron flowing through the intestinal lumen, for example increased lipid peroxidation, can not be ignored [26–29]. Furthermore it is most likely of little value to fortify a diet with low bioavailability. Thus, the best course of action for interventions designed to improve iron status must therefore firstly be to improve the bioavailability of the diets' native iron content and secondly to use a type of fortificant with high bioavailability.

The same elemental iron powders provided by SUSTAIN have recently been analysed for physiochemical properties and evaluated for RBV using the rat depletion method by AOAC [10]. With this method Swain et al. observed significant different RBV values in rats ranging from 0.21 to 0.64. The lack of significant different human RBV values in this present paper does not allow a proper comparison with the findings in rat by Swain et al. Nevertheless, the finding that both our human RBV and the AOAC based RBV in rats shares indicate that the reduced powder, Atomet 95SP, is considerably lower than the others.

The enhancing effect of AA on $FeSO_4$ absorption is well known, but for elemental iron, to the best of our knowledge, has only been observed twice before [2, 30]. The administered AA and electrolytic iron in the present study gives a molar ratio of ~1:6 (AA:Fe) which is below what is generally considered necessary to achieve optimal positive effect on iron absorption when AA is given together with $FeSO_4$, i. e. 2:1 [31]. Despite this, the addition of 50 mg AA, to 100 mg electrolytic iron powder A-131 had a marked effect on the iron absorption. Ascorbic acid not only enhanced the total uptake but also accelerated the early uptake.

In summary, based on double-blinded cross-over studies in humans with a validated methodology using healthy male regular blood donors we have shown that the evaluated elemental iron powders currently available for commercial use are significantly less well absorbed compared to $FeSO_4 \cdot H_2O$. The results indicate that the reduced iron powder Atomet 95SP was absorbed to a lower extent compared to the other iron powders

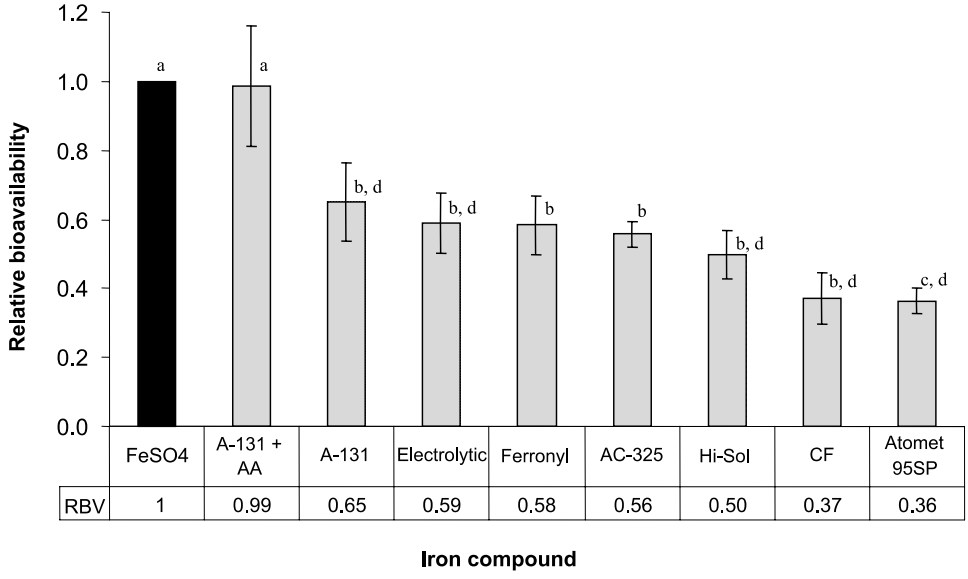

Fig. 2 Bioavailability relative to $FeSO_4$ (ratio $FeSO_4 = 1$) based on the area under the curve for induced increase in serum iron concentration in healthy male blood donors (mean ± SEM). Values are means ± SEM. Relative bioavailability values without a common superscript letter differs ($p < 0.05$)

and only 36 % compared to $FeSO_4 \cdot H_2O$. Absorption of the electrolytic iron powder A-131 with 50 mg ascorbic acid was as well absorbed as $FeSO_4 \cdot H_2O$. Thus, since RBV of elemental iron seems low this suggests that an alternative could be co-fortifying with AA when using elemental iron as fortificant. However, before applying this concept further studies are needed.

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