Eur J Nutr (2007) 46:95–102
DOI 10.1007/s00394-006-0637-4

ORIGINAL CONTRIBUTION

Huanmei Zhang
Gunilla Önning
Rickard Öste
Elisabeth Gramatkovski
Lena Hulthén

Improved iron bioavailability in an oat-based beverage: the combined effect of citric acid addition, dephytinization and iron supplementation

Received: 30 March 2006
Accepted: 21 November 2006
Published online: 16 January 2007

H. Zhang · G. Önning (✉)
Biomedical Nutrition, P.O. Box 124
Centre for Chemistry and Chemical Engineering
Lund University
22100 Lund, Sweden
E-Mail: gunilla.onning@kc.lu.se

R. Öste
Applied Nutrition and Food Chemistry
Centre for Chemistry and Chemical Engineering
Lund University
Lund, Sweden

E. Gramatkovski · L. Hulthén
Dept. of Clinical Nutrition
Sahlgrenska Academy at Göteborg University
Göteborg, Sweden

Abstract *Background* Iron deficiency in children is a major worldwide nutritional problem. An oat beverage was developed for 1- to 3-year-old children and different treatments were used to improve the iron bioavailability. *Aim of the study* To investigate the effects of citric acid addition, phytase treatment and supplementation with different iron compounds on non-heme iron absorption in human from a mineral-supplemented oat-based beverage. *Methods* A 240 g portion of a ^{55}Fe-labeled test product (*T*) or a ^{59}Fe-labeled reference dose (*R*) was served as breakfast after overnight fasting on four consecutive days in the order of TRRT. On day 18 the retention of ^{59}Fe was measured by a whole-body counter and the erythrocytes uptake of ^{55}Fe and ^{59}Fe by a liquid-scintillation counter. Forty-two healthy subjects (men and women) were randomized into four study groups, members of each being given one of the studied four products (A, B, C, and D) supplemented with Fe (1.3 mg/portion), Zn, Ca, Se and P. Ferric ammonium citrate (FeAC) was added to products A, B, and C and ferric pyrophosphate (FePP) to product D. Citric acid (60 mg/portion) was added to products B, C, and D and phytase treatment applied to products C and D. *Results* Citric acid improved iron absorption by 54% from 3.9% in product A to 6.0% in product B ($p = 0.051$). Phytase treatment increased iron absorption by 78% (from 6.0 to 10.7%, $p = 0.003$) by reducing the phytate-phosphorus content per portion from 16.3 mg in product B to 2.8 mg in product C. The two compounds gave similar iron absorption rates ($p = 0.916$). *Conclusions* A combination of citric acid addition, dephytinization and iron supplementation significantly increased the iron absorption in an oat-based beverage. Such a beverage can be useful in the prevention of iron deficiency in 1- to 3-year-old children.

Key words iron absorption – iron supplementation – phytase – citric acid – adults

EJN 637

Introduction

Iron deficiency and the anemia associated with it have long been a major nutritional problem throughout the world [1]. WHO [2001] has estimated that 39% of the children 0–4 years of age in non-industrialized countries and 20% of those in industrialized countries have iron deficiency, using anemia as an indicator [2]. To combat nutritional iron deficiency, a strategy is to add iron to food vehicles that are targeted to segments of a population for whom the risk of it is greatest, such as infants, school children and fertile women [3]. However, iron is regarded as the most difficult mineral to add to foods and to ensure adequate absorption because of the dilemma between the inhibitory effect of some dietary components and bioavailability of iron salt and between bioavailability of iron and undesirable sensory changes in the fortified foods [4, 5].

Oat-based liquid foods have gained increasingly interests on the market. Among these foods, a beverage has been developed earlier and been supplemented with calcium and vitamins present in dairy products (CEBA Foods). It could also serve as a vehicle for iron supplementation. However, such oat products as porridge and oat bran inhibit iron absorption markedly due to their high content of phytate, which is known to interfere with the absorption of such minerals as iron and zinc [6–8]. The reduction in iron absorption that phytate brings about has been shown to be dose-dependent [9]. The inactivation of the endogenous phytase of oats by the heat treatment usually employed to prevent oats from being rancid during storage can also lower iron absorption indirectly.

Several methods, including the enzymatic hydrolysis of phytate and the addition of organic acids and extra iron, have been used to increase iron absorption. Treatment of oat products by exogenous phytase (from *Aspergillus niger*) can effectively degrade phytic acid [10]. Enzymatic treatment of this sort has also been found to produce an increase in iron absorption from oat porridge in humans [11]. Addition of citric acid has been reported to significantly increase the iron absorption from a rice meal in humans [12, 13]. Ferric ammonium citrate (FeAC) and ferric pyrophosphate (FePP) are two of the iron compounds commonly used for food fortification in Europe. The advantage of the two iron salts is that they cause less adverse color or flavor changes in food vehicles [14, 15].

To increase the nutritional value of oat-based beverages, a mineral-supplemented oat beverage which is targeted at 1- to 3-year-old children has been developed, one which contains iron as FeAC or FePP together with zinc, calcium, phosphorus and selenium. The aim of the present study was to investigate the non-heme iron absorption from this iron-supplemented oat-based beverage and to assess the role and importance of citric acid addition, phytase treatment and supplementation with different iron compounds.

Previous studies have indicated that iron absorption values obtained in adults can be used to evaluate the effect of enhancers and inhibitors of iron absorption in infants [16]. By using adults, more sensitive and precise measurements of iron absorption using radioactive indicators could be applied in the present study. The study was done on adults to rank these treatments and the results can later be used to select a study product which will be used in a large study on children.

Subjects and methods

Subjects

Forty-five subjects, 23 men and 22 women, were recruited from the student population of the University of Göteborg by placing advertisements for the experiment on campus. At the time of recruitment, none of those accepted were consuming nutrient supplements containing iron or zinc. The subjects, aged 19–35 years, were healthy volunteers, none of whom reported having had any infections during the 4 weeks prior to the study. Three to four subjects in each group were regular blood donors, providing a reasonable range of inter-subject variation in Fe absorption. Blood donation during the study was not allowed and no volunteer had donated blood within the 2 months before the study. Subjects were provided with oral and written information on the aims of the study and the procedures employed. The project was approved by the Ethics Research Committee of the University of Göteborg and the Isotope Committee of Sahlgrenska University Hospital in Göteborg.

While the experiment period was in progress, two of the subjects caught a cold and one subject was found to have an extremely high serum ferritin level. Their data was excluded and thus forty-two subjects were included in the final data analysis. The iron status of the subjects were: hemoglobin levels of 121–166 (mean 143) g/l, a transferrin saturation of 13–46 (mean 27) %, and a serum ferritin concentration of 9–191 (mean 35) μg/l.

Experimental design

The absorption studies were performed in adults to examine the effect on iron absorption from an oat-

based beverage by adding citric acid, phytase treatment and supplementation of different iron compounds. The studies comprised 4 trials. In trial 1 the iron absorption from product A by supplementation of iron as FeAC was examined. In trial 2 the iron absorption from product B by citric acid addition and FeAC supplementation was examined. In trial 3 the iron absorption from product C by citric acid addition, phytase treatment and FeAC supplementation was examined. In trial 4 the iron absorption from product D by citric acid addition, phytase treatment and FePP supplementation was examined. An identical study design was employed in all the trials according to Hallberg [17].

Subjects were divided into four test groups of 10–12 subjects each. In each trial, a portion (240 g) of the test product (*T*) or a reference dose (*R*) was served to the subjects as breakfast on alternate mornings after overnight fasting on four consecutive days in the order of TRRT. Each test product was labeled with ^{55}Fe (Perkin Elmer Life Sciences, USA) as $FeCl_3$, which was added before serving. The reference dose consisted of a solution of 10 ml 0.01 M HCl containing 30 mg ascorbic acid and 3 mg Fe as $FeSO_4$ labeled with ^{59}Fe (Perkin Elmer Life Sciences, USA) as $FeCl_3$. Each subject received a total of 74 kBq ^{55}Fe from the test products and 37 kBq ^{59}Fe from the two reference doses. No consumption of food or drink was allowed during the 3 h after ingesting of the reference dose or the test product. Two weeks after the last breakfast of the study, a whole body counting of retained ^{59}Fe was made. At that time a blood sample (120 ml) was also drawn for determination of the ^{55}Fe content and of the ^{59}Fe content in the erythrocytes.

Measurement of iron absorption

Analysis of ^{55}Fe and ^{59}Fe in erythrocytes was performed by use of a liquid-scintillation counter employing a modified version of the method described by Eakins and Brown [18]. The absorption of ^{55}Fe was calculated from the retention of ^{59}Fe from the whole body counting, and the relative incorporation of the two tracers into the erythrocytes. The methodology has been described previously by Björn-Rasmussen et al. [19] and by Hallberg [17].

Expressing the results of the iron absorption measurements

The relative bioavailability of non-heme Fe in the meal was expressed as the ratio (*T*:*R*) of the absorption of non-heme Fe from the test meal (*T*) to that absorbed from the reference dose (*R*) [20]. There is a normal distribution of these ratio values in a group of subjects, and the mean and their SDs were multiplied by 40 to obtain the percentage absorption that corresponds to a 40% reference-dose absorption ($T_{40\%}$). Absorption values adjusted to a 40% absorption from reference doses were chosen because they correspond to the absorption expected in subjects who are borderline Fe deficient [18, 19]. Results in different subjects can thus be adjusted to the same iron status, facilitating the comparisons between measurements, individual and groups.

Table 1 Basic nutrient content of a 240 g portion of the mineral-supplemented oat-based beverage[1]

Nutrient	Total content
Energy	418.4 kJ/100 kcal
Protein	2.4 g
Fat	3.6 g
Saturated fatty acids	0.4 g
Carbohydrate	15.6 g
Mono-, disaccharides	9.6 g
Dietary fiber	1.9 g
β-glucan	1.0 g
Na	0.14 g
Fe	1.3 mg[2]
Zn	1.8 mg[2]
Ca	0.15 g
P	0.08 g
Se	2.5 μg[3]

[1]The native content of Na, Fe, Zn, Ca, P and Se was 20, 0.1, 0.3, 7, 52 mg and <1.8 μg, respectively
[2]The added amounts of Fe and Zn were 1.56 mg and 1.8 mg, respectively
[3]Amount added

Study products

The iron-supplemented oat-based beverages were produced in a pilot plant at CEBA Foods AB, Sweden. They were prepared from steam-heated oats (*Avena Sativa*), water, and rapeseed oil on the basis of a European patent (EP 0731646). Each 240 g portion of the beverage was supplemented by 1.56 mg Fe either as ferric ammonium citrate (Fluka, 09714) or as ferric pyrophosphate (Sigma, P6526, lot 022K0163), together with 1.8 mg Zn as zinc chloride (Merck, GR), 144 mg Ca, and other P and Se additives. Ferric pyrophosphate (FePP) or ferric ammonium citrate (FeAC) was directly soluble into water and the resulting solution was added after heat treatment. The total nutrient content in the oat-based beverage is shown in Table 1.

Four test products were produced: A, B, C and D. Ferric ammonium citrate (FeAC) was added to products A, B, and C and ferric pyrophosphate (FePP) to product D. Also, 60 mg citric acid (BDH, England) per 240 g beverage was added to products B, C, and D. In addition, products C and D were treated at 60°C for

Table 2 Inositol phosphates, iron, zinc and citric acid content and pH per 240 g portion of the different test products

Product	Treatment[1]	IP3[2] (mg)	IP4 (mg)	IP5 (mg)	IP6 (mg)	IP4–IP6 (mg)	Phy-P[3] (mg)	Fe[4] (mg)	Zn[4] (mg)	Citric acid (mg)	pH[5]
A	FeAC	UD[6]	1.15	8.46	44.86	54.47	16.3	1.27	1.77	—	6.66
B	FeAC + CA	UD	1.00	8.58	42.21	51.79	16.3	1.33	1.80	60	6.45
C	FeAC + CA + Phytase	UD	UD	UD	UD	UD	2.8	1.33	1.74	60	6.46
D	FePP + CA + Phytase	UD	UD	UD	UD	UD	2.8	1.25	1.28	60	6.48

[1]FeAC, Ferric ammonium citrate; CA, Citric acid; FePP, Ferric pyrophosphate
[2]IP3, IP4, IP5 and IP6 are referred to as inositol tri-, tetra-, penta- and hexa-phosphate, respectively
[3]Total phytate phosphorus
[4]Measured level
[5]pH measured at the time of consumption
[6]Undetectable

2 h by exogenous phytase (EC 3.1.3.8, Novozymes, Danmark).

Analysis of test products

Phytate analysis

Analysis of total phytate phosphorus (Phy-P) in the test products was conducted using an ion-exchange method as described by Harland and Oberleas [21]. The inositol triphosphate (IP3), inositol tetraphosphate (IP4), inositol pentaphosphate (IP5) and inositol hexaphosphate (IP6) content of the test samples was measured using an HPLC method [22].

Fe and Zn measurement

Analysis of Fe was conducted by Steins laboratorium AB, Sweden using a microwave digestion and ICP method. Analysis of Zn in the tested samples and in the reference sample (measured mean value was 57.6 μg/g DM, which was in accordance with the certified range of 56.5 ± 1.7 μg/g, Whole meal Flour BCR 189, Community Bureau of Reference, Brussels) involved microwave digestion in a CEM MARS 5 and flame atomic absorption spectrometry on a Perkin Elmer Aanalyst 800 under the recommended conditions.

Statistical analysis

Mean $T_{40\%}$ values between products were compared for significance by Student's *t*- test using SPSS version 11.5. Differences were considered significant at $p < 0.05$.

Results

Composition of test products

The phytate content of each of the test products is listed in Table 2. The Phy-P content of the untreated oat-based beverages (products A and B) was 16.3 mg per portion. Phytase treatment reduced the level to 2.8 mg per portion (for products C and D) after 2 h of incubation. Analysis of the different inositol phosphates showed that phytase treatment reduced the IP6, IP5 and IP4 content from 43.5, 8.5, and 1.1 mg per portion, respectively, to undetectable levels.

The Fe and Zn content of the four products (Table 2) was measured as 1.3 and 1.8 mg per portion, respectively. The measured Fe content was lower than the intended addition level (Table 1). The addition of citric acid decreased the pH of the products from 6.7 to 6.5.

Iron absorption

Iron absorption from the four oat-based beverage products (A, B, C, and D) is shown in Table 3. The addition of citric acid increased the Fe absorption ($T_{40\%}$) from 3.9% in product A to 6.0% in product B ($p = 0.051$). When the phytate content was decreased (in product C), the Fe absorption was increased still more to 10.7% in comparison with product B ($p = 0.003$). Thus, the iron absorption was improved 2.7-fold in product C by the citric acid addition and phytase treatment as compared with the untreated oat beverage, product A. No significant difference in iron absorption between the two iron compounds was found, that of ferric ammonium citrate in product C (10.7%) and ferric pyrophosphate in product D (10.6%) ($p = 0.916$).

Discussion

The present results clearly showed that using a combination of dephytinization, citric acid addition and iron supplementation can improve the iron absorption several folds from an oat-based beverage. Especially had the phytase treatment a marked enhancing effect on the absorption of iron. This agrees with findings reported by Hallberg et al. [9]

Table 3 Iron absorption by healthy human subjects given different test products[1]

Product	Treatment[2]	*n*	Iron absorption indexes			Iron absorption				Bioavailable nutrient density[4] mg Fe/1000 kcal
			Hemoglobin g/l	Transferrin saturation %	Serum ferritin µg/l	Test Meal (*T*) %	Reference Dose (*R*) %	*T/R*	$T_{40\%}$ %	
A	FeAC	11(m2,f9)	135.1 ± 6.7	25.8 ± 7.9	31.8 ± 18.2	3.5 ± 2.6	35.8 ± 15.1	0.10 ± 0.05	3.9 ± 1.8 [a]	0.50
B	FeAC + CA	8 (m6,f2)	148.4 ± 10.4	24.6 ± 5.0	40.4 ± 61.1	8.4 ± 6.5	52.6 ± 21.0	0.15 ± 0.07	6.0 ± 2.6 [a]	0.80
C	FeAC + CA + Phytase	12 (m10,f2)	147.6 ± 12.4	28.1 ± 11.2	41.7 ± 27.1	10.0 ± 4.8	37.2 ± 15.8	0.27 ± 0.08	10.7 ± 3.1 [b]	1.42
D	FePP + CA + Phytase	11 (m4,f7)	141.2 ± 8.4	29.5 ± 8.7	27.2 ± 22.7	15.5 ± 10.0	55.9 ± 29.4	0.26 ± 0.09	10.6 ± 3.4 [b]	1.33

[1]Mean ± SD, except for the bioavailable nutrient density
[2]FeAC, Ferric ammonium citrate; CA, Citric acid; FePP, Ferric pyrophosphate
[3]$T_{40\%}$: Mean individual absorption ratios (*T/R*) were multiplied by 40 to obtain the percentage absorption of iron corresponding to a 40% reference-dose absorption. $T_{40\%}$ values having different superscript letters (a, b) were significantly different ($p < 0.05$)
[4]$T_{40\%}$ absorption values adjusted for the energy content of the meals

showing phytates inhibit non-heme iron absorption in a dose-dependent way, most markedly at the dose of 2–10 mg Phy-P per portion, a slight additional inhibiting effect being attained at dose of 10–250 mg Phy-P per portion. In the present study, since the phytate content of the untreated products was rather high, 16.3 mg Phy-P per portion (240 g), a significant inhibitory effect could be expected. Phytase treatment reduced the level to 2.8 mg Phy-P per portion and the Fe absorption from the oat-based beverage was significantly increased by 78%. IP6 and IP5 are the strongest inhibitors of iron absorption but IP4 and IP3 also inhibit it [23, 24]. In the present study, IP6, IP5 and IP4 were effectively degraded to an undetectable level, IP3 being undetectable all the way. Degradation of these phytates led to the improved iron absorption.

Some investigations of the effect of dephytinization on non-heme Fe absorption in oats have been carried out [11, 25, 26]. Larsson et al. [25] examined the effects that the dephytinization by malting and soaking had on Fe absorption from a breakfast porridge containing 30 g oats per portion. They found that there was a reduction in IP6 from 340 mg to 83 mg by the treatments and an increase in Fe absorption from 0.15 mg to 0.2 mg. Although the improved absorbed Fe was high by virtue of the high native Fe content (3.5 mg Fe/30 g oats/portion), the processes of malting and soaking were not completely effective since the level of IP6 was still high afterwards. In the present study, if prepared from the same amount of oats, a beverage would contain 1.63 mg Fe, dephytinization increased absorbed Fe up to 0.17 mg. This level was similar to that absorbed from the malted-oat porridge, but the phytate content was much lower so that the inhibition of phytates on the absorption of Fe from other foods, if consumed together with the beverage, would not take place. The inhibition of phytates on the absorption of other minerals, such as Ca and Zn [6, 7], both from the beverage and from other food source could also be prevented. From the point view of processing techniques, the liquid suspension of finely ground oats used in the present study facilitated the phytase activity, advantageous over the malting and soaking processing of Larsson et al. [25] did, assuring an efficient degradation of the phytates.

The FePP in the oat-based beverage provided iron absorption similar to that of FeAC. FeAC is water-soluble whereas FePP is generally considered water-insoluble [15]. The commonly used iron pyrophosphate for fortification of foods and drinks is $Fe_4(P_2O_7)_3{\cdot}9H_2O$ [27]. However, the FePP used in the present study contained not more than one water molecule. According to the certificate of analysis [28], 5 g of FePP dissolved in 100 g water at room temperature produces a clear to very slightly hazy yellow-green solution. Since the level of Fe added per portion is only equal to 5.7 mg FePP per 100 g liquid suspension, FePP can be considered to be water-soluble. In practice, a clear concentrated aqueous solution of FePP, 10 times less than the solubility, was prepared and used for the production of the fortified beverage in the study. The soluble FePP form allows for the application of the extrinsic labeling method which is suggested not appropriate for monitoring iron absorption from a diet that contains insoluble forms of iron [29].

The present study reports for the first time the iron absorption of FePP added into a liquid food. The iron bioavailability from FePP added to foods in the particle form or in the micronised and dispersible form has been evaluated in humans [32–35]. FePP in these forms was mainly applied to semi-solid or solid food vehicles and the micronised and dispersible form enabled a higher iron absorption than the particle one [30–33]. However, it is difficult to compare the iron absorption of FePP in our study directly with in these

studies because different study designs and presentation of the iron absorption results were used. Namely, in our study ferrous sulphate was added to the reference dose whereas it was directly added to the investigated food vehicle in these studies [30–32]. Also, the Fe absorption was expressed in a way corresponding to the absorption expected in the people who are borderline Fe deficient in our study, whereas it was expressed as the absorption of one iron compound in relation to ferrous sulphate in the other studies.

The iron bioavailability of iron added as FeAC has been studied in food vehicles such as fish sauce and sugar or in iron supplements [34–38]. These studies indicate that consumption at low level of iron as FeAC in food fortification would give better absorption than at high dose [34]. The present study showed that FeAC was well-absorbed in phytase treated oat-based beverages in which FeAC was evaluated at the level of 1.3 mg compared to that of 4. 5 mg in fish sauce, 5 mg in sugar and 60 mg in iron supplement, respectively [34–36], Meanwhile, FeAC caused no obvious sensory changes.

It has been suggested that thermal processing can affect the bioavailability of iron. Hurrell et al. [39] observed that the relative bioavailability of FePP in humans from chocolate-drink powder was decreased from 75% to 21% when the compound was added prior to processing which included vacuum drying at 100°C. The effects of thermal processing were avoided in our studies, however, since FePP or FeAC was added after heat treatment.

Earlier studies have demonstrated the profound effect of citric acid on non-heme Fe absorption from food. Citric acid added at 1 g and 36 mg per serving to a basic rice meal was reported to improve iron absorption by three- and two-fold, respectively [12]. Also, citric acid added at 750 mg per serving to a similar rice meal together with ascorbic acid significantly improved iron absorption by 67% [13]. In contrast, 1 g of citric acid reduced iron absorption by about two-third in a simple Latin American-type meal composed of maize chapattis, rice and black beans, one that contained 250 mg of Phy-P and 4.3 mg of native iron [40]. Citric acid added at 36 mg per serving to fish sauce had no effect on iron absorption [34]. In the present study, the addition of 60 mg of citric acid was shown to increase Fe absorption from 1.3 mg Fe as FeAC from an oat-based beverage by 54%, the increase not being significantly although it was nearly so ($p = 0.051$). Use of differing amounts of citric acid, the presence of various factors both of inhibitory and of conducive in complex meals, and differences in the experimental setup may lead to conflicting data. Further studies are needed to clarify the role of citric acid.

Metal ions can interact with each other in a way that inhibits the absorption of each. This is one of the principles used in the present study for deciding what the supplementation level of Fe, Zn, Ca, Se and P to employ, in addition to RDA [41] principles, the Nordic Nutrition Recommendations [42], and the Swedish Food Agency regulations [43, 44]. Calcium has been shown to have an inhibitory effect on Fe absorption between 40 and 300 mg in a dose-dependent way [45]. The calcium content of the test products was 0.15 g per portion, which could be expected to have a negative effect on iron absorption. Since reducing the calcium content could further increase the bioavailabity of Fe, it would be advantageous for the iron absorption rate to be higher than 10.7%. However, it is desirable to have calcium in the product since children need both iron and calcium for body growth and metabolism.

According to Lönnerdal [46], a 1:1 molar ratio of Fe to Zn is able to prevent the interaction between Fe and Zn if given together as a supplement. Rossander-Hulthén et al. [47] showed that neither zinc fortification in a meal nor consumption of diets high in zinc interferes with iron absorption. Furthermore, some studies have shown there is no effect of iron fortification on zinc absorption [48, 49]. Nevertheless, the recommended 1:1 ratio of Fe to Zn was adopted in the present study and the studied oat beverage combined with citric acid addition, phytase treatment and a fortificant mixture of Fe, Ca, Zn and others provided iron absorption as high as 10.7%.

One measure to express iron absorption in relation to iron requirements in 1–3 years old children is by expressing the absorption results per unit energy. For 95% of the children 1–3 years of age who consume 1,300 kcal/d, absorption of 0.6 mg Fe/d or 0.45 mg Fe/1,000 kcal is needed in order to ensure the iron absorption requirements [50]. In the present investigation, the three combined treatments resulted in an iron absorption of 1.3–1.4 mg/1,000 kcal (Table 3), which is higher than the value mentioned above and also higher than 1.08 mg Fe/1,000 kcal, a figure obtained in a study where a weaning gruel (based on milk powder and cereals) was consumed together with meat and ascorbic acid [51]. It should be noted that the iron requirements of women of fertile age and teenagers is in the range of 0.4–1.5 mg Fe/1,000 kcal. Obviously, the iron-dense beverage investigated here with tested techniques could provide highly bioavailable iron for children between 1–3 years of age and also for other groups who are with high iron requirements. Their Zn absorption would likewise be improved since the inhibitory effect of phytate would be lessened and the possible interference by iron is attenuated.

Although our study represents a short-term experiment, the results appear to have clear dietary implications since no intestinal adaptation to a high-phytate diet with respect to iron absorption occurs [52]. At the same time, it would be of interest to investigate the long-term effects of the techniques employed here on the absorption of iron, zinc and other minerals in humans.

In conclusion, a combination of citric acid addition, phytase treatment and iron supplementation significantly increased the iron absorption in an oat-based beverage. Such a beverage appears potentially useful in the prevention of iron deficiency in 1- to 3-year-old children.

Acknowledgement This work was supported by CEBA Foods AB, Sweden. We thank all subjects for their cooperation; Angie Öste Triantafyllou for enzyme preparation and Benny Wraae for the production of the studied products.

References

1. UNICEF/UNU/WHO/MI (1998) Technical Workshop Preventing iron Deficiency in Women and Children: Background and Consensus on Key Technical issues and Resources for Advocacy, Planning, and Implementing National Programmes. UNICEF, New York, NY
2. WHO/UNICEF/UNU (2001) Iron deficiency anaemia: assessment, prevention, and control. Geneva, World Health Organization. (WHO/NHD/01.3), pp 15–17
3. Cook JD, Skikne BS, Baynes RD (1994) Iron deficiency: the global perspective. Adv Exp Med Biol 356:219–228
4. Hurrell RF (1999) Iron. In: Hurrell RF (ed) The mineral fortification of foods. Leatherhead Publishing. Surrey, UK, pp 54–93
5. Hurrell RF (2002) Fortification: overcoming technical and practical barriers. J Nutr 132 (suppl):806–812
6. Rossander-Hulthén L, Gleerup A, Hallberg L (1990) Inhibitory effect of oat products on non-haem iron absorption in man. Eur J Clin Nutr 44:783–791
7. Reddy NR (2002) Occurrence, distribution, content and dietary intake of phytate. In: Reddy NR, Sathe SK (eds) Food Phytates. London, UK: CRC Press, pp 30–32
8. Sandstrom B, Almgren A, Kivisto B, Cederblad A (1987) Zinc absorption in humans from meals based on rye, barley, oatmeal, triticale and whole wheat. J Nutr 117(11):1898–1902
9. Hallberg L, Brune M, Rossander L (1989) Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. Am J Clin Nutr 49 (1):140–144
10. Fretzdorff B (1992) Phytic acid in cereals - current status and possibilities of their reduction. Getreide, Mehl und Brot 46(6):180–185
11. Hurrell RF, Reddy MB, Juillerat MA, Cook JD (2003) Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. Am J Clin Nutr 77(5):1213–1219
12. Gillooly M, Bothwell TH, Torrance JD, MacPhail AP, Derman DP, Bezwoda WR, Mills W, Charlton RW (1983) The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables. Br J Nutr 49:331–342
13. Ballot D, Baynes RD, Bothwell TH, Gillooly M, Macfarlane BJ, MacPhail AP, Lyons G, Derman DP, Bezwoda WR, Torrance JD, Bothwell JE (1987) The effect of fruit juices and fruits on the absorption of iron from a rice meal. Br J Nutr 57(3):331–343
14. Wang CF, King RL (1973) Chemical and sensory evaluation of iron-fortified milk. J Food Sci 38:938–940
15. Hurrell RF (1997) Preventing iron deficiency through food fortification. Nutr Rev 55(6):210–212
16. Hurrell RF, Davidsson L, Reddy M, Kastenmayer P, Cook JD (1998) A comparison of iron absorption in adults and infants consuming identical infant formulas. Br J Nutr 79(1):31–36
17. Hallberg L (1980) Food iron absorption. In: Cook JD (ed) Methods in hematology. London: Churchill Livingstone, pp 116–133
18. Eakins JD, Brown DA (1966) An improved method for the simultaneous determination of iron-55 and iron-59 in blood by liquid scintillation counting. Inter J Appl Radia Iso 17:391–397
19. Björn-Rasmussen E, Hallberg L, Isaksson B (1974) Food iron absorption in man. Application of the two-pool extrinsic tag method to measure heme and non-heme iron absorption from the whole diet. J Clin Invest 53:247–255
20. Magnusson B, Björn-Rasmussen E, Hallberg L, Rossander L (1981) Iron absorption in relation to iron status: model proposed to express results to food iron absorption measurements. Scand J Haematol 27:208–210
21. Harland BF, Oberleas D (1977) A modified method for phytate analysis using an ion-exchange procedure: application to textured vegetable proteins. Cereal Chem 54(4):827–832
22. Sandberg AS, Ahderinne R (1986) HPLC method for determination of inositol tri-, tetra-, penta-, and hexaphosphates in foods and intestinal contents. J Food Sci 51:547–550
23. Rossander L, Sandberg A-S, Sandström B (1992) The influence of dietary fibre on mineral absorption and utilization. In: Schweizer TF, Edwards CA (eds) Dietary fibre - a component of food. Nutritional function in health and disease. Springer-Verlag, pp 198–214
24. Sandberg AS, Brune M, Carlsson NG, Hallberg L, Skoglund E, Rossander-Hulthen L (1999) Inositol phosphates with different numbers of phosphate groups influence iron absorption in humans. Am J Clin Nutr 70:240–246
25. Larsson M, Rossander-Hulthén L, Sandström B, Sandberg AS (1996) Improved zinc and iron absorption from breakfast meals containing malted oats with reduced phytate content. Br J Nutr 76:677–688
26. Davidsson L, Galan P, Cherouvrier F, kastenmayer P, Juillerat MA, Hercberg S, Hurrell RF (1997) Bioavailability in infants of iron from infant cereals: effect of dephytinization. Am J Clin Nutr 65:916–920
27. Richardson DP (1983) Iron fortification of foods and drinks. Chem Ind 13:498–501
28. Schulz L (2005) Iron (III) pyrophosphate (P6526, Lot 022K0163). In Sigma-Aldrich Certificate of Analysis. St. Louis, Missouri USA
29. Consaul JR, Lee K (1983) Extrinsic tagging in iron bioavailability research: a critical review. J Agric Food Chem 31(4):684–689

30. Davidsson L, Kastenmayer P, Szajewska H, Hurrell RF, Barclay D (2000) Iron bioavailability in infants from an infant cereal fortified with ferric pyrophosphate or ferrous fumarate. Am J Clin Nutr 71(6):1597–1602
31. Fidler MC, Walczyk T, Davidsson L, Zeder C, Sakaguchi N, Juneja LR, Hurrell RF (2004) A micronised, dispersible ferric pyrophosphate with high relative bioavailability in man. Br J Nutr 91(1):107–112
32. Fidler MC, Davidsson L, Zeder C, Walczyk T, Marti I, Hurrell RF (2004) Effect of ascorbic acid and particle size on iron absorption from ferric pyrophosphate in adult women. Int J Vitam Nutr Res 74(4):294–300
33. Zimmermann MB, Wegmueller R, Zeder C, Chaouki N, Rohner F, Saissi M,Torresani T, Hurrell RF (2004) Dual fortification of salt with iodine and micronized ferric pyrophosphate: a randomized, double-blind, controlled trial. Am J Clin Nutr 80(4):952–959
34. Walczyk T, Tuntipopipat S, Zeder C, Sirichakwal P, Wasantwisut E, Hurrell RF (2005) Iron absorption by human subjects from different iron fortification compounds added to Thai fish sauce. Eur J Clin Nutr 59(5):668–674
35. Layrisse M, Martinez-Torres C, Renzi M (1976) Sugar as a vehicle for iron fortification: further studies. Am J Clin Nutr 29(3):274–279
36. Heinrich Hc, Gabbe EE, Bruggemann J, Optiz KH (1974) Effect of fructose on ferric and ferrous iron-absorption in man. Nutr Metab 17:236–248
37. Gonzalez H, Mendoza C, Viteri FE (2001) Absorption of unlabeled reduced iron of small particle size from a commercial source. A method to predict absorption of unlabeled iron compounds in humans. Arch Latinoam Nutr 51:217–224
38. Hahn PF, Jones E, Lowe RC, Meneely GR, Peacock WE (1945) The relative absorption and utilization of ferrous and ferric iron in anemia as determined with the radioactive isotope. Am J Physiol 143:191–202
39. Hurrell RF, Reddy MB, Dassenko SA, Cook JD (1991) Ferrous fumarate fortification of a chocolate drink powder. Br J Nutr 65(2):271–283
40. Hallberg L, Rossander L (1984) Improvement of iron nutrition in developing countries: comparison of adding meat, soy protein, ascorbic acid, citric acid, and ferrous sulphate on iron absorption from a simple Latin American-type of meal. Am J Clin Nutr 39:577–583
41. National Research Council (1989) Recommended dietary allowance. 10th. edn. Washington, D.C: National Academy Press
42. Nordic Nutrition Recommendations (1996) Scand J Nutr 40:161–165
43. Statens livsmedelsverks föreskrifter om livsmedel för speciella medicinska ändamål (SLVFS) (Swedish Food Agency Regulation on foods for special medical uses) (2000):15 (H377)
44. Livsmedelsverkets föreskrifter om berikningsmedel i livsmedel för särskilda näringsändamål. LIVSFS (Swedish Food Agency Regulation on Food Supplements for special dietary uses) (2002):18 (H378)
45. Hallberg L, Brune M, Erlandsson M, Sandberg AS, Rossander-Hulten L (1991) Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. Am J Clin Nutr 53(1):112–119
46. Lönnerdal B (2000) Dietary factors influencing zinc absorption. J Nutr 130(suppl):1378–1383
47. Rossander-Hulthén L, Brune M, Sandström B, Lönnerdal B, Hallberg L (1991) Competitive inhibition of iron absorption by manganese and zinc in humans. Am J Clin Nutr 54:152–156
48. Fairweather-Tait SJ, Wharf SG, Fox SE (1995) Zinc absorption in infants fed iron-fortified weaning food. Am J Clin Nutr 62:785–789
49. Davidsson L, Almgren A, Sandstrom B, Hurrell RF (1995) Zinc absorption in adult humans: the effect of iron fortification. Br J Nutr 74(3):417–425
50. FAO/WHO Report (2002) Human vitamin and mineral requirements. Report of a joint FAO/WHO expert consultation, Bangkok, Thailand
51. Hallberg L, Hoppe M, Andersson M, Hulthén L (2003) The role of meat to improve the critical iron balance during weaning. Pediatrics 11:864–870
52. Brune M, Rossander L, Hallberg L (1989) Iron absorption: no intestinal adaptation to a high-phytate diet. Am J Clin Nutr 49(3):542–545