390 *J. Agric. Food Chem.* **2002**, *50*, 390−395

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Inhibition of Iron Uptake by Phytic Acid, Tannic Acid, and $ZnCl_2$: Studies Using an In Vitro Digestion/Caco-2 Cell Model

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The objective of this study was to document the effects of phytic acid, tannic acid, and zinc on iron uptake in an in vitro digestion/Caco-2 cell culture model. The effects of phytic acid and tannic acid on iron uptake were measured at increasing molar ratios of $FeCl_3$ to phytic acid or tannic acid. Maximal inhibition of iron uptake by phytic acid occurred at a 1:10 ratio of Fe to phytic acid. Dialyzable Fe decreased with a low Fe to phytic acid ratio but increased with Fe:phytic acid ratios greater than 1:3 indicating that more iron was soluble at higher phytic acid levels but less available. As in human studies, heme iron was less inhibited by phytic acid than nonheme iron. Tannic acid was a more potent inhibitor of nonheme iron uptake, as maximal inhibition (97.5%) of iron uptake occurred at a ratio of 1:1 or less. The addition of $ZnCl_2$ to the digest at ratios of 1:0.5 and 1:1 decreased iron uptake by 57 and 80%, respectively. Overall, the results agree qualitatively with studies in humans and demonstrate the relative effects of these compounds on iron uptake in this model system. This study provides key information for determining iron availability under more complex meal conditions.

KEYWORDS: Iron; zinc; bioavailability; phytic acid; tannic acid; in vitro digestion; Caco-2

INTRODUCTION

The in vitro digestion/Caco-2 cell culture model developed by Glahn et al. (*1*, *2*) shows promise as a rapid and cost-effective tool to predict iron uptake by humans. It combines simulation of human digestion with nutrient uptake by Caco-2 cells, a human intestinal epithelial cell line. This model has been specifically developed for the measurement of iron availability from foods using Caco-2 cell ferritin formation as the marker of iron uptake. The use of ferritin formation negates the need for radioisotopic labeling of the food thus making it more useful for a broad range of users. In general, the system is able to address experimental objectives not feasible or affordable to study in vivo.

As with any in vitro model, validation of the system relative to human trials is critical. This model system has demonstrated qualitatively similar results with human studies under a multitude of conditions. For example, iron uptake from digests of beef, chicken, and fish was 300−400% of the iron uptake from a digest containing casein (*1*). In other studies, investigators demonstrated the benefits of human milk vs a bovine milk-based infant formula on iron availability (*3*). The enhancing effects of meat and ascorbic acid on iron availability have also been demonstrated in this system (*2*, *4*, *5*). Also, in comparing several commercial iron supplements, the in vitro iron availability from a polysaccharide−Fe complex and an $FeSO_4$ preparation was qualitatively identical to a closely matched human study (*5*, *6*). Taken together, the above studies impart confidence in the use of this system as the literature abounds with similar effects on iron availability to humans.

The applications for this model system are numerous. It can be used to improve the bioavailable Fe from commercial food products such as ready-to-eat breakfast cereals, infant cereals, and infant formula, etc. It can be used as a screening tool for determining relative iron availability from varieties of staple food crops such as rice, wheat, corn, and beans, thereby identifying those with improved or poor nutritional quality. In addition to evaluating individual foods, the system can be used to determine Fe availability from meals or specific food and drink, the combinations of which are endless. It represents a means to systematically study or screen numerous factors, compounds, or conditions and thereby refine and improve the design of the more expensive and definitive human trial. For all of these applications, it is imperative that appropriate amounts of food and iron be considered when designing the experiments so as not to overload the system. In general, the standard conditions developed for this system are very sensitive to small changes in iron availability and capable of handling a broad range of foods (*1*−*5*).

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10.1021/jf011046u CCC: $22.00 © 2002 American Chemical Society
Published on Web 12/13/2001

The objective of the present study was to provide information on the effects of known inhibitors of iron uptake (i.e., phytic acid, tannic acid, and Zn) in this model system and their relative potency in the absence of other ingredients present in a food or meal matrix. Documentation of these effects will be valuable to other users of this model as it will help provide an understanding of specific factors affecting iron availability in a food matrix.

MATERIALS AND METHODS

Chemicals, Enzymes, and Hormones. Unless otherwise stated, all chemicals, enzymes, and hormones were purchased from Sigma Chemical Co. (St. Louis, MO). The source of $FeCl_3$ was a 1040 μg Fe/mL solution in 1% HCl (SIGMA #I-9011).

Cell Culture. Caco-2 cells were obtained from the American Type Culture Collection (Rockville, MD) at passage 17 and used in experiments at passage 25−33. Cells were seeded at a density of 50 000 cells/cm^2 in collagen-treated 6-well plates (Costar Corp., Cambridge, MA). The cells were grown in Dulbecco's Modified Eagle Medium (GIBCO, Grand Island, NY) with 10% v/v fetal calf serum (GIBCO), 25 mmol/L HEPES, and 1% antibiotic antimycotic solution (GIBCO). The cells were maintained at 37 °C in an incubator with a 5% CO_2 and 95% air atmosphere at constant humidity, and the medium was changed every 2 days. The cells were used in the iron uptake experiments at 13 days post seeding. Under these conditions, the amount of cell protein measured in each well was found to be highly consistent from well to well within each culture plate.

In Vitro Digestion. Porcine pepsin (Sigma #P-7000, 800−2500 units/mg protein), pancreatin (Sigma #P-1750, activity = 4 × U. S. P. specifications), and bile extract (Sigma #B-8631, glycine and taurine conjugates of hyodeoxycholic and other bile salts) were used. Further preparation of the pepsin, pancreatin, and bile extract was performed as follows. Shortly before use, 0.2 g of pepsin was dissolved in 5 mL of 0.1 mol/L HCl, added to 2.5 g of Chelex-100 (Catalog #142-2842, Bio-Rad Laboratories, Hercules, CA), and shaken on a rotating titer plate tabletop shaker (Lab Line Instruments, Melrose Park, IL) for 30 min. The Chelex was removed by filtration (1.6 cm diameter filtration column) from the pepsin solution. An additional 5 mL of 0.1 mol/L HCl was added to the column, and the filtrate was collected into the pepsin solution. The final total volume of the eluted pepsin solution was 8 mL.

For the intestinal digestion, 0.05 g of pancreatin and 0.3 g of bile extract were dissolved in 25 mL of 0.1 mol/L $NaHCO_3$. Chelex-100 (12.5 g) was added, and the resulting mixture was shaken for 30 min on the rotating titer plate tabletop shaker. The mixture was then poured into a 1.6 cm diameter filtration column to filter out the Chelex. An additional 10 mL of 0.1 mol/L $NaHCO_3$ was added to the column, and the filtrate was collected into the pancreatin/bile solution. The final total volume of the pancreatin/bile solution was 27 mL. Treatment of the pepsin and pancreatin/bile solutions via the methods described above did not affect the activity of the enzymes.

Peptic and intestinal digestions were carried out on a rocking platform shaker (Reliable Scientific, Inc., Hernando, MS) in an incubator at 37 °C with a 5% CO_2 and 95% air atmosphere maintained at constant humidity. The intestinal digestion was carried out in the upper chamber of a two-chamber system in 6-well plates, with the cell monolayer attached to the bottom surface of the lower chamber (Figure 1). The upper chamber was formed by fitting the bottom of an appropriate-sized Transwell insert ring (Costar Corp., Cambridge, MA) with a 15 000 molecular weight cutoff dialysis membrane (Spectra/Por 2.1, Spectrum Medical Industries, Inc., Gardena, CA). The membranes were soaked in deionized water for at least 12 h prior to use. The dialysis membrane was held in place with a silicone ring (item no. 2-215-S604, Web Seal, Inc., Rochester, NY). After the dialysis membrane was fastened to the insert ring, the entire unit was sterilized in 70% ethanol and then kept in sterile water until use.

To start the peptic digestion, each sample was placed in a 50 mL screw cap culture tube containing 10 mL of 130 mmol/L NaCl, 5 mmol/L KCl, and 5 mM PIPES at pH 2.0 (adjusted with 1.0 mol/L HCl). Then, 0.5 mL of the pepsin solution was added. The tube was capped, placed horizontally, and incubated for 60 min on the rocking shaker (55 oscillations per minute). For the intestinal digestion step, the pH of the sample (also referred to as the "digest") was raised to pH 6 by adding 1 mol/L $NaHCO_3$ dropwise. Then, 2.5 mL of the pancreatin/bile extract mixture was added. The pH was adjusted to pH 7 with 1 mol/L $NaHCO_3$, and the volume was brought to 15 mL with 120 mmol/L NaCl and 5 mmol/L KCl. This mixture was referred to as the "intestinal digest".

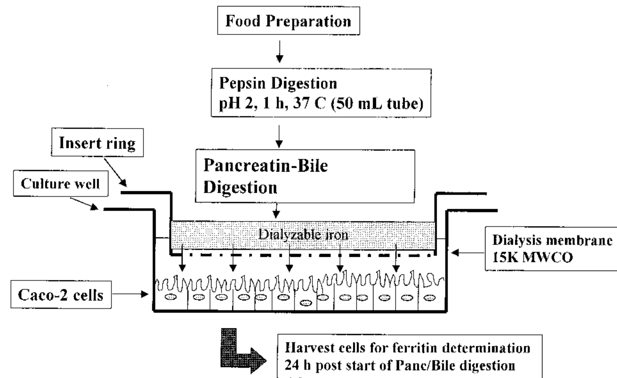

Figure 1. Diagram of in vitro digestion/Caco-2 cell culture model.

Preparation of the 6-Well Culture Plates with Cell Monolayers. Immediately before the intestinal digestion period, the growth medium was removed from each culture well, and the cell layer was washed twice with 37 °C Minimum Essential Media (MEM, #41500; GIBCO, Inc., Grand Island, NY) at pH 7. This MEM was chosen because it contained no added iron and, upon formulation with the following ingredients, was always found to contain less than 80 μg iron/L. The MEM was supplemented with 10 mmol/L PIPES (piperazine-*N*,*N*′-bis-[2-ethanesulfonic acid]), 1% antibiotic/antimycotic solution (Sigma #A-9909), hydrocortisone (4 mg/L), insulin (5 mg/L), selenium (5 μg/L), triiodothyronine (34 μg/L), and epidermal growth factor (20 ug/L). A fresh 1.0 mL aliquot of MEM covered the cells during the experiment. A sterilized insert ring, fitted with a dialysis membrane, was then inserted into the well, thus creating the two-chamber system. Then, a 1.5 mL aliquot of the intestinal digest was pipetted into the upper chamber. The plate was then covered and incubated on the rocking shaker at 6 oscillations per minute for 120 min.

When the intestinal digestion was terminated, the insert ring and digest were removed. The solution in the bottom chamber was allowed to remain on the cell monolayer, and an additional 1 mL of MEM was added to each well. The cell culture plate was then returned to the incubator for an additional 22 h, after which the cells were harvested for analysis.

To determine the amount of iron that diffused into the bottom chamber during the intestinal digestion period, plates without cells were used and treated identically as those with cells for each replication of the experiment. At the end of the intestinal digestion period, the entire volume of solution in the bottom chamber was collected for measurement of total iron.

Harvesting of Caco-2 Cell Monolayers 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 PIPES 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.

Experimental Design. Five series of experiments were conducted. In each series, 6 samples or "digests" were compared simultaneously using standard 6 well plates. Two plates were used for each replication of an experiment, thus allowing for duplicate measurements of cell

iron uptake from each sample. The average of the duplicates was used as the value for that replication. Replications of an experiment were performed on separate days.

In the first series of experiments, $FeCL_3$ was added at concentrations of 0, 5, 10, 20, 50, and 100 μmol/L to 10 mL of 140 mmol/L NaCl, 5 mmol/L KCl, and 10 mmol/L PIPES, set to pH 2 with 5 mol/L HCl. Each solution was then subjected to the digestion process described previously.

In the second series of experiments, 1 μmol of $FeCL_3$ (i.e., 53.7 μL of the 1040 μg Fe/mL 1% HCl stock solution) was combined with varying amounts of a 10 mmol/L phytic acid (SIGMA #P-3168, Type V) solution to yield Fe:Phytic acid molar ratios of 1:0, 1:1, 1:3, 1:10, 1:20, and 1:40. Then, 10 mL of 140 mmol/L NaCl, 5 mmol/L KCl, and 10 mmol/L PIPES, set to pH 2, was added to each. Each solution was then subjected to the digestion process described previously. As each digest was adjusted to 15 mL volume (by weight, 1 g approximates 1 mL) following the pepsin digestion, the Fe concentration at the start of the intestinal digestion period was 67 μmol/L.

The third series of experiments was designed to compare the effect of phytic acid on heme Fe (hemoglobin) vs nonheme Fe ($FeCl_3$). Each digest contained 1 μmol of iron from a nonheme ($FeCl_3$; Sigma #I-9011) or heme (bovine hemoglobin; Hb; Sigma #H-2500) standard. Digests with and without phytic acid (10 μmol; Sigma #P-3168) were prepared and subjected to in vitro digestion as described above.

The fourth series of experiments was conducted in an identical fashion as the second, except that tannic acid (Sigma #T-0125) was used in place of phytic acid.

The fifth series of experiments was designed to determine the effects of Zn on Caco-2 cell Fe uptake and to determine if preexposure to high levels of Zn alters Caco-2 cell Fe uptake. For these experiments, stock solutions of $FeCl_3$ and $ZnCl_2$ were combined at pH 2 and then added to 10 mL of 140 mmol/L NaCl, 5 mmol/L KCl, and 10 mmol/L PIPES, at pH 2, to yield a constant Fe concentration of 50 μmol/L and Zn concentrations of 0, 25, and 50 μmol/L in each digest. In addition, 3 of the 6 cell monolayers in each plate were exposed to serum free minimum essential media (GIBCO, cat. #41500−067) containing 50 μmol/L $ZnCl_2$ for 48 h prior to each experiment.

Analyses. All glassware used in the sample preparation and analyses was acid-washed. Caco-2 cell protein was measured on samples that had been solubilized in 0.5 mol/L NaOH, using a semi-micro adaptation of the Bio-Rad DC protein assay kit (Bio-Rad Laboratories, Hercules, CA). A one stage, 2 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. Pilot studies had determined that centrifugation of the Caco-2 cell sample prior to sampling was not necessary for accurate ferritin measurement. Analysis of the iron content of the experimental solutions and digests was conducted using an inductively coupled plasma emission spectrometer (ICAP model 61E Trace Analyzer, Thermo Jarrell Ash Corporation, Franklin, MA).

Statistics. Statistical analysis of the data was performed using the software package GraphPad Prism (GraphPad Software, San Diego, CA). Statistical analyses were conducted according to the methods of Motulsky (*7*). Prior to analysis, data were log-transformed to achieve equal variance. As each replication of an experiment in our study was a paired comparison, a repeated measures ANOVA was performed with the Tukey's post test to compare the various means of each series of experiments. Means were considered significantly different if *p* values were less than or equal to 0.05. Variance within treatment groups is expressed as standard error of the mean (SEM).

RESULTS

Figure 2 documents the ferritin formation in response to increasing amounts of $FeCl_3$ added to the system. The results indicate that in the absence of added foods or other potential food components, iron uptake under these conditions is maximal between 20 and 50 μmol/L.

The effects of phytic acid on iron uptake are summarized in Figure 3. An equimolar ratio of Fe and phytic acid resulted in a 70% inhibition of Fe uptake. A 1:3 ratio of phytic acid decreased Fe uptake by 79%, and maximal inhibition of iron uptake (85%) occurred at a 1:10 ratio of Fe to phytic acid. Dialyzable Fe decreased in the presence of a 1:1 and 1:3 Fe to phytic acid ratio but increased at Fe:phytic acid ratios greater than 1:3 indicating that more iron was soluble at higher phytic acid levels but less available.

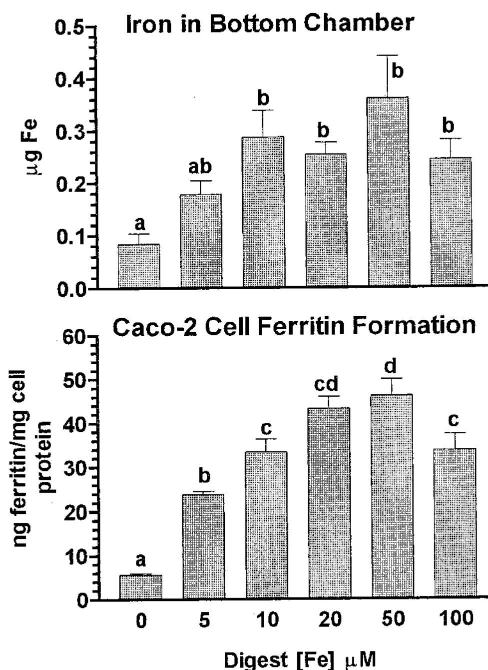

Figure 2. Measured variables for digests containing $FeCl_3$ (0–100 μmol/L). The top panel represents the measured amount of Fe that dialyzed into the bottom chamber during the 2 h intestinal digestion period. The lower panel represents the Caco-2 cell ferritin formation in response to the iron of the digests. Values represent mean ± SEM, $n = 5$. Bar values with no letters in common are significantly different ($p < 0.05$).

Figure 4 documents the inhibitory effect of phytic acid on nonheme iron uptake similar to that observed in Figure 3 for a 1:10 Fe to phytic acid molar ratio. The percent inhibition of phytic acid on nonheme Fe uptake was 88%. This inhibitory effect of phytic acid was attenuated for heme Fe, where we observed an inhibition of only 31%.

Figure 5 summarizes the effects of tannic acid on iron uptake. Tannic acid produced maximal inhibition (92%) of iron uptake at a ratio of 1:0.1, the lowest ratio studied. The total bottom chamber Fe was not affected by increasing amounts of tannic acid.

The effects of Zn on iron uptake are summarized in Figure 6. The addition of $ZnCl_2$ at ratios of 1:0.5 and 1:1 decreased iron uptake by 58 and 82%, respectively. Pretreatment of the cell cultures with $ZnCl_2$ at levels of 25 and 50 μmol/L for 48 h did not significantly affect iron uptake. Bottom chamber Fe was unaffected by Zn content in the digest (data not shown).

DISCUSSION

The present study clearly documents the inhibitory effects of phytic acid, tannic acid, and Zn on Caco-2 cell iron uptake, all of which have been shown to inhibit iron absorption in humans (*8*−*11*). In order for an in vitro digestion/Caco-2 cell model to be a useful predictor of iron availability to humans, it is necessary to document in this system the effects of known inhibitors and promoters observed in human studies. It is important to note that the ratios of inhibitors to Fe used in this

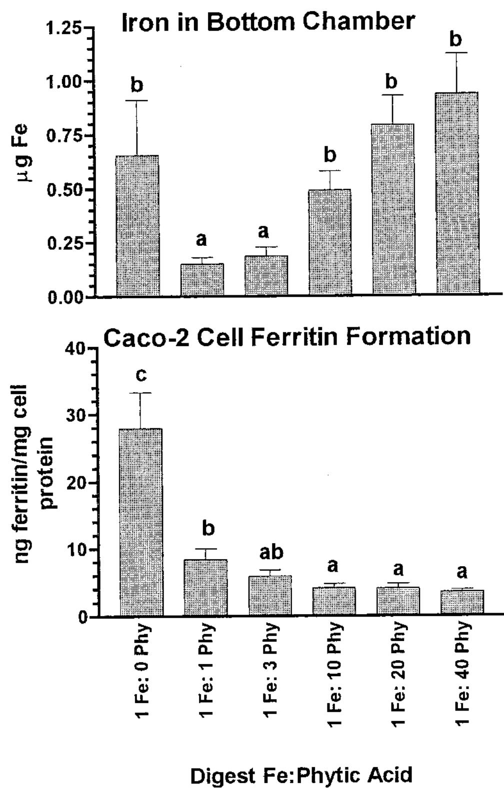

Figure 3. Measured variables for digests containing $FeCl_3$ (1 µmol/15 mL; i.e., 67 µmol/L) plus phytic acid at various molar ratios. The top panel represents the measured amount of Fe that dialyzed into the bottom chamber during the 2 h intestinal digestion period. The lower panel represents the Caco-2 cell ferritin formation in response to the iron of the digests. Values represent mean ± SEM, $n = 5$. Bar values with no letters in common are significantly different ($p < 0.05$).

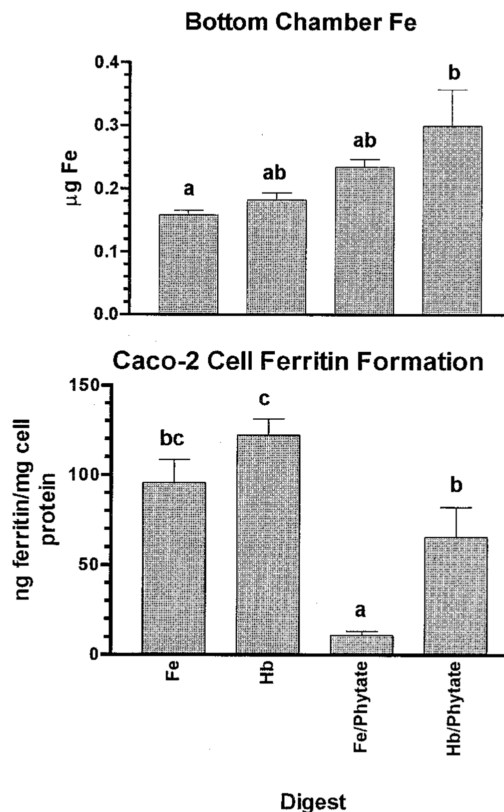

Figure 4. Effect of phytate (Phy) on nonheme (Fe) and heme (hemoglobin; Hb) iron availability. The top panel represents the amount of iron measured in the bottom chamber with no cells present at the end of the intestinal digestion period. The bottom figure represents Caco-2 cell ferritin formation measured 24 h post start of the intestinal digestion period. Bar values with no letters in common are significantly different ($p < 0.05$). Values are means + SEM; $n = 5$.

study are similar in range to those found in foods and meal conditions. Our primary goal for this study was to document the effects of these compounds on iron uptake in this in vitro model. Documentation of these effects furthers the validation of this system and provides useful information for applications of this system in food development.

This model system demonstrates the ability to determine a specific molar ratio of Fe:phytic acid that results in a specific level of effect. For example, the results of Figure 3 indicate that molar ratios of Fe to phytic acid of 1:10 or greater result in maximal inhibition of iron uptake. Inhibition is less than maximal at some point between a 1:3 and 1:10 Fe to phytic acid ratio. This type of research would not be cost-effective to perform in human subjects due to the high cost of multiple treatment groups. One should note that these ratios may only apply to the specific conditions of this study as the addition of foods or other compounds are likely to modify these results.

Heme iron was less affected by phytic acid relative to nonheme iron (Figure 4). Given the 1:10 ratio of iron to phytate in this study, the results support observations that heme iron is less susceptible to the inhibitory effects of phytate (*12*). It is thought that heme Fe is absorbed by the enterocyte as an intact metalloporphyrin following liberation from globin by proteolytic enzymes (*13*). Presumably, it is this porphyrin ring structure that shields heme Fe from phytate as well as other enhancers and inhibitors in the diet. It should be noted that the absorption of heme Fe is not entirely unaffected by dietary components. For example, meat has been shown to enhance heme Fe absorption (*12*, *14*). Furthermore, hemin, a purified heme fraction, forms high molecular weight aggregates at neutral pH that have been shown to be highly unabsorbable (*13*, *15*). The addition of globin, however, has been shown to prevent heme aggregation, thereby increasing absorption (*15*).

Evidence abounds in the literature indicating that polyphenolic compounds inhibit iron absorption (*16*, *17*). In this in vitro study, the results indicate that tannic acid is a more potent inhibitor of iron uptake relative to phytic acid or Zn. The results indicate that a 1:0.1 molar ratio of Fe to tannic acid produces maximal or near maximal inhibition of iron uptake under these simple conditions. In more complex meal conditions, the potency of tannic acid will likely be different. For example, Brune et al. (*18*) compared the effects of increasing amounts of tannic acid added to bread rolls prepared from unfortified white wheat flour. An inverse dose−response relationship was observed between Fe absorption and tannic acid content in the human subjects. Maximal inhibition of Fe uptake in vivo was 88% and occurred at or near a 1:1 molar ratio of Fe to tannic acid. Brune et al. also observed that gallic acid inhibited Fe absorption to the same extent as tannic acid (per mole galloyl groups) and that chlorogenic acid inhibited Fe absorption to a lesser extent. They observed no inhibition of Fe uptake when catechin was added to the test meal. They concluded that the content of Fe binding galloyl groups is a major determinant of Fe availability in the diet.

The inhibition of Fe absorption by phenolic compounds is likely due to the binding of Fe, thus making the iron unavailable for absorption. Tannic acid binds Fe with relatively high affinity (*19*). As with phytic acid, addition of other compounds or food would likely modify the inhibitory effects. Very little is known

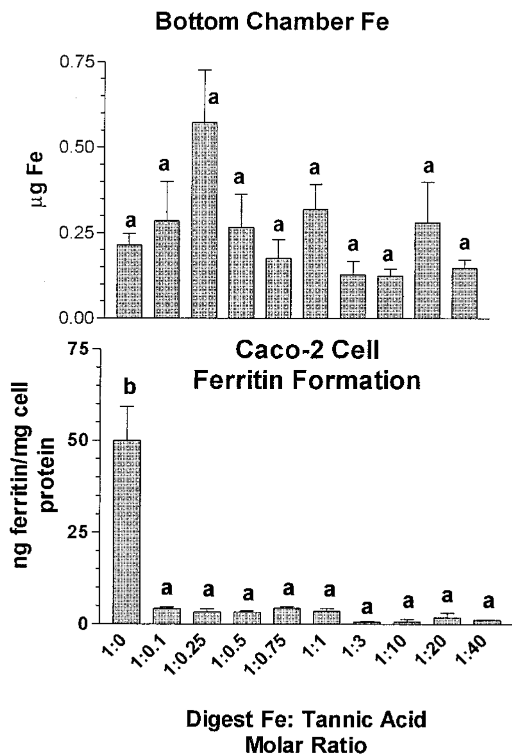

Figure 5. Effect of tannic acid on nonheme Fe availability. The top panel represents the amount of iron measured in the bottom chamber with no cells present at the end of the intestinal digestion period. The bottom figure represents Caco-2 cell ferritin formation measured 24 h post start of the intestinal digestion period. Bar values with no letters in common are significantly different ($p < 0.05$). Values are means + SEM; $n = 4$–6.

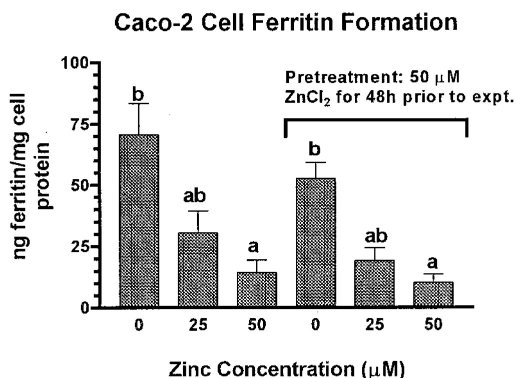

Figure 6. Effect of $ZnCl_2$ on nonheme Fe availability. $ZnCl_2$ was added at concentrations of 0, 25, and 50 umol/L to a digest containing $FeCl_3$ (50 umol/L). In addition, 3 of the 6 cell monolayers in each plate were exposed to serum free minimum essential media containing 50 umol/L $ZnCl_2$ for 48 h prior to each experiment. Bar values with no letters in common are significantly different ($p < 0.05$). Values are means + SEM; $n = 4$.

of the effects of specific polyphenols on iron availability; however, we believe this model system represents a way to effectively investigate this class of compounds with regard to iron availability. It will require a systematic approach in identification and isolation of compounds coupled with the measurement of effects using this in vitro system.

Given the high fortification level of many food products, the interactions of iron and zinc in foods and supplements represent critical issues in nutrition. Iron has been shown to inhibit intestinal Zn absorption (*9*−*11*). This effect of iron has primarily been shown to occur under conditions where iron is consumed as a supplement rather than incorporated into a meal (*20*). A recent study by Donangelo et al. (*21*) indicates that zinc supplements may also inhibit iron absorption from the diet. In that study, the effects of Zn supplementation on iron metabolism of 11 healthy, nonanemic women with low iron reserves were examined. The subjects ingested the zinc supplements in the evening 2 h after dinner. The results indicated that Zn supplementation improved indices of zinc status but produced changes in iron metabolism suggesting cellular iron deficiency and further depletion of iron stores. A possible cause of the iron loss could be inadequate iron absorption due to the presence of the supplemental zinc in the intestinal lumen. In the present study, we tested for a similar effect and observed that zinc inhibited iron uptake. Our study conditions would be most similar to a situation where humans ingest iron and zinc simultaneously as supplements. Pretreatment of the cells with 50 μmol/L Zn for 48 h did not affect iron uptake. Clearly, the results suggest that more work should be done to investigate the interactions of Zn and Fe in various food matrixes.

Considerable research efforts are directed at increasing the nutritional quality of foods and the effectiveness of supplement programs throughout the world. The bulk of these efforts is aimed at improving the iron status of children and women of reproductive age. This research requires the study of iron availability and nutrient interactions under a broad range of food and meal conditions. An in vitro model that accurately predicts effects in humans appears to be the only cost-effective way to directly study meal conditions and improve food quality. The results of this study and the overall body of literature concerning this model indicate that this system is useful for this field of research. Defining how closely this model can predict human Fe absorption will require simultaneous measurement of samples drawn from the same foods or meals in both in vitro and human trials. At present, there are studies underway by the authors of this paper comparing in vitro predictions of Fe uptake from both short term (single meal) and longer term studies (multiple meal or diet regimen) with the results from studies involving human subjects.

ACKNOWLEDGMENT

The authors thank Jean Hsu, Borys Polon, Olivia A. Lee, and Matthew I. Goldman for their fine technical assistance.

LITERATURE CITED

(1) Glahn, R. P.; Wien, E. M.; Van Campen, D. R.; Miller, D. D. Caco-2 cell iron uptake from meat and casein digests parallels in vivo studies: Use of a novel in vitro method for rapid estimation of iron bioavailability. *J. Nutr.* **1996**, *126*, 332−339.

(2) Glahn, R. P.; Lee, O. A.; Yeung, A.; Goldman, M. I.; Miller, D. D. Caco-2 Cell Ferritin Formation Predicts Nonradiolabeled Food Iron Availability in an In vitro Digestion/Caco-2 Cell Culture Model. *J. Nutr.* **1998**, *128*, 1555−1561.

(3) Glahn, R. P.; Lai, C.; Hsu, J.; Thompson, J. F.; Van Campen, D. R. Decreased Citrate Improves Iron Availability From Infant Formula: Application of an In vitro Digestion/Caco-2 Cell Culture Model. *J. Nutr.* **1998**, *128*, 257−264.

(4) Glahn, R. P.; Lee, O. A.; Miller, D. D. In vitro Digestion/Caco-2 Cell Culture Model to Determine Optimal Ascorbic Acid to Fe Ratio in Rice Cereal. *J. Food Sci.* **1999**, *64*, 925−928.

(5) Glahn, R. P.; Rassier, M.; Goldman, M. I.; Lee, O. A.; Cha, J. A Comparison of Iron Availability From Commercial Iron Preparations Using An In vitro Digestion/Caco-2 Cell Culture Model. *J. Nutr. Biochem.* **2000**, *11*, 62−68.

(6) Tinawi, M.; Martin, K. J.; Bastani, B. Oral iron absorption test in patients on CAPD: Comparison of ferrous sulfate and a polysaccharide ferric complex. *Nephron* **1996**, 74: 291−294.

(7) Motulsky, H. Comparing Three Or More Means. In *Intuitive Biostatistics*; Motulsky, H., Ed.; Oxford University Press: Oxford, NY, 1995; pp 255−262.
(8) Benito, P.; Miller, D. D. Iron absorption and bioavailability: An updated review. *Nutr. Res.* **1998**, *18* (3), 581−603.
(9) Krebs, N. F. Overview of zinc absorption and excretion in the human gastrointestinal tract. *J. Nutr.* **2000**, *130*, 1374S−1377S.
(10) Lonnerdal, B. Dietary factors influencing zinc absorption. *J. Nutr.* **2000**, *130*, 1378S−1383S.
(11) O'Brien, K. O.; Zavaleta, N.; Caulfield, L. E.; Wen, J.; Abrams, S. A. Prenatal iron supplements impair zinc absorption in pregnant Peruvian women. *J. Nutr.* **2000**, *130*, 2251−2255.
(12) Hallberg, L.; Bjorn-Rasmussen, E.; Howard, L.; Rossander, L. A discussion of possible mechanisms for the absorption promoting effect of meat and for the regulation of iron absorption. *Scand. J. Gastroenterol*. **1979**, *14*, 769−779.
(13) Conrad, M. E.; Cortell, S.; Williams, H. L.; Foy, A. I. Polymerization and intraluminal factors in the absorption of hemoglobin-iron. *J. Lab. Clin. Med.* **1966**, *68*, 659−668.
(14) Martinez-Torres, C.; Layrisse, M. Iron absorption from veal muscle. *Am. J. Clin. Nutr.* **1971**, *24*, 531−540.
(15) Conrad, M. E.; Benjamin, B. I.; Williams, H. I.; Foy, A. I. Human absorption of hemoglobin-iron. *Gastroenterology* **1967**, *53*, 5−10.
(16) Layrisse, M.; Garcia-Casal, M. N.; Solano, L.; Baron, M. A.; Arguello, F.; Llovera, D.; Ramirez, J.; Leets, I.; Tropper, E. Iron bioavailability in humans from breakfasts enriched with iron bisglycine chelate, phytates and polyphenols. *J. Nutr.* **2000**, *130*, 2195−2199.
(17) Zijp-Itske, M.; Korver, O.; Tijburg-Lilian, B. M. Effect of tea and other dietary factors on iron absorption. *Crit. Rev. Food Sci. Nutr*. **2000**, *40* (5), 371−398.
(18) Brune, M.; Rossander, L.; Hallberg, L. Iron absorption and phenolic compounds: Importance of different phenolic structures. *Eur. J. Clin. Nutr.* **1989**, *43*, 547−558.
(19) South, P. K.; Miller, D. D. Iron binding by tannic acid: Effects of selected ligands. *Food Chem.* **1998**, *63*, 167−172.
(20) Whittaker, P. Iron and zinc interactions in humans. *Am. J. Clin. Nutr.* **1998**, *68* (Suppl.), 442S−446S.
(21) Donangelo, C. M.; Woodhouse, L. R.; Mertz, S. D.; Viteri, F. E.; King, J. C. Effect of zinc supplementation on iron metabolism in young women with low iron reserves. *FASEB J*. **2000**, *14* (4), A483.

Received for review August 6, 2001. Revised manuscript received November 1, 2001. Accepted November 1, 2001.

JF011046U