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Effect of Dietary Ligands and Food Matrices on Zinc Uptake in Caco-2 Cells: Implications in Assessing Zinc Bioavailability

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The kinetics, depletion/repletion of zinc, and effects of dietary ligands/food matrices on ^{65}Zn uptake was studied in Caco-2 cells. The uptake of zinc showed a saturable and nonsaturable component, depending upon the media zinc concentrations. Intracellular depletion increased zinc uptake, whereas zinc loading did not. Phytic acid and histidine inhibited zinc uptake, while tannic acid, tartaric acid, arginine, and methionine increased zinc uptake. Tannic acid at a 1:50 molar ratio promoted zinc uptake from wheat- and rice-based food matrices. Further, Caco-2 cells responded similarly with zinc and iron uptake when fed Indian bread prepared from low- and high-extraction wheat flour, representing low and high phytate content. However, inclusion of tea extract or red grape juice as a source of polyphenols enhanced the uptake of zinc while decreasing that of iron. These results suggest that the Caco-2 cells predict the correct direction of response to dietary ligands even from complex foods.

KEYWORDS: Zinc; iron; dietary ligands; uptake; food matrix; Caco-2 cells

INTRODUCTION

Zinc is an essential element and functions as a cofactor for many enzymes and proteins. It is known that, similar to iron, poor bioavailability of zinc from vegetarian diets leads to deficiency and that supplementation or fortification of zinc has been associated with increased linear growth, reduction in diarrheal disease, and enhanced immune function (*1*). Biofortification of staple crops through marker-assisted selection and breeding is now being considered as an emerging strategy to increase the density of zinc (*2*). However, ensuring substantial bioavailability of zinc from biofortified staple crops is crucial for the intended beneficial outcome.

Assessment of bioavailability of zinc in humans or animal models is cumbersome and cannot be used as a screening tool (*3, 4*). *In vitro* methods based on measuring solubility or dialysability of iron and zinc are convenient screening tools, but the lack of a biological component precludes its use (*5, 6*). Simulated *in vitro* digestion coupled with the Caco-2 cell model has been extensively used for assessing the bioavailability of iron, carotenoids, and other minerals (*5, 7*−*9*). The usefulness of the Caco-2 cell line to predict the magnitude of the effect of zinc absorption modifiers on its bioavailability has been demonstrated in commercial human milk fortifiers (*10, 11*). Although the *in vitro* method based on the Caco-2 cell line predicts the correct direction of response to phytate (*3, 12, 13*), limited data are available with other dietary ligands and from more complex foods/meals to use this model as a reliable screening tool for assessing the bioavailability of zinc (*5, 14*).

The aim of the present study is, therefore, to systematically investigate the direction of the effect of various dietary ligands on zinc uptake in Caco-2 cells and then to extend these studies to more complex foods/meals.

MATERIALS AND METHODS

Materials. Minimum essential medium (MEM), Dulbecco's modified Eagle's medium (DMEM), *N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN), and all of the other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO), except methionine, which was purchased from SRL Chemicals, Mumbai, India. Radioactive ^{65}Zn (zinc chloride, 3 Ci/g) and ^{59}Fe (ferric chloride, carrier free) were purchased from BRIT, Mumbai, India.

Cell Culture. Caco-2 cells were procured from the National Center for Cell Sciences (NCCS), Pune, India, and cultured in 6-well plates as described previously (*9, 15*). Briefly, Caco-2 cells were maintained in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS), 1% non-essential amino acids, 2 mmol/L L-glutamine, and 1% antibiotic−antimycotic solution, at 37 °C in an incubator with a 5% CO_2/95% air atmosphere at constant humidity. All of the uptake experiments were conducted 12−14 days postconfluence.

Preparation of Dietary Ligands. Stock solutions (10 mmol/L) of phytic acid as inositol hexaphosphate (IP_6), tannic acid (gallotannin), L-ascorbic acid, L-arginine, L-methionine, L-histidine monohydrochloride

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monohydrate, and L-(+)-tartaric acid were prepared in MEM immediately prior to use, except (+)-catechin, which was made in absolute ethanol and further diluted with MEM. The above stock solutions were diluted to 25, 125, and 250 μmol/L final concentrations in MEM containing 25 μmol/L $ZnSO_4$ (traced with 1 μCi ^{65}Zn) to achieve 1:1, 1:5, and 1:10 molar ratios of zinc/dietary factor, respectively, and allowed to stand for 1 h at 37 °C for equilibration. The differentiated Caco-2 cells were then incubated with 2 mL of above mixtures for a period of 2 h.

Time and Dose Effect. To study time-dependent uptake, 2 mL of fresh MEM supplemented with 25 μmol/L Zn (traced with 1 μCi of ^{65}Zn) was added to Caco-2 cells and incubated for 0−240 min. Dose-dependent uptake of zinc was monitored by incubating the cells with 0−200 μmol/L $ZnSO_4$ traced with ^{65}Zn for a period of 2 h. Nonspecific binding of the tracer was assessed with 100-fold molar excess of nonradioactive zinc in the form of $ZnSO_4$.

Effect of Repletion/Depletion of Zinc on Caco-2 Cell Zinc Uptake. To study the effect of repletion or depletion of zinc on zinc uptake, cells were pretreated with either 25 μmol/L Zn or 10 μmol/L TPEN (a specific intracellular zinc chelator), respectively, for a period of 3 h. The pretreated cells were then supplemented with fresh MEM containing 25 μmol/L $ZnSO_4$ (traced with ^{65}Zn), and the uptake of zinc was assessed at the end of 2 h.

Measurement of Cellular ^{65}Zn. The medium was aspirated at the end of the incubation, and the monolayer washed 3 times with 10 mmol/L *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer at pH 7.2, containing 1 mmol/L EDTA, to remove nonspecifically bound zinc. The cells were scraped in 400 μL of PBS (50 mmol/L sodium phosphate-buffered saline at pH 7.2) with a rubber policeman, and the cell-associated radioactivity was counted in a γ ray spectrometer (GRS-201 L, PLA electro appliances, Mumbai, India).

Preparation of Tea Extract and Red Grape Juice and Estimation of Polyphenols. Tea extract was prepared by boiling 220 mg of commercially available tea leaf powder with 31 mL of milli-E water for 10 min, cooled, and filtered, and the filtrate was used for the study. Red grape juice with no additives was purchased from a local supermarket. The polyphenol content was determined colorimetrically using catechin as the standard (*16*).

Wheat-Based Indian Bread (Chapatti). High-extraction (>90%) (HEWF) and low-extraction (<70%) (LEWF) wheat flour (obtained from a local flour mill) based Indian bread (chapatti) was prepared as follows. Dough was prepared by adding approximately 80 mL of milli-E water to 100 g of the flour. Dough (50 g) was then rolled manually with a wooden roller on a wooden platform and baked over direct gas flame. The chapattis were then sliced into small pieces, homogenized using a kitchen blender to prepare a 20% (w/v) homogenate in saline, and used immediately for *in vitro* digestion/Caco-2 cell uptake studies.

Preparation of the Rice Matrix. Polished rice obtained from the local market was cooked by pressure-cooking for 10 min with a 1:2 ratio of rice/water. The cooked rice was homogenized, freeze-dried by lyophilization (to remove the excessive moisture), and powdered in a kitchen blender. A 20% homogenate was made as described above and used immediately for *in vitro* digestion/Caco-2 cell uptake studies.

***In Vitro* Digestion.** *In vitro* digestion was performed as described earlier, with the modification of using extrinsically labeled zinc and iron (*9, 15*). The zinc and iron content of LEWF was restored to that of HEWF by the addition of $ZnSO_4$ or $FeSO_4$ to normalize the extraction losses. A 5 g portion of the homogenates of HEWF, LEWF, and rice was traced with either 5 μCi of ^{65}Zn or 3.37 μCi of ^{59}Fe and diluted to 9 mL with saline, and the pH was adjusted to 2.0 with HCl. Then, 0.5 mL of pepsin (40 mg/mL) was added and incubated for 1 h at 37 °C in a shaking water bath. For intestinal digestion, the pH of all of the samples was raised to 6.0 with 1 M $NaHCO_3$ and then 2.5 mL of pancreatin/bile extract mixture (0.05 g of pancreatin and 0.3 g of bile extract/25 mL 0.1 M $NaHCO_3$) was added to the samples. Finally, the pH of all of the samples was adjusted to 6.7 with 1 M NaOH, and the final volume of all of the samples was made to 15 mL with saline and immediately added to the upper chamber of the transwell insert as described below. A set of controls without food matrix containing the equal quantities of zinc or iron to that of diet samples were also processed simultaneously to serve as respective references.

Zinc Uptake in Caco-2 Cells. The spent medium from differentiated Caco-2 cell monolayers was replaced with 2 mL of MEM, and an insert ring fitted with dialysis membrane (12 kDa, Spectrapore) was introduced into the wells, thus creating upper and lower chambers. A total of 2 mL of the "intestinal digesta" was introduced into the upper chamber and incubated in a humidified CO_2 incubator at 5% CO_2 at 37 °C for a period of 2 h with continuous shaking at 150 rpm. At the end of this, the upper chamber was removed and the plates were further incubated for 1 h (without shaking). The medium was aspirated, and the monolayers were washed 3 times, scraped into 400 μL of PBS, and ^{65}Zn counted as described above. No apparent toxicity was observed because of the presence of food digest and ^{65}Zn as assessed by microscopic examination of cell monolayers and the LDH release assay.

Estimation of Iron, Zinc, Phytate, and Protein. Iron and zinc content was estimated by atomic absorption spectrometry following microwave digestion (Mars Xpress). Phytate was estimated using an anion-exchange method (*17*). The protein concentration in the cell lysates was estimated using BCA kit (Sigma).

Calculation of the Percentage of Uptake. The CPM obtained was subtracted from the nonspecific CPM to obtain the specific CPM and DPM (counting efficiency of 57 and 55% for ^{65}Zn and ^{59}Fe, respectively) for each well. Because the specific activity of ^{65}Zn was constant in all of the wells, uptake was derived as a percentage of control, considering control as 100%.

Statistical Analysis. All of the experiments were performed in triplicates and repeated at least once to generate six observations. The percentage of uptake was calculated assuming the respective controls (either zinc or iron in the absence of food matrix) as 100% throughout the paper. Descriptive statistics, such as mean and standard deviation (SD), were calculated using Microsoft Excel, and the kinetic constants V_{max} and K_m were calculated using Sigma Plot (version 7.0). One-way analysis of variance (ANOVA) followed by a least significant differences (LSD) posthoc test was performed using the SPSS7 statistical package to assess the effect of treatments, and a p value < 0.05 was considered significant.

RESULTS

Kinetics of Zinc Uptake. Zinc uptake increased as a function of time and plateaued after 100 min (**Figure 1**A). Therefore, all of the subsequent uptake studies were carried out for 2 h to ensure maximum uptake of zinc. Nonlinear regression analysis of the data revealed both saturable (0−50 μmol/L) and nonsaturable (>50 μmol/L) uptake as a function of the media Zn concentration (solid line in **Figure 1**B). Therefore, the diffusion component was calculated by extrapolation of initial linear data points (dashed line in **Figure 1**B), and the mediated component represents the difference between the diffusion component and actual data points (dotted lines in **Figure 1**B). The mediated component was then fitted into a double-reciprocal plot to obtain kinetic parameters. The K_m and V_{max} values obtained were 65 μmol and 93 nmol (mg of protein)$^{-1}$ 2 h^{-1}, respectively (inset in **Figure 1**B).

Effect of Intracellular Zinc Depletion and Repletion. Repletion of zinc did not significantly influence the uptake of zinc in Caco-2 cells, while depletion resulted in a significant increase by about 69% above the control ($p < 0.05$; **Figure 2**).

Effect of Dietary Ligands on Zinc Uptake. The effect of dietary ligands on the uptake of zinc is summarized in **Figure 3**. Phytic acid dose-dependently inhibited zinc uptake, while histidine inhibited the uptake only at a 1:10 molar ratio. Tannic acid, tartaric acid, and methionine increased the uptake of zinc dose-dependently. However, catechin and ascorbic acid did not influence the uptake of zinc even at a 1:10 molar ratio in Caco-2 cells (data not shown).

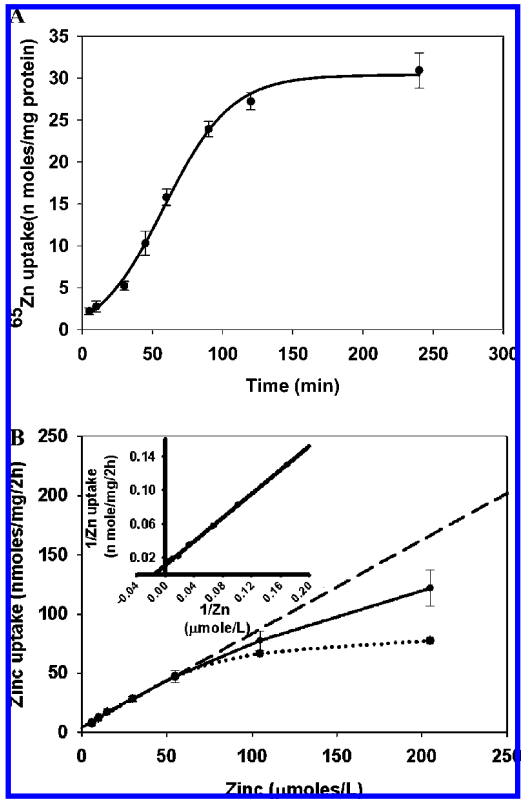

Figure 1. Time- and dose-dependent zinc uptake in Caco-2 cells. A total of 25 or 0−200 μmol of Zn/L was traced with ^{65}Zn, and the uptake was measured as a function of (A) time or (B) dose, respectively. (B) Solid line represents actual data points (both mediated and diffusion component) with mean ± SD (n = 6). The dashed line is the extrapolation of the initial linear data points, representing the diffusion component. The mediated component was derived by subtracting the diffusion component from the actual data (dotted line). (Inset) Double-reciprocal plot of mediated zinc uptake.

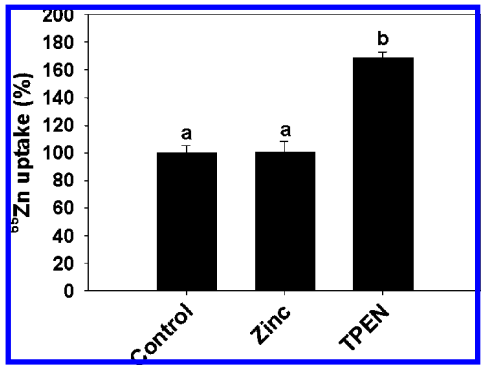

Figure 2. Effect of zinc depletion or repletion on Caco-2 cell zinc uptake. Caco-2 cells were pre-incubated in the absence or presence of 25 μmol of Zn/L or TPEN (10 μmol/L) for a period of 3 h. The uptake was carried out for 2 h with 25 μmol of Zn/L traced with ^{65}Zn. The bars represent mean + SD, and bars with different superscript letters differ significantly (p < 0.05, n = 6).

Zinc, Iron, Phytate, and Polyphenol Contents of Food Matrices and Beverage Extracts. The zinc and iron contents of HEWF (4.58 ± 0.1 and 4.04 ± 0.13 mg/100 g, respectively) were higher than in LEWF (1.26 ± 0.06 and 1.04 ± 0.03 mg/100 g, respectively) and rice (1.3 ± 0.1 and 4.0 ± 0.2 mg/100 g, respectively). The phytate content of HEWF (581 ± 28.3 mg/100 g) was 5.3 times higher than in LEWF (109 ± 14.0 mg/100 g) and 3.5 times higher than in rice (160.3 ± 6.65 mg/100 g). Polyphenol content was highest in HEWF (11.88 ± 2.79 mg/100 g), followed by rice (7.91 ± 1.596 mg/100 g) and LEWF (3.23 ± 0.22 mg/100 g). Grape juice contained higher amounts of polyphenols (101.5 ± 7.0 mg/100 mL) than tea (51.9 ± 13.90 mg/100 mL).

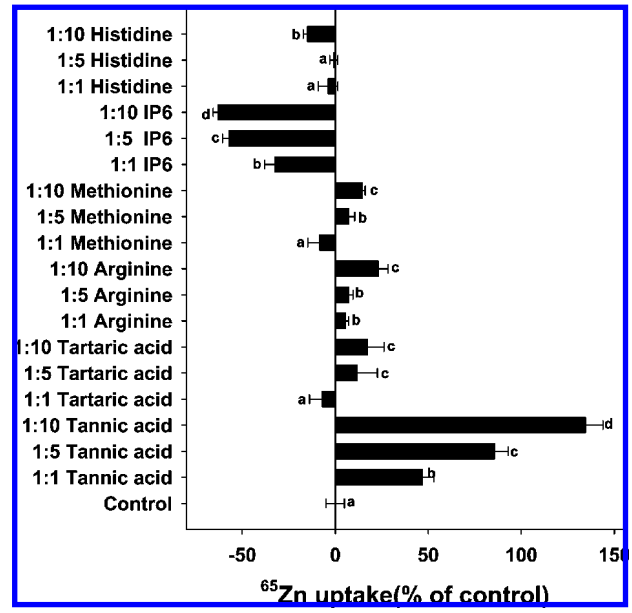

Figure 3. Effect of dietary ligands on Caco-2 cell zinc uptake. Zinc uptake was measured either in the absence or presence of 1:1, 1:5, and 1:10 molar ratio of zinc/dietary ligands. The bars represent mean ± SD, and bars with different superscript letters differ significantly compared to the control and within each dietary ligand (p < 0.05, n = 6). IP_6 = Inositol hexaphosphate (phytic acid).

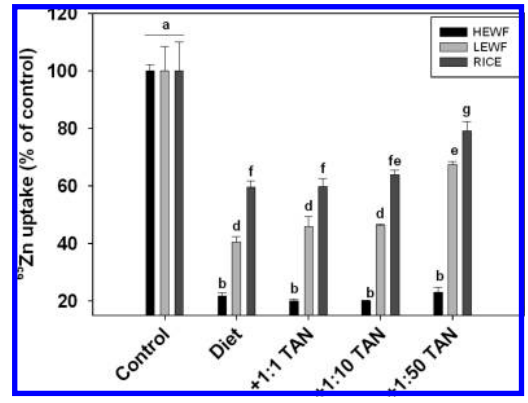

Figure 4. Effect of the food matrix and tannic acid on zinc bioavailability. Zinc uptake was measured from HEWF, LEWF, and rice matrices in the absence and presence of 1:1, 1:10, and 1:50 molar ratios of zinc/tannic acid. The bars represent mean + SD, and bars with different superscript letters differ significantly compared to respective controls and food matrices (p < 0.05, n = 6).

Effect of Tannic Acid on Zinc Uptake from Rice- and Wheat-Based Meals. The uptake of zinc was highest from rice (59.6 ± 2.17%), followed by LEWF (40.44 ± 1.88%) and HEWF (21.86 ± 0.89%) (**Figure 4**). Tannic acid significantly increased the uptake of zinc from LEWF and rice matrices at a 1:50 molar ratio but not at lower molar concentrations.

Effect of the Wheat Flour Extraction Rate and Polyphenolic Beverages on Zinc and Iron Uptake. To validate the direction of response of Caco-2 cells to phytate and polyphenols, the uptake of zinc and iron were studied from wheat-flour-based meals in the presence and absence of polyphenol-rich beverages under identical conditions (**Figure 5**). The direction of response

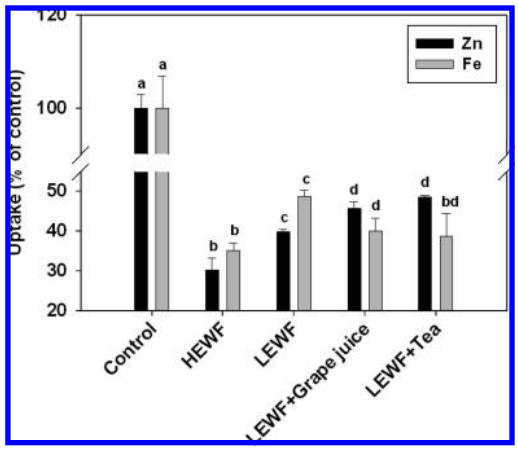

Figure 5. Effect of wheat flour extraction, tea, and grape juice on zinc and iron uptake. Zinc and iron uptake was measured from HEWF and LEWF in the absence and presence of red grape juice or tea. The bars represent mean + SD, and bars with different superscript letters differ significantly ($p < 0.05$, $n = 6$).

to the amount of phytate in Caco-2 cells was similar for both zinc and iron. The uptake of both minerals from LEWF was significantly higher compared to HEWF ($p < 0.05$). The addition of tea extract or red grape juice to LEWF significantly ($p < 0.05$) increased the uptake of zinc; at the same time, the uptake of iron was inhibited significantly.

DISCUSSION

The outcome of approaches aimed at reducing the zinc deficiency greatly depends upon ensuring substantial bioavailability of zinc from fortified foods. This entails screening for zinc bioavailability and selection of fortified foods or breeder lines for improved bioavailability. Although the coupled *in vitro* digestion/Caco-2 cell line model has been used for screening iron and carotenoid bioavailability (*7*−*9*), limited data are available to reliably use this model for screening the bioavailability of zinc, particularly from whole foods and meals. Recent reviews highlighted the inadequate characterization of this model for zinc bioavailability studies (*5, 14*). Herein, we studied the kinetics, effects of zinc depletion/repletion, and various dietary ligands on Caco-2 cell zinc uptake. The data suggest that the direction of response of various dietary ligands either in their pure form or from a typical meal is similar to that of studies in humans and animals (**Tables 1** and **2**). Further, we demonstrated that tannic acid or polyphenolic beverages increase the uptake of zinc from either zinc salt or typical meals in Caco-2 cells.

Understanding the kinetics, response to intracellular pools, and various dietary ligands is the first step necessary for the characterization of a model for screening the bioavailability of nutrients. The uptake of zinc increased as a function of time and is saturated at about 2 h. However, there appears to be a diffusion component (nonmediated) at higher concentrations of zinc. Although the exact mechanism of zinc uptake by the enterocytes is not clear, the kinetic parameters suggest the possibility of an initial membrane binding of zinc for its subsequent rapid uptake, as evidenced by sigmoidal kinetics. Similar kinetics of zinc absorption was reported in Caco-2 cells grown on transwell plates (*18*).

Previous studies demonstrated the homeostatic regulation of zinc absorption in human subjects and cultured cells (*19*−*22*). Although the exact mechanism of intestinal zinc absorption has not been elucidated, the presence of specific zinc transporters in the enterocyte and modulation of their expression/function by zinc status have been reported in the intestine of patients that have undergone ileostomy and in various cell types (*19, 20*). In tune with these results, intracellular depletion of zinc with TPEN significantly increased the uptake of zinc, while zinc uptake in repleted cells remained unchanged, suggesting that the absorption of zinc is attuned to the cellular zinc status. Similarly, zinc absorption has been reported to be higher in subjects restricted with dietary zinc (*23*). Together, these data suggest that the Caco-2 cells possess the absorption and regulatory mechinary for zinc absorption that closely resembles that of humans, an essential feature required for an intestinal cell model.

Various dietary ligands, such as phytates, polyphenols, organic acids, and amino acids, are known to modulate the absorption of nutrients in humans, particularly that of iron and zinc. The effect of methionine, tartaric acid, ascorbic acid, and tannic acid on zinc uptake is in qualitative agreement with published literature (**Table 1**). Bioavailability of zinc from the zinc−methionine complex in humans and chicks was reported to be higher than that of $ZnSO_4$, implying efficient absorption of zinc from the zinc−methionine complex (*24*). Similarly, supplementation of the zinc−methionine complex has been reported to improve growth and plasma zinc levels compared to $ZnSO_4$ in mice (*25*). However, contrary to the results in humans, histidine at higher concentrations (1:10 molar ratio) decreased zinc uptake in Caco-2 cells (*26*). Similar inhibitory effects of histidine on the uptake of zinc in freshwater rainbow trout and isolated rat enterocytes have also been reported (*27, 28*). Arginine, a guanidine group containing amino acid, enhanced the uptake of zinc in Caco-2 cells, which has not been tested thus far in either animals or humans.

Dietary polyphenols are known to interact with iron, leading to inhibition of its absorption. It has been reported that consumption of polyphenol-rich foods reduces iron status without adversely affecting the zinc status in humans and animals (*29, 30*). Between the two representative polyphenols tested, catechin did not influence the uptake of zinc (data not shown). In contrast, tannic acid enhanced the zinc uptake in Caco-2 cells from either zinc salt or rice- and wheat-based meals. However, a much higher amount of tannic acid is required to enhance the uptake of zinc from meals compared to that of zinc salt in the absence of food matrix, suggesting its interaction with other components of the food matrix. It is possible that the large difference in phytic acid between LEWF and HEWF influenced the uptake of zinc. Further, the zinc uptake from the rice-based matrix was higher than that of LEWF and could be due to a higher polyphenol content in rice matrix (refer to the Results). In tune with these results, ingestion of green tea, rich in polyphenols, has been reported to increase the zinc status and tibia zinc concentrations and decrease the iron status in rats (*31, 32*), suggesting a preferential absorption enhancing effect of polyphenols on zinc nutrition. An intriguing finding is the varied effects of catechin (no effect on zinc uptake, data not shown) and tannic acid on zinc uptake. It is possible that specific structural polyphenols (possibly similar to that of tannic acid) chelate/bind zinc and thereby increase the uptake. Recently, the interaction of zinc with epigallo-catechin gallate and potentiation of its anticancer properties in the presence of zinc have been demonstrated (*33*), suggesting a direct role for polyphenols on zinc absorption/metabolism.

The effect of various dietary ligands on the bioavailability of iron in Caco-2 cells has been well-documented and is reported to be in qualitative and quantitative agreement with human studies (*7, 10, 15, 34*). Therefore, we tested the effect of wheat

Table 1. Qualitative Comparison of the Effect of Dietary Ligands on Zinc Uptake in Caco-2 Cells in the Present Study and Published in the Literature

study	phytic acid	polyphenols[a]	methionine	tartaric acid	ascorbic acid	histidine
human	inhibitor(*35*[b],*36*[c])[d]	no effect (*30*)	enhancer (*24*)	enhancer (*1*)	no effect (*37*)	enhancer (*26*)
animal	inhibitor(*3*[e])	no effect (*29*) enhancer (*31, 32*)	enhancer (*25*)			inhibitor (*27*)
present Caco-2 cells[c]	inhibitor	enhancer catechin, no effect tannic acid, enhancer	enhancer	enhancer	no effect	mild inhibition at high concentrations

[a] Polyphenol source: tea extract. [b] Whole and dephytinized meal. [c] High- and low-extraction wheat flour. [d] Indicates respective reference. [e] Flour and bread.

Table 2. Comparison of Zinc Absorption Ratios in Caco-2 Cells (Present Study) and in Humans (Literature) from High and Low Phytate Meal

			ratio	
study	phosphorus (mg/g)	absorption (%)	phosphorus (B/A)	absorption (B/A)
whole soy meal (A)[a]	4.0	22.8	0.0075	1.51
dephytinized soy meal (B)	0.03	34.6		
whole bread (A)[b]	2.85	8.2	0.292	1.60
white bread (B)	0.833	13.2		
HEWF (A)[c]	5.81	0.62	0.187	1.46
LEWF (B)	1.09	0.91		

[a] From ref *35*. [b] From ref *36*. [c] Present study; HEWF, high-extraction wheat flour; LEWF, low-extraction wheat flour.

flour with 2 levels of extraction and polyphenol-rich beverages on the uptake of zinc and iron simultaneously from the same meal, which allowed us to validate the direction of response in Caco-2 cells. The absorption of both zinc and iron were higher from LEWF (70% extraction; 109 mg of phytate/100 g) compared to that of HEWF (90% extractions; 581 mg of phytate/100 g). In tune with these observations, the bioavailability of zinc was reported to be higher from white bread (low phytate) compared to that of whole wheat bread (high phytate) and dephytinized soy meal in humans (*35, 36*). These observations support our findings that Caco-2 cells predict the correct direction of response toward dietary phytate from wheat-based typical meals. Interestingly, the zinc absorption ratio between LEWF- and HEWF-based meals (1.46) in the present study was similar to that derived from the literature between white and whole wheat bread (1.60, *36*) or between dephytinized soy and whole soy meal (1.51, *35*) in humans (**Table 2**). Although the absorption ratio of zinc remained similar in all of the studies (including the present study), the phosphorus ratio varied with the source of the food matrix. It is evident from **Table 2** that the soy-based meal showed higher zinc absorption either before (22.8%) or after (34.6%) dephytnization. This could be due to the high protein content of the soy-based meal contributing to higher absorption of zinc compared to wheat-based meal (*1*). However, the phosphorus and zinc absorption ratio of the wheat-based meal in the present study are comparable to that of the reported study (*36*). These results further suggest that Caco-2 cells may also be used for quantitatively predicting the bioavailability of zinc in humans. Inclusion of tea or grape juice as polyphenol sources reduced the uptake of iron, in tune with previous observations (*7, 34*). In contrast, similar to that of tannic acid, tea and grape juice enhanced the uptake of zinc from wheat-flour-based meals, implying the enhancing effect of polyphenols on zinc uptake. It can also be argued that the factors other than polyphenols, such as tartaric acid and other organic acids present in tea or grape juice, might enhance the zinc absorption. However, ascorbic acid did not influence the uptake even at the highest concentrations tested, which is in tune with previous studies in humans and Caco-2 cells (*11, 37*).

In conclusion, Caco-2 cells respond to intracellular zinc status, and the uptake of zinc is modulated by dietary ligands in a manner similar to that reported in humans and animals. Therefore, the coupled *in vitro* digestion/Caco-2 cell model appears to be a promising tool for assessing the zinc bioavailability from whole foods and meals. However, further studies are needed to understand the mechanism of enhanced zinc uptake in the presence of polyphenols and its possible significance in human zinc nutrition.

ABBREVIATIONS USED

MEM, minimum essential medium; DMEM, Dulbecco's modified eagle's medium; TPEN, *N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)ethylenediamine; HEWF, high-extraction wheat flour; LEWF, low-extraction wheat flour.

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LITERATURE CITED

(1) Lonnerdal, B. Dietary factors influencing zinc absorption. *J. Nutr.* **2000**, *130*, 1378S–1383S.
(2) Stein, A. J.; Nestel, P.; Meenakshi, J. V.; Qaim, M.; Sachdev, H. P.; Bhutta, Z. A. Plant breeding to control zinc deficiency in India: How cost-effective is biofortification. *Public Health Nutr.* **2007**, *10*, 492–501.
(3) Lonnerdal, B.; Sandberg, A.-S.; Sandstrom, B.; Kunz, C. Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. *J. Nutr.* **1989**, *119*, 211–214.
(4) Mazariegos, M.; Hambidge, K. M.; Krebs, N. F.; Westcott, J. E.; Lei, S.; Grunwald, G. K.; Campos, R.; Barahona, B.; Raboy, V.; Solomons, N. W. Zinc absorption in Guatemalan schoolchildren fed normal or low-phytate maize. *Am. J. Clin. Nutr.* **2006**, *83*, 59–64.
(5) Fairweather-Tait, S.; Lynch, S.; Hotz, C.; Hurrell, R.; Abrahamse, L.; Beebe, S.; Bering, S.; Bukhave, K.; Glahn, R.; Hambidge, M.; Hunt, J.; Lonnerdal, B.; Miller, D.; Mohktar, N.; Nestel, P.; Reddy, M.; Sandber, A. S.; Sharp, P.; Teucher, B.; Trinidad, T. P. The usefulness of *in vitro* models to predict the bioavailability of iron and zinc: A consensus statement from the Harvest Plus expert consultation. *Int. J. Vitam. Nutr. Res.* **2005**, *75*, 371–374.
(6) Nayak, B.; Nair., K. M. *In vitro* bioavailability of iron from wheat flour fortified with ascorbic acid, EDTA and sodium hexametaphosphate, with or without iron. *Food Chem.* **2000**, *80*, 545–550.
(7) Au, A. P.; Reddy, M. B. Caco-2 cells can be used to assess human iron bioavailability from a semi-purified meal. *J. Nutr.* **2000**, *130*, 1329–1334.
(8) Garrett, D. A.; Failla, M. L.; Sarama, R. J. Development of an *in vitro* digestion method to assess carotenoid bioavailability from meals. *J. Agric. Food Chem.* **1999**, *47*, 4301–4309.
(9) Satyanarayana, B.; Raghu, P.; Ravinder, P.; Nair, K. M. Gastric digestion of pea ferritin and modulation of its iron bioavailability

by ascorbic and phytic acids in Caco-2 cells. *World J. Gastroenterol.* **2007**, *13*, 2083–2088.
(10) Etcheverry, P.; Wallingford, J. C.; Miller, D. D.; Glahn, R. P. Calcium, zinc, and iron bioavailabilities from a commercial human milk fortifier: A comparison study. *J. Dairy Sci.* **2004**, *87*, 3629–3637.
(11) Etcheverry, P.; Wallingford, J. C.; Miller, D. D.; Glahn, R. P. The effect of calcium salts, ascorbic acid and peptic pH on calcium, zinc and iron bioavailabilities from fortified human milk using an *in vitro* digestion/Caco-2 cell model. *Int. J. Vitam. Nutr. Res.* **2005**, *75*, 171–178.
(12) Han, O.; Failla, M. L.; Hill, A. D.; Morris, E. R.; Smith, J. C., Jr. Inositol phosphates inhibit uptake and transport of iron and zinc by a human intestinal cell line. *J. Nutr.* **1994**, *124*, 580–587.
(13) Hansen, M.; Sandström, B.; Lönnerdal, B. The effect of casein phosphopeptides on zinc and calcium absorption from high phytate infant diets assessed in rat pups and Caco-2 cells. *Pediatr. Res.* **1996**, *40* (4), 547–552.
(14) Hotz, C. Evidence for the usefulness of *in vitro* dialyzability, Caco-2 cell models, animal models, and algorithms to predict zinc bioavailability in humans. *Int. J. Vitam. Nutr. Res.* **2005**, *75*, 423–435.
(15) Glahn, R. P.; Lee, O. A.; Yeung, A.; Goldman, M. I.; Miller, D. D. Caco-2 cell ferritin formation predicts non-radiolabeled food iron availability in an *in vitro* digestion/Caco-2 cell culture model. *J. Nutr.* **1998**, *128*, 1555–1561.
(16) Price, M. L.; Scoyoc, S. V.; Butler, L. G. A critical evaluation of the vanillin reaction as an assay for tannins in sorghum grain. *J. Agric. Food Chem.* **1978**, *26*, 1214–1218.
(17) Harland, B. F.; Oberleas, D. Anion exchange method for determination of phytate in foods: Collaborative study. *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 667–670.
(18) Raffaniello, R. D.; Lee, S. Y.; Teichberg, S.; Wapanir, R. A. Distinct mechanisms of zinc uptake at the apical and basolateral membranes of Caco-2 cells. *J. Cell. Physiol.* **1992**, *152*, 356–361.
(19) Cragg, R. A.; Phillips, S. R.; Piper, J. M.; Varma, J. S.; Campbell, F. C.; Mathers, J. C.; Ford, D. Homeostatic regulation of zinc transporters in the human small intestine by dietary zinc supplementation. *Gut* **2005**, *54*, 469–478.
(20) McMohan, R. J.; Cousins, R. J. Mammalian zinc transporters. *J. Nutr.* **1998**, *128*, 667–670.
(21) Tran, C. D.; Miller, L. V.; Krebs, N. F.; Lei, S.; Hambidge, K. M. Zinc absorption as a function of the dose of zinc sulfate in aqueous solution. *Am. J. Clin. Nutr.* **2004**, *80*, 1570–1573.
(22) Krebs, N. F. Overview of zinc absorption and excretion in the human gastrointestinal tract. *J. Nutr.* **2000**, *130*, 1374S–1377S.
(23) Lee, D. Y.; Prasad, A. S.; Hydrick-Adair, C.; Brewer, G.; Johnson, P. E. Homeostasis of zinc in marginal human zinc deficiency: Role of absorption and endogenous excretion of zinc. *J. Lab. Clin. Med.* **1993**, *122* (5), 549–556.
(24) Rosado, J. L.; Munoz, E.; Lopez, P.; Allen, L. H. Absorption of zinc sulfate, methionine, and polyascorbate in the presence and absence of a plant-based rural Mexican diet. *Nutr. Res.* **1993**, *13*, 1141–1151.
(25) Yu, Z. P.; Le, G. W.; Shi, Y. H. Effect of zinc sulfate and zinc methionine on growth, plasma growth hormone concentration, growth hormone receptor and insulin-like growth factor-I gene expression in mice. *Clin. Exp. Pharmacol. Physiol.* **2005**, *32*, 273–278.
(26) Schölmerich, J.; Freudemann, A.; Köttgen, E.; Wietholtz, H.; Steiert, B.; Löhle, E.; Haussinger, D.; Gerok, W. Bioavailability of zinc from zinc-histidine complexes. I. Comparison with zinc sulfate in healthy men. *Am. J. Clin. Nutr.* **1987**, *45*, 1480–1486.
(27) Glover, C. N.; Hogstrand, C. Amino acid modulation of in vivo intestinal zinc absorption in freshwater rainbow trout. *J. Exp. Biol.* **2002**, *205*, 151–158.
(28) Raffaniello, R. D.; Wapnir, R. A. Zinc uptake by isolated rat enterocytes: Effect of low molecular weight ligands. *Proc. Soc. Exp. Biol. Med.* **1989**, *192*, 219–224.
(29) Afsana, K.; Shiga, K.; Ishizuka, S.; Hara, H. Reducing effect of ingesting tannic acid on the absorption of iron, but not of zinc, copper and manganese by rats. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 584–592.
(30) Ganji, V.; Kies, C. V. Zinc bioavailability and tea consumption. Studies in healthy humans consuming self-selected and laboratory-controlled diets. *Plant Foods Hum. Nutr.* **1994**, *46*, 267–276.
(31) Hamdaoui, M. H.; Chahed, A.; Ellouze-Chabchoub, S.; Marouani, N.; Ben Abid, Z.; Hédhili, A. Effect of green tea decoction on long-term iron, zinc and selenium status of rats. *Ann. Nutr. Metab.* **2005**, *49*, 118–124.
(32) Greger, J. L.; Lyle, B. J. Iron, copper and zinc metabolism of rats fed various levels and types of tea. *J. Nutr.* **1988**, *118*, 52–60.
(33) Sun, S. L.; He, G. Q.; Yu, H. N.; Yang, J. G.; Borthakur, D.; Zhang, L. C.; Shen, S. R.; Das, U. N. Free Zn^{2+} enhances inhibitory effects of EGCG on the growth of PC-3 cells. *Mol. Nutr. Food Res.* **2008**, *52* (4), 465–471.
(34) Boato, F.; Wortley, G. M.; Liu, R. H.; Glahn, R. P. Red grape juice inhibits iron availability: Application of an in vitro digestion/Caco-2 cell model. *J. Agric. Food Chem.* **2002**, *50*, 6935–6938.
(35) Egli, I.; Davidsson, L.; Zeder, C.; Walczyk, T.; Hurrell, R. Dephytinization of a complementary food based on wheat and soy increases zinc, but not copper, apparent absorption in adults. *J. Nutr.* **2004**, *134*, 1077–1080.
(36) Sandstrom, B.; Arvidsson, B.; Cederblad, A.; Bjorn-Rasmussen, E. Zinc absorption from composite meals. The significance of wheat extraction rate, zinc, calcium, and protein content in meals based on bread. *Am. J. Clin. Nutr.* **1980**, *33*, 739–745.
(37) Sandstrom, B.; Cederblad, A. Effect of ascorbic acid on the absorption of zinc and calcium in man. *Int. J. Vitam. Nutr. Res.* **1987**, *57*, 87–90.

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