Original Communication

Ascorbic Acid can Reverse the Inhibition of Phytic Acid, Sodium Oxalate and Sodium Silicate on Iron Absorption in Caco-2 cells

Wanling He, Xiaoli Li, Ke Ding, Yuanxiao Li, and Wang Li

College of Animal Science and Technology, Henan University of Science and Technology, Luoyang, People's Republic of China

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Abstract: The objective of the present study is to determine the effect of phytic acid (PA), sodium oxalate (SO) and sodium silicate (SS) on non-heme iron bioavailability in both the presence and absence of ascorbic acid (AA) using an in vitro digestion/Caco-2 cell model, and the levels of AA needed to promote Fe absorption from Fe complexed with PA, SO or SS were also determined. The results indicated that adding PA at 1:1, 3:1, 5:1 and 10:1 molar as compared to Fe decreased ferrous iron uptake by 55.80 %($P < 0.05$), 72.33 % ($P < 0.05$), 73.32 % ($P < 0.05$), and 73.26 % ($P < 0.05$), respectively. Adding SS at 1:1, 3:1, 5:1 and 10:1 molar as compared to Fe also decreased ferrous iron uptake by 51.40 % ($P < 0.05$), 66.12 %($P < 0.05$), 60.19 % ($P < 0.05$) and 45.11 % ($P < 0.05$), respectively. Adding SO at 5:1 and 10:1 molar as compared to Fe decreased ferrous iron uptake by 40.81 % ($P < 0.05$) and 33.14 % ($P < 0.05$), respectively. When adding AA to iron plus organic acid medias reached molar ratios of 5:5:1 AA:PA:Fe, 3:5:1 AA:SO:Fe and 5:5:1 AA:SS:Fe, iron absorption from FeSO4 were significantly increased ($P < 0.05$). However, no significant effect was observed in iron absorption from FeCl3 when adding AA to the media. The results showed that PA, SS or SO decreases iron uptake from ferrous Fe, and AA can counteract their inhibiting effect on ferrous iron absorption and thus increase ferrous iron uptake. The results may be important for elucidating factors affecting iron bioavailability in the small intestine and for the development of foods with improved iron bioavailability.

Keywords: Caco-2 cell model, iron uptake, phytic acid, sodium oxalate, sodium silicate, ascorbic acid

Introduction

Iron deficiency anemia remains the most prevalent nutritional deficiency in developed and developing countries[1, 2]. Iron deficiency adversely affects the cognitive development of children [3, 4], increases maternal and infant mortality, and reduces physical work capacity in adulthood [5]. Diets characterized by low iron bioavailability, are one of the main causes of iron deficiency anemia [6]. Bioavailability can be defined as the extent to which a nutrient is capable of being absorbed to be utilized within the body. A large number of factors are likely to influence the proportion of iron absorbed from a particular food. These can be related to the form of iron (heme or non-heme), iron status, characteristics of the foods(presence of inhibitors such as phytic acid (PA), polyphenolic compounds, or enhancers like ascorbic acid (AA), and gastrointestinal conditions [7].

The main source of iron in our diet is in the form of non-heme iron, which has a strong tendency to interact with other compounds in the meal. PA is found in cereals and legumes and has been shown to inhibit Fe absorption in humans [8] and in cell culture models [9]. Polyphenols (e.g., tannic acid), which are present in tea, red wine, etc., are also potent inhibitors of ferrous Fe uptake [10]. In contrast, AA has been shown to enhance Fe uptake in humans and in cell culture models [10].

Several organic acids have previously been shown to influence the availability and absorption of nonheme iron. For instance, Hazell and Johnson [11] showed that ascorbic, citric, and malic acid all increased the diffusible iron in white wheat flour. Gillooly and co-workers [12] observed a significant increase in intestinal iron absorption from a rice meal supplemented with ascorbic, citric, tartaric, or malic acid. However, effects of organic acids such as sodium oxalate (SO) or sodium silicate (SS) on

ferric and ferrous iron uptake that occurs naturally in foods, such as fruits, vegetables, and lactic fermented foods is scarce. The effect of the interaction between AA and SO, or between AA and SS on the bioavailability of non-heme iron, is not yet fully understood.

The Caco-2 cell line is a human adenocarcinoma cell line that has proven to be a useful model for studying iron absorption from foods and iron fortificants [13,14]. The cells differentiate into polarized monolayers with characteristics such as a brush border membrane containing the enzymes present in normal absorptive epithelial enterocytes. Ferritin formation by the Caco-2 cells following exposure to an iron sources has been used as a marker for iron uptake [15].

Thus, the present study used an in vitro Caco-2 cell model to reveal the effect of PA, SO and SS on iron absorption in both the presence and absence of AA.

Material and Methods

Chemical, enzymes, and hormones

Unless otherwise stated, all chemicals, enzymes, and hormones were purchased from sigma chemical Co. (St. Louis, MO, USA).

Materials

The Caco-2 cell line was obtained from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). The growth medium was the glucose-rich Dulbecco's modified Eagle's medium (DMEM w/4.5 mg/L glucose) obtained from Gibco (Grand Island, NY, USA), containing 10 % fetal bovine serum (FBS), 1 % non-essential amino acids, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. Trypsin-EDTA and Hanks-buffered salt solution (HBSS, pH 7.2–7.4) used in transport studies were purchased from Gibco (Grand Island, NY, USA). Type I rat tail collagen was supplied by Sigma (St. Louis, MO, USA). Transwell-Clear inserts (six-well plate, clear polyester membrane, 0.4 μm pore size, 4.7 cm2 growth area) were purchased from Corning Incorporated (Corning, Canton, NY, USA).

Cell culture

The Caco-2 cells were grown in 25 cm2 tissue culture flasks with 5 mL of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % FBS, 4mL/L antibiotic mixture, 25 mg/L amphotericin B, and 100 μM nonessential amino acids. Medium was replaced every 2–3 days and cells grew to approximately 80 % confluency before subculturing. Cells used in the uptake experiment were seeded on Transwell inserts at a density of 2.5× 105 cells per insert (4.7 cm2) and grown for 21–22 days in supplemented DMEM. Medium (1.5mL apical, 2.5 mL basolateral) was changed every other day for 14 days and then daily for another 7 days prior to use for uptake studies on day 21. Formation of a monolayer was monitored by measuring the transepithelial electrical resistance (TEER) with a Millicell electrical resistance system (Millipose, Bedford, MA). Cell layers were used only after TEER had increased to greater than 590 Ohms.cm2, a level indicating the formation of an intact monolayer [16]. All cells were maintained at 37 ˚C in a culture incubator (Thermo, Waltham, MA, USA) with 5 % CO2 and 90 % relative humidity.

Intestinal digestion simulation treatments

Porcine pepsin (Sigma # P-7000, 800–2500 units/mg protein), pancreatin (Sigma # P-1750, activity) 4× AU.S. P. specifications), and bile extract (Sigma # B- 8631, glycine and taurine conjuates of hyodeoxycholic and other bile salts) were purchased from Sigma Chemicals. The preparation of gastric and enteric (pepsin, pancreatin, and bile extract) digestion solutions were performed as previously published [9].

Experimental design

In Vitro digestion

To simulate gastric and enteric digestion, different iron forms were treated as previous described. A certain amount of FeCl3 as well as FeSO4 were weighted and dissolved in 5mL of pepsin solution at pH 2.0 (pepsin content = 25 mg/mL) for 60 min on the rocking shaker (55 oscillation/min), respectively. The pH of these solutions were adjusted to 6.0 with 1 mol/L NaHCO3, then added to 25 mL of pancreatin/bile extract (pancreatin 2 mg/mL, bile extract 10 mg/mL) and adjusted the pH to 7.0 with 1mol/L NaHCO3, and incubated for 120 min on the rocking shaker (55 oscillation/min). The digestion solutions were made to 100 mL volume with a pH 7.0 mixture solution including 120 mmol/L NaCl and 5 mmol/L KCl. To inhibit the protease, the intestinal digest was heated for 4 min at 100 ˚C in a water bath, and then the gastrointestinal digest solution was cooled in an ice bath and centri-

fuged at 4000 rpm at 4 °C for 5 min, and the supernatants were used in the following Caco-2 cell absorption experiment (ESM 1, ESM 2).

For the Caco-2 cell iron uptake experiment, the cell transport plates were used. A fresh 1.0 mL aliquot of previously prepared supernatant was mixed with 9 mL of minimum essential medium (MEM) to obtain the cell culture medium. The bottom chamber was filled with 2.5 mL of HBSS and the upper chamber filled with 1.5 mL of cell culture medium. The cell transport plates were then returned to the incubator for an additional 22 h, at 37 °C , after which time the cells were harvested for analysis. Experiments that were always conducted in the same fashion for every set of experiments to reduce day-to-day variations.

PA, SO or SS treatment in the absence or presence of AA

To study the effects of Phytate acid (PA, C6H17NaO24P6, MW = 682.02), sodium oxalate (SO, C2Na2O4, MW = 134), or sodium silicate (SS, Na2O3Si, MW = 122.06) on iron bioavailability from FeSO4 and FeCl3 both in the absence or presence of AA, the final concentration of two iron compounds was 16 μmol/L according to our previous study[17], and both FeSO4 and FeCl3 (the amounts are calculated on the basis of pure Fe, with each being 0.9 mg of Fe) were dissolved in 25 mL of 50 % (v/v) HCl solution for 60 min on the rocking shaker (55 oscillation/min). A prepared PA, SO or SS solutions (PA:Fe, SO:Fe or SS:Fe = 0:1, 1:1, 3:1, 5:1 or 10:1 molar) in the absence of AA or (AA: PA:Fe, AA:SO:Fe or AA:SS:Fe were 0:5:1, 1:5:1, 3:5:1 and 5:5:1, respectively) in the presence of AA was added to the pepsin solution, and the mixed solutions were freshly shaken for 30 min. Then 120 mmol/L NaCl and 5 mmol/L KCl was added to the mixed solution to 1 mL and the pH adjusted to 7.0 with 1mol/L NaHCO3 and incubated for 120 min on the rocking shaker (55 oscillations per minute) to obtain 100 mL of mixed solution. All treatment solutions were removed from the cells after 2 h of incubation and replaced with fresh MEM without added iron, PA, SO or SS. Cells were incubated for an additional 22 h and then harvested. Cellular ferritin and total protein were then analyzed and compared among all treatments.

Harvesting of Caco-2 cells for ferritin analysis

The cell monolayers were harvested 24 h after the start of the intestinal digestion period. To harvest the cells, the media covering the cells were removed, and the cells were washed twice with a 2 mL volume of a rinse solution containing 140 mmol/L NaCl, 5 mmol/L KCl, and 10 mmol Piperazome-1,4 bis(2-ethanesulfonic acid) (PIPES) (Sigma, # CB-054) at pH 7. After the monolayers were rinsed, 2 mL of deionized water was placed on each monolayer. The plates were then placed on a rack with the bottom of each plate in contact with the water of a benchtop sonicator, which was kept in a cold room at 4 °C. The cells were sonicated for 15 min and then scraped from the plate surface and harvested, along with the 2 mL volume of water in each well, and stored at –20 °C . The ferritin concentration and total protein concentration were determined on an aliquot of the harvested cell suspension with a one-stage sandwich immunoradiometric assay (FERIRON II Ferritin Assay, Ramco Laboratories, Houston, TX) and a colorimetric assay (Bio-Rad DC Protein Assay, Bio-Rad, Hercules, CA), respectively. Caco-2 cells synthesize ferritin in response to increases in intracellular iron concentration. Therefore, the ratio of ferritin/total protein expressed as ng ferritin/mg protein was used as an index of the cellular iron uptake.

Analysis

The cells were harvested after 24h in the intestinal digestion period. The Caco-2 cell protein was measured on samples that had been solubilized in 0.5 mol/L NaOH, using a semimicro adaptation of the Bio-Rad DC protein assay kit (Bio-Rad Laboratories, Hercules, CA). A one-stage, two-site immunoradiometric assay was used to measure Caco-2 cell ferritin content (FER-Iron II Ferritin Assay, RAMCO Laboratories, Houston, TX). A 10 μL sample of the sonicated Caco-2 cell monolayer, harvested in 2 mL of water was used for each ferritin measurement. Total iron content in digest solutions and in the bottom chamber was determined by inductively coupled argon plasma-Mass spectrometer (ICP-MS Agilent 7500a America).

Statistical analysis

Statistical analysis of the data was performed using the software package DPS6.0. Statistical analysis was conducted according to the methods of Motulsky [18]. Prior to analysis and when appropriate, data were log transformed to achieve equal variance. The figures were created using the software Sigmaplot 10.0. One-way ANOVA was performed with Tukey's post test to compare the various means of each series of experiments. Means were considered to be significantly different if p values were ≤ 0.05.

Results

Effect of PA on ferritin formation in the absence of AA

Effect of PA on ferritin concentrations in cells treated with the two iron sources are shown in Figure 1. In general, iron uptake from FeSO4 was significantly higher than that from FeCl3 in the absence of PA, however, the addition of PA to the Caco-2 cell cultures decreased ferritin formation for both FeSO4 and FeCl3 sources. When PA was added to the Caco-2 cell culture system at molar ratio (PA:Fe) of 1:1, 3:1, 5:1, and 10:1, cell ferritin formation w as significantly decreased by 55.80 %($P < 0.05$), 72.33 % ($P < 0.05$), 73.32% ($P < 0.05$) and 73.26 % ($P < 0.05$) for FeSO4 source. However, for FeCl3 source, the addition of PA to the media made no significant differences ($P < 0.05$).

Effect of PA on ferritin formation in the presence of AA

Effect of PA on ferritin concentrations in cells treated with the two iron sources in the presence of AA are shown in Figure 2. Regardless of the iron source, the patterns of ferritin formation were qualitatively comparable, with increased iron uptake as the addition of AA to the PA plus iron media increased. For FeSO4 treatment, when the addition of AA to the PA plus iron media with molar ratio of AA:PA:Fe being 5:5:1, cell ferritin formation were significantly higher ($p < 0.05$) than that of the AA free group. For FeCl3 treatment, the addition of AA to the PA plus iron media had no significant effects ($p < 0.05$) on iron uptake.

Effect of SO on ferritin formation

Effect of SO on ferritin concentrations in cells treated with the two iron sources are shown in Figure 3. In general, the addition of SO to the Caco-2 cell cultures decreased ferritin formation for both FeSO4 and FeCl3 sources. When SO

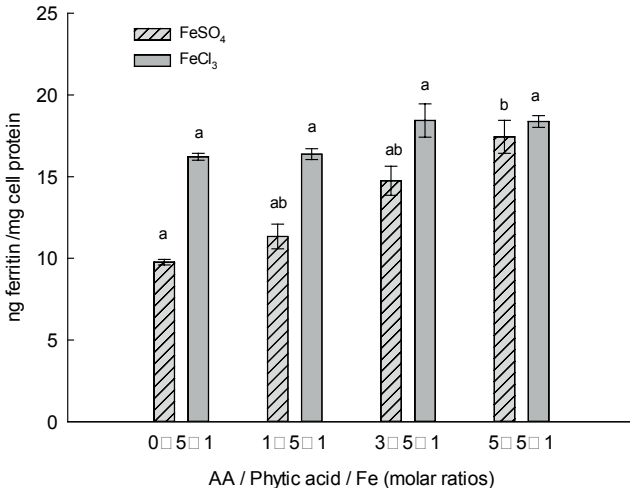

Figure 2. Effect of phytic acid on ferritin formation in response to $FeCl_3$ and $FeSO_4$, in the presence of ascorbic acid (AA). Bars within an iron compound with no letters in common are significantly different ($p < 0.05$). Values represent the mean ± SD of six replicate wells per experiment.

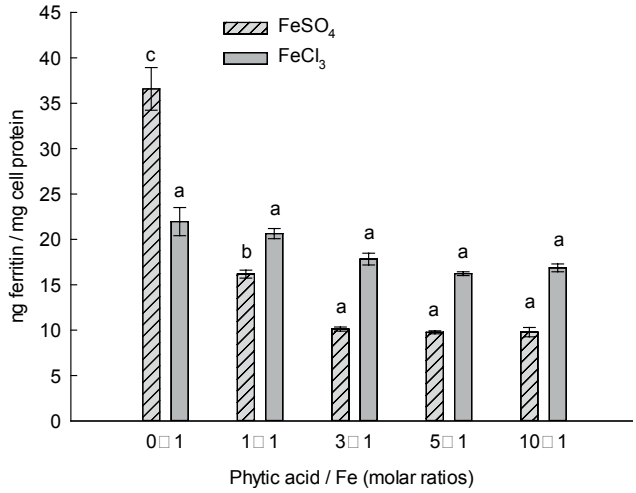

Figure 1. Effect of phytic acid on ferritin formation in response to $FeCl_3$ and $FeSO_4$. Values with no letters in common within the same Fe source are significantly different ($p < 0.05$). Values represent the mean ± SD of six replicate wells per experiment.

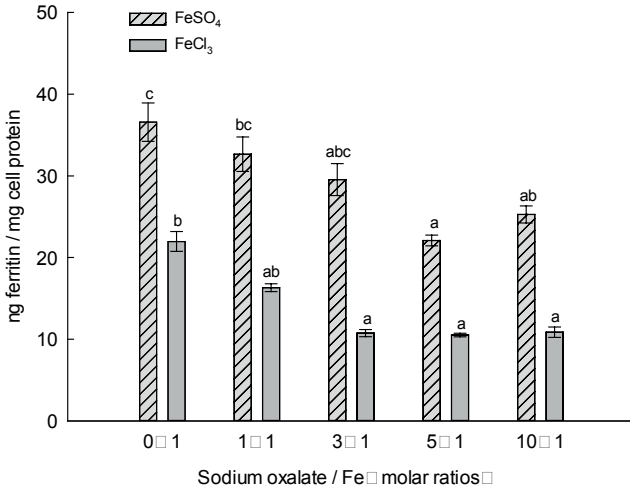

Figure 3. Effect of oxalate on ferritin formation in response to $FeCl_3$ and $FeSO_4$. Values with no letters in common within the same Fe source are significantly different ($p < 0.05$). Values represent the mean ± SD of six replicate wells per experiment.

was added to the Caco-2 cell culture system at molar ratio (SO:Fe) of 5:1 and 10:1, cell ferritin formation were decreased by 40.81% ($P < 0.05$) and 33.14% ($P < 0.05$) for FeSO4, respectively. For FeCl3 source, the addition of SO at molar ratio (SO:Fe) of 3:1, 5:1, and 10:1 all had significant ($P < 0.05$) inhibition on cell ferritin formation when compared to the control group. However, no significant differences ($P < 0.05$) were found among the above three molar ratio addition groups.

Effect of SO on ferritin formation in the presence of AA

Effect of SO on ferritin concentrations in cells treated with the two iron sources in the presence of AA are shown in Figure 4. For FeSO4 treatment, when the addition of AA to the SO plus iron media with molar ratio of AA:SO:Fe were 3:5:1 and 5:5:1, cell ferritin formation were significantly higher ($p < 0.05$) than that of the AA free group. For FeCl3 treatment, the addition of AA to the SO plus iron media had no significant effects ($p < 0.05$) on iron uptake.

Effect of SS on ferritin formation

Effect of SS on ferritin concentrations in cells treated with the two iron sources are shown in Figure 5. For FeSO4 source, the addition of SS to the media significantly decreased ($P < 0.05$) cell ferritin formation. Compared to the control group, the addition of SS with molar ratios of (SS:Fe) 1:1, 3:1, 5:1, and 10:1, cell ferritin formation were decreased by 51.40% ($P < 0.05$), 66.12% ($P < 0.05$), 60.19% ($P < 0.05$) and 45.11% ($P < 0.05$), respectively. For FeCl3 treatment, the addition of SS to the media had no significant effects ($p < 0.05$) on iron uptake.

Effect of SS on ferritin formation in the presence of AA

Effect of SS on ferritin concentrations in cells treated with the two iron sources in the presence of AA are shown in Figure 6. Regardless of the iron source, the patterns of ferritin formation were qualitatively comparable, with increased iron uptake as the addition of AA to the SS plus iron media increased. For FeSO4 treatment, when the addition of AA to the SS plus iron media with molar ratio of AA:SS:Fe was 5:5:1, cell ferritin formation was significantly higher ($p < 0.05$) than that of AA-free group. For FeCl3 treatment, the addition of AA to the SS plus iron media had no significant effects ($p < 0.05$) on iron uptake.

Discussion

The availability of nonheme iron for absorption in the intestinal lumen is considered the most important and most limiting factors to meet the daily requirement of iron. Majority of dietary non-heme iron enters the gastrointestinal tract in the ferric form. However, Fe3+ is thought to be essentially non-bioavailable and, therefore, it must be converted to ferrous iron prior to absorption

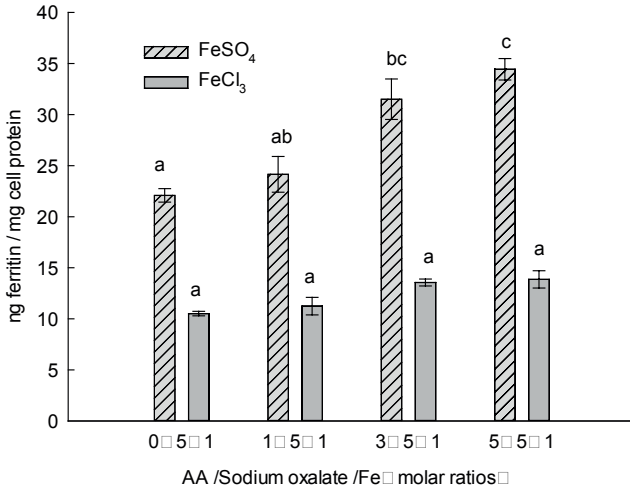

Figure 4. Effect of oxalate on ferritin formation in response to $FeCl_3$ and $FeSO_4$, in the presence of ascorbic acid (AA). Bars within an iron compound with no letters in common are significantly different ($p < 0.05$). Values represent the mean ± SD of six replicate wells per experiment.

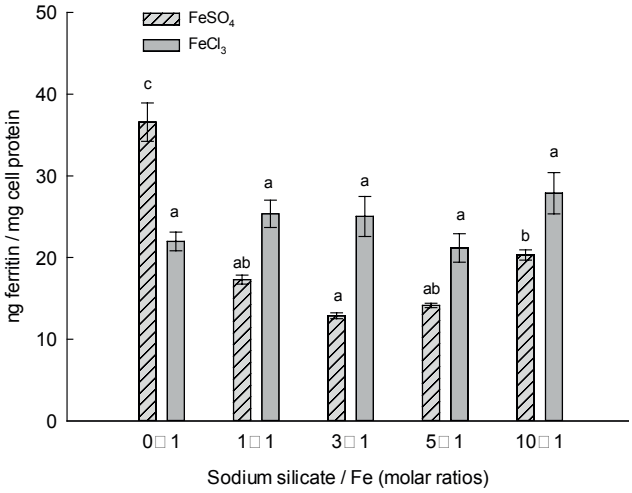

Figure 5. Effect of sodium silicate (SS) on ferritin formation in response to $FeCl_3$ and $FeSO_4$. Bars within an iron compound with no letters in common are significantly different ($p < 0.05$). Values represent the mean ± SD of six replicate wells per experiment.

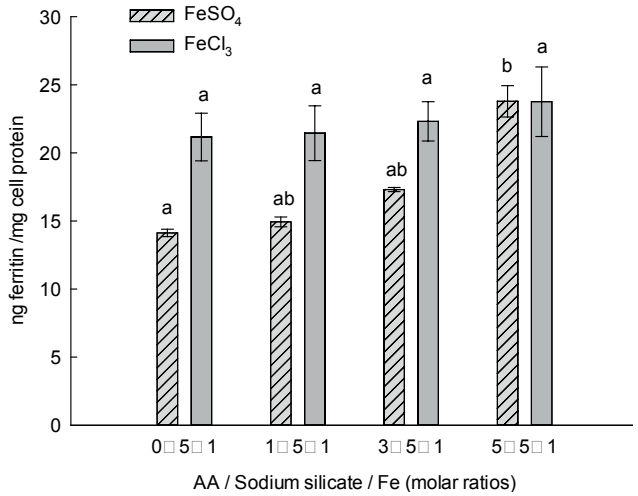

Figure 6. Effect of sodium silicate (SS) on ferritin formation in response to $FeCl_3$ and $FeSO_4$, in the presence of ascorbic acid (AA). Bars within an iron compound with no letters in common are significantly different ($p < 0.05$). Values represent the mean ± SD of six replicate wells per experiment.

[19]. Reduction of ferric iron is a prerequisite for its uptake at the enterocyte through the divalent metal ion transporter-1 (DMT1), apart from solubility [20]. In addition, iron availability is, affected by the dietary components and their composition, which may have promoting or inhibiting properties. Furthermore, the strength of the binding, solubility, and concentration of the complex in the food or meal play a major role in iron bioavailability, as these factors influence the degree to which iron can interact with other compounds and the luminal iron transporter. However, to our knowledge, studies on the interaction between organic acids on iron uptake are very limited. Therefore, we investigate the effect of organic acids such as PA, SO and SS on iron uptake from ferrous and ferric iron both in the absence and presence of AA.

Phytic acid is a significant inhibitor of iron absorption, which has been shown to inhibit iron uptake in humans [9]. Several studies indicate a reduction of non-heme iron absorption at marginal dietary iron supply due to phytic acid, in humans [21] and other monogastric animals [22]. Phytic acid and iron form insoluble complexes that are not available for absorption under the pH conditions of the small intestine. Phytic acid strongly inhibit iron absorption in dose-dependent fashion and even rather small amounts of phytic acid have a marked effect. In a study by Hallberg et al. [21], iron absorption in human subjects was inhibited by 18% to 82% when different concentrations of phytate were added to wheat rolls. In studies by Brune et al. [23] and Siegenberg et al. [24], a similar inhibitory effect was found. Other researchers [8] reported that maximal inhibition of Fe uptake occurs when molar ratio of PA:Fe is greater than 10, and the results of the Pallauf et al. [25] study demonstrated that the PA in the diet significantly decreased apparent iron absorption in growing rats fed semisynthetic diets containing molar PA/iron ratios of 17 and above. In the present study, we observed significant effects of different dose of phytic acid on iron uptake from FeCl3 and FeSO4 sources by Caco-2 cells. The results indicated that phytic acid significantly reduced ferrous Fe uptake when the PA to Fe molar ratios in the media ranged from 1 to 10, whereas these effects were not observed for FeCl3 source. This antinutritive effect of PA on iron absorption is in agreement with previous data in vitro and in vivo [22,23,26]. Phytic acid mostly binds Fe2+ but not others; therefore, it is possible that a majority of Fe2+ from FeSO4 was chelated by phytic acid only a few Fe2+ for uptake, and in contrast, Fe3+ from FeCl3 was not affected by the addition of phytic acid.

In the present study, we also observed effects of different dose of SO and SS on iron uptake from FeCl3 and FeSO4 sources in both the presence and absence of AA in Caco-2 cells. The results indicated that SO significantly decreased iron uptake from ferrous iron when the molar ratio of SO:Fe reached or exceeded 5:1. For FeCl3 source, the significant decrease of iron uptake emerged when the molar ratio of SO:Fe reached 3:1. Under conditions such as in the present study, in which no foods were present in the in vitro digests, we propose that SO primarily inhibits nonheme iron absorption by binding the iron and thereby restricting exchange or interaction of iron with the brush border surface and the Fe2+ transporter. An explanation for the difference of impact between lower and higher SO on ferric and ferrous iron absorption can be that at higher concentrations of SO the molecules may form larger complexes and render the ferric or ferrous iron even less available for absorption. The effects of SS on iron uptake by Caco-2 cells differed between ferric and ferrous iron sources. It can clearly be seen that SS significantly reduced ferrous iron uptake for all the molar ratio levels in the absence of AA, however, the effect was not detectable for ferric iron, indicating that ferric iron does not form complexes with SS as readily as the ferrous iron. It is worth noticing that the findings of a recently published study on the dose-dependent effect of several organic acids on iron uptake into Caco-2 cells provide some insights into the complex mechanisms involved [27]. Uptake was measured from samples containing a range of molar ratios of iron to organic acid of between 1:1 and 1:400. Of interest was the identification of a group-specific effect when organic acids were classified according to chemical structure, which suggested that differences in dose-response are most likely related to the number of hydroxyl and carboxyl groups of these acids. Accordingly, four-

carbon dicarboxylic acids, such as tartaric, malic, succinic, and fumaric acid, showed a positive effect on both ferric and ferrous iron absorption in the cells, but to varying degrees. The number of hydroxyl groups was shown to be important. Citric, lactic, and oxalic acid (2-, 3-, and 5-carbon carboxylic acids) had a similar and very negative effect on ferrous iron and a positive effect on ferric iron absorption. Acetic and propionic acids, which are simple 2- or 3-carbon monocarboxylic acids, showed a positive effect on ferrous iron and no effect on ferric iron uptake. The negative effect of SO on ferrous iron absorption in the present study is also in agreement with previous data in vitro[27].

Several studies indicate that it is possible to overcome inhibitor's impact on iron absorption by addition of reasonable levels of absorption enhancers (i.e., promoters). South and Miller[28] reported that iron binding by tanic acid could be reduced by addition of AA in vitro, although this effect was dependent on the sequence of addition. When consumed simultaneously with tea, ascorbic acid can prevent the formation of an iron-tannic complex and thus counteract the inhibiting effect of tea on iron absorption. Several human studies have also provided evidence that the effects of phytate and polyphenolics can be at least partially overcome by the addition of AA [21]. The present study of the interaction between PA, SO or SS and Fe indicates that reasonable dietary levels of AA can partially reverse the inhibitory effects of PA, SO or SS. Initiation of reversal of inhibition by PA, SO or SS began at a molar ratio of 5:5:1 AA:PA:Fe, 3:5:1 AA:SO:Fe and 5:5:1 AA:SS:Fe, respectively. The process by which AA increases iron uptake has previously been ascribed to the reductive properties of AA that increase the conversion of ferric to ferrous iron [29], the latter then being a substrate for the membrane transporter DMT1 that is specific for ferrous iron [19, 30]. Another added benefit of AA was proposed as a weak ligand to form a complex with iron, which increases the solubility of iron by stabilizing it from oxidation and precipitation at near neutral pH [31]. The present study again proved the function of AA in counteracting the inhibitor's impact on iron absorption, However, the exact mechanism by which AA prevent the inhibition effect of inhibitors such as some organic acids on iron absorption need to be further studied.

In summary, the results from the present study support the usefulness of Caco-2 cells for investigating the effects of dietary factors on iron cellular uptake. The data also clearly demonstrate that the efficiency of ferrous iron uptake can be decreased by PA, SS and SO, and AA can counteract their inhibiting effect on ferrous iron absorption and thus increase ferrous iron uptake, which provides a reference and guidance for reasonable dietary collocation. However, the inhibition mechanism of PA, SS and SO on iron absorption as well as the exact mechanism by which AA prevent the inhibition effect of organic acid on iron absorption remains unclear.

Conclusions

To conclude, the impact of chelating agents, such as organic acids, on iron uptake is a complex combination of different forces and mechanisms. The types of complexes, the solubility of complex, the concentration of the Fe complex, the strength of the bonds in complex and the interaction of the complex with other food ingredients, the affinity of complex to the uptake proteins and tendency of complex to deliver iron to uptake proteins are significant factors affecting Fe bioavailability. However, it is still uncertain what happens with the organic acids and iron in a complex meal, containing other confounding factors, that has to pass through the digestive system before it reaches the site of absorption. More studies are needed for a broader understanding of the effects from adding specific acids in a meal.

Acknowledgements

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Conflict of interest

The authors declare no conflict of interest.

Electronic supplementary materials

The electronic supplementary material is available with the online version of the article at http://dx.doi.org/10.1024/0300-9831/a000503.

ESM 1. Table.
Iron content in supernatants of digestion solutions (organic acids with iron) in the absence of AA.

ESM 2. Table.
Iron content in supernatants of digestion solutions(organic acids with iron) in the presence of AA (mg/L)

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Wanling He, Xiaoli Li
College of Animal Science and Technology
Henan University of Science and Technology
No.263 Kaiyuan Road
Luolong District
Luoyang 471023
People's Republic of China

lxlhwlhh@163.com

