Ferrous iron uptake by rat duodenal brush border membrane vesicles: Effects of dietary iron level and competing minerals (Zn^{+2}, Mn^{+2}, and Ca^{+2})

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We sought to confirm a recent report that Fe^{+2} uptake into rat brush-border membrane vesicles is markedly increased by short-term consumption of iron-deficient diet, with no additional enhancement as the animal becomes functionally iron-deficient with continuing dietary Fe deprivation. In addition, we investigated whether previously observed in vivo absorption interactions of iron, zinc, and manganese occur in the brush border membrane vesicles uptake process, and whether short-term or long-term consumption of an iron-deficient diet affects the interaction at the uptake level. We did not observe any differences in Fe^{+2} uptake between normal and iron-deficient brush border membrane vesicles, even when the iron status contrast was intensified by feeding a high iron versus iron-deficient diet for 3 weeks. Equimolar Zn^{+2} and Mn^{+2} decreased Fe^{+2} uptake by 29 to 50% and 11 to 39%, respectively. Iron deficiency did not alter these effects. Equimolar Fe^{+2} decreased Zn^{+2} uptake by 13 to 22%. Calcium, included as a negative control, did not affect Fe^{+2} uptake. Thus, some competition between Fe^{+2} and similar divalent cations does occur at the level of the brush border membrane; the exact nature of this competition remains to be determined. (J. Nutr. Biochem. 5:571–577, 1994.)

Keywords: rat; intestine; brush border membrane; iron; zinc; manganese

Introduction

Iron homeostasis in mammals is controlled mainly through regulation of iron absorption because very little absorbed iron is excreted.[1] There are two distinct barriers at which regulation may occur: uptake of iron from the intestinal lumen into the mucosal cell, and its subsequent transport out of the mucosal cell through the basolateral membrane into plasma. Evidence exists that both sites respond to iron deprivation[2]; however, there remains considerable controversy as to the relative importance of each of these steps in the regulation of iron status. More recent studies using in vivo experimental models indicate that the basolateral transport step is not enhanced in iron deficiency,[3] and iron binding by basolateral membranes is not increased in response to increased need (hypoxia).[4] Computer modeling of iron absorption kinetics in dogs indicates it is the uptake step that is markedly enhanced in iron deficiency.[3] However, iron uptake by intestinal brush border membrane vesicles (BBMV) prepared from mice and rats has not been consistently enhanced in parallel with enhanced iron absorption.[5–9] The difference in results may be due to the means by which iron absorption was enhanced (hypoxia versus dietary iron deficiency),[5,8] and the region(s) of the small intestine from which the vesicles were prepared.[9]

In vitro and in vivo studies indicate that under both adequate and iron-deficient conditions, trace elements with similar physicochemical properties, such as iron, zinc, copper, and manganese, may interact at the site of uptake.[8,10,11] In vivo studies have shown that the absorption of Zn and Mn

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are also enhanced during Fe deprivation.[12,13] Intestinal BBMV have been widely used as a simplified model capable of representing the in vivo function of the apical membrane.[14,15] In the present study, we sought to utilize rat BBMV to confirm a recent report[6] that Fe^{+2} uptake into rat BBMV is markedly increased by short-term (24 hr) consumption of an iron-deficient diet, with no additional enhancement as the animal becomes functionally iron-deficient from prolonged dietary Fe deprivation (2 wk). Our additional objectives were to determine if iron, zinc, and manganese interact in an uptake process at the level of the brush border, and to determine whether short-term or long-term consumption of an iron-deficient diet affects the interaction at the uptake level.

Methods and materials

Rats

Sprague-Dawley male rats, 151 to 175 g, were obtained from Camm Research Institute (Wayne, NJ USA). They were housed individually in stainless-steel wire cages in a temperature- (20 to 22° C) and light- (0600 to 1800 hr) controlled room. Body weight was measured upon arrival and weekly thereafter. The rats had ad libitum access to the diet and deionized water. On the day of the experiment, each rat, weighing 230 to 350 g, was anesthetized with an intramuscular injection of a 2:1 (vol/vol) mixture of ketamine hydrochloride (Ketaset, Fort Dodge Laboratories, Fort Dodge, IA USA) and xylazine (Rompun, small animal formulation, Mobey Corp., Shawnee, KS USA). As soon as the intestinal tissue, liver, and blood samples were removed, the rat was killed by severing the heart and thoracic arteries. The protocol was approved by the Institutional Animal Care and Use Committee, Cornell University. Animal care complied with the Guide for the Care and Use of Laboratory Animals.[16]

Diets

A custom-mixed low iron diet was obtained commercially (ICN Biochemicals, Cleveland, OH USA). This diet was modified from the AIN-76A diet and has been described previously.[17] Iron-adequate diet was prepared by adding ferric citrate to the iron-deficient diet. By analysis, the iron-deficient diet contained 7 to 8 mg Fe/kg diet; in experiments 1 and 2, the normal diet contained 36 to 43 mg Fe/kg diet. In experiment 3, the normal diet contained 98 mg Fe/kg diet. In all experiments, all rats were started on the iron-adequate diet. Then, 4 days (experiment 1), 2 weeks (experiment 2), or 3 weeks (experiment 3) before each experimental day, two rats were switched to the iron-deficient diet.

BBMV preparation

On each experimental day, BBMV from both treatment groups were prepared and analyzed in parallel. With a few modifications, BBMV were prepared by the methods of Topham et al.[6] The mucosal tissue from the first 10 cm of the duodenum was scraped with a glass slide, weighed, and placed immediately in ice-cold homogenization buffer (50 mmol/L mannitol, 5 mmol/L HEPES, pH = 7.4). Each BBMV preparation used the combined tissue of two rats, which resulted in approximately 0.85 to 1.25 g wet tissue. To this amount of tissue, 200 mL of ice-cold homogenization buffer was added, and the contents homogenized for 2.5 minutes at high speed in a Waring blender (Waring Products Division, New Hartford, CT USA). Throughout the BBMV preparation, the tissue samples were maintained on ice and were centrifuged at 0 to 2° C. Sufficient 1.0 mol/L $MgCl_2$ was added to the homogenates to bring the concentration to 10 mmol/L, and the homogenates were allowed to stand on ice for 20 min with occasional stirring. The homogenates were then centrifuged at 3000*g* for 15 min. The supernatants were re-centrifuged at 20,000*g* for 30 min. The pellets from this centrifugation were resuspended via passage through a 25-ga needle in 15 mL of a solution containing 100 mmol/L NaCl, 100 mmol/L mannitol, 20 mmol/L HEPES, 0.1 mmol/L $MgSO_4$, and pH = 7.4. Three strokes of a Teflon pestle homogenizer was then used to homogenize the resuspended pellets. $MgCl_2$ was added to a concentration of 10 mmol/L, and the samples were allowed to incubate on ice for 20 min with occasional stirring. The samples were then centrifuged at 3000*g* for 15 min. The supernatants were then centrifuged at 20,000*g* for 30 min. The 100 mmol/L NaCl, 100 mmol/L mannitol, 20 mmol/L HEPES, 0.1 mmol/L $MgSO_4$, pH = 7.4 solution was used to resuspend the final pellet at a protein concentration of 4 to 5 μg/μL. Freshly prepared vesicles were used for each experiment.

Transport media

Mineral-ascorbate solutions were prepared immediately before use from mineral stock solutions ($^{59}FeCl_3$, $^{65}ZnCl_2$, $^{45}CaCl_2$, 120 mmol/L $FeCl_3$, 120 mmol/L $MnCl_2$, 120 mmol/L $ZnCl_2$, 120 mmol/L $CaCl_2$) and freshly prepared 32.8 mmol/L ascorbic acid. The radioisotope stock solutions were in 0.1 mol/L HCl; other mineral stock solutions were adjusted to pH 1.8 with HCl. Aliquots of 500 mmol/L mannitol, 1 mol/L NaCl, and 100 mmol/L HEPES (pH = 7.4) were added to the mineral-ascorbate solutions to achieve final concentrations of 100 mmol/L mannitol, 100 mmol/L NaCl, 20 mmol/L HEPES, pH = 7.4. The final concentration of the minerals and ascorbic acid in the transport media was always 0.2 and 4.0 mmol/L, respectively. Measurement of the ferrous iron concentration in the media, as the ferrozine complex,[18] confirmed that the ascorbate reduced the iron and maintained it as Fe^{+2} throughout the transport experiments.

Transport measurement

A 50 μL volume of transport media was added to 5 μL of BBMV, vortexed, and placed in a shaking water bath at 37° C for 10 min. Mineral uptake was terminated by the addition of 2.5 mL of an ice cold 154 mmol/L NaCl, 5 mmol/L HEPES (pH = 7.4) "stop" solution, and rapidly filtered through a 0.2 μm filter (Gelman Sciences, Ann Arbor, MI USA) on a fritted-glass support. An additional 2.5-mL volume of the "stop" solution was then washed over the filter. The filter was removed and placed in a scintillation vial for later quantification of radioisotope via gamma counting (^{59}Fe, ^{65}Zn) or liquid scintillation counting (^{45}Ca). Uptake values for each mineral were calculated from the specific activity (cpm per pmol of the mineral) and expressed as pmol · μg protein^{-1} · 10 min^{-1}. Only Fe^{+2}, Ca^{+2}, and Zn^{+2} uptake could be determined, as a radioactive tracer for Mn^{+2} was not available at the time of the study.

Protein assay

Samples were solubilized in 0.5 mol/L NaOH and assayed with a semi-micro adaptation (sample volume = 25 μL) of the Bio-Rad DC protein assay kit (Bio-Rad Laboratories, Hercules, CA USA).

Iron status

Percent serum transferrin iron saturation was calculated as the ratio (serum iron to total serum iron binding capacity) × 100.[19,20] Liver storage iron was measured by the method of Torrance and Bothwell.[21]

Marker enzymes for subcellular fractions

Alkaline phosphatase (EC 3.1.3.1; a brush border membrane marker) was measured by a modification of the method of Forstner et al.[22] The modifications were: (1) total assay volume was 1.0 mL; (2) 1.0 mmol/L $CaCl_2$ was added to the assay reagent; (3) the time course of the enzyme reaction was followed spectrophotometrically; and (4) the samples were solubilized in 17 mmol/L β-D-glucopyranoside detergent (final BDG concentration in assay = 0.85 mmol/L).

Sucrase (EC 3.2.1.48; a brush border membrane marker) was measured by a semi-micro adaptation (sample volume = 60 μL) of the method of Messer and Dahlqvist.[23] Because the glucose oxidase (Sigma type X-S, Sigma, St. Louis, MO USA) contained a small amount of sucrase activity, separate standard curves were run for the blanks and assays.

Ouabain-sensitive (Na^+-K^+)-stimulated, Mg^{+2}-requiring ATPase (Na^+/K^+ transporting ATPase; EC 3.6.1.37), a marker for the basolateral membranes, was measured by the method of Quigley and Gotterer,[24] except that the phosphate released was determined by a modification of the Fiske-Subbarow method described for a glucose-6-phosphatase assay,[25] and 0.56 mol/L ascorbic acid was the reducing agent.[26]

Electron microscopy

Samples were selected at random from five experiments and were prepared for electron microscopy according to the methods of Kessler et al.[27] A Philips Model 201 Transmission Electron Microscope (Philips Electronic Inst., Mahwah, NJ) was used.

Statistical analysis

The Minitab Statistical Software, release 6.1.1 (Minitab, State College, PA USA) was used. The iron status measurements for the normal and −Fe groups were compared by one-tailed *t* tests. Other comparisons were made using two-tailed *t* tests.[28]

Results

Iron status of the rats

The iron status was assessed by measuring the storage (nonheme) iron in the liver and transferrin saturation in the serum *(Table 1)*. In experiment 1, consumption of the −Fe diet for 4 days decreased transferrin saturation, but liver iron stores were not significantly changed. In experiment 2, consumption of the −Fe diet for 14 days decreased transferrin saturation but again not liver iron stores. In both experiments 1 and 2, the liver iron stores in the normal rats were lower than values for control rats reported by others.[6,29] In experiment 3, when the iron level of the normal diet was more than doubled to 98 mg Fe per kg diet, and rats were fed either the normal or the −Fe diet for 21 days, both the mean transferrin saturation and liver iron stores were significantly lower in the −Fe group. Transferrin saturation values for the −Fe rats in all three experiments were in the <0.16 range, which is generally considered indicative of iron deficiency in humans[30]; we are not aware of a similar range established for rats.

Membrane purity

Enrichment of brush-border membrane markers in the BBMV fractions was approximately 22 fold for sucrase and 17 to 18 fold for alkaline phosphatase *(Table 2)*. In a preliminary experiment, ouabain-sensitive (Na^+-K^+)-stimulated, Mg^{+2}-requiring ATPase was enriched 0.2 to 2.0 fold in the BBMV, indicating only a small amount of contamination with basolateral membranes (data not shown).

Electron microscopy

Vesicle formation was confirmed by electron microscopy, and the appearance of the vesicles was nearly identical to that reported by Kessler et al.[27]

Influence of iron status on BBMV uptake of Fe^{+2}, Zn^{+2}, and Ca^{+2}

Feeding an iron-deficient diet for 4, 14, or 21 days did not affect uptake of Fe^{+2} by BBMV *(Table 3)*. Regardless of iron status, the BBMV took up similar amounts of Fe^{+2} and Zn^{+2} and about one-tenth as much Ca^{+2} *(Table 3)*. The correlation (*r* value) over all preparations, between Fe^{+2} and Zn^{+2} uptake was 0.77 ($P < 0.01$); between Fe^{+2} and Ca^{+2} it was 0.24 (nonsignificant, $P > 0.05$).

Inhibition of Fe^{+2} uptake by equimolar Zn^{+2}, Mn^{+2}, or Ca^{+2}

In all three experiments, adding equimolar Zn^{+2} to the media decreased Fe^{+2} uptake by 30 to 50%, and the inhibition was similar in BBMV from normal and iron-deficient rats *(Fig-*

Table 1 Iron status measurements in normal rats and rats which had been fed an iron-deficient diet for 4, 14, or 21 days

Experiment no.	Fe status	(No. of rats)	Serum transferrin saturation	Liver non-heme iron
				nmol Fe/g
1	Normal	(6)	0.369 ± 0.120	393 ± 75
	−Fe 4 days	(6)	0.090 ± 0.031*	247 ± 14
2	Normal	(4)	0.458 ± 0.120	435 ± 151
	−Fe 14 days	(4)	0.056 ± 0.010*	225 ± 10
3	Normal	(10)	0.471 ± 0.031	929 ± 70
	−Fe 21 days	(10)	0.037 ± 0.003**	227 ± 12**

Values are mean ± SEM. Means for rats fed the iron-deficient diet are significantly lower than the normal rats in the same experiment. *$P < 0.05$; **$P < 0.01$.
The normal and −Fe diets contained 35 to 40 mg Fe and 7 to 8 mg Fe per kg diet, respectively, in experiments 1 and 2.
The normal and −Fe diets contained 98 and 7 mg Fe per kg diet, respectively, in experiment 3.

Table 2 Marker enzyme specific activity, yield, and enrichment in duodenal brush-border membrane vesicles (BBMV) from normal rats and rats fed an iron-deficient (−Fe) diet for 4 days

Parameter	Alkaline phosphatase		Sucrase	
	Normal	−Fe	Normal	−Fe
Homogenate Sp. Act.*	4.29 ± 0.20	4.90 ± 0.23	.0269 ± .0029	.0231 ± .0019
BBMV Sp. Act.	75.3 ± 2.9	82.3 ± 3.9	.603 ± .054	.504 ± .031
Yield in BBMV†	20.8 ± 1.8	19.1 ± 1.6	26.1 ± 0.9	24.8 ± 1.4
Enrichment in BBMV‡	17.7 ± 0.8	16.9 ± 0.8	22.9 ± 1.5	22.2 ± 0.8

Values are mean ± SEM. n = 8 preparations; each preparation was made with the mucosa from two rats. The protein yields were 1.2 ± 0.3% and 1.1% ± 0.2% in the normal and −Fe samples, respectively.
*Enzyme specific activity is expressed as $\mu mol \cdot min^{-1} \cdot mg\ protein^{-1}$.
†Percent of homogenate activity.
‡(Sp. Act. in BBMV)/(Sp.Act. in homogenate).

Table 3 Fe^{+2}, Zn^{+2}, and Ca^{+2} uptake into duodenal brush-border membrane vesicles (BBMV) from non-fasted normal rats and rats that had been fed an iron-deficient (−Fe) diet for 4, 14, or 21 days

Experiment no.	Fe status	Fe^{+2} uptake	Zn^{+2} uptake	Ca^{+2} uptake
1	Normal*	50.71 ± 10.58 (13)	53.64 ± 5.71 (9)	5.54 ± 0.57 (6)
	−Fe 4 days	50.62 ± 6.75 (13)	54.18 ± 4.43 (9)	5.81 ± 0.61 (6)
2	Normal*	49.96 ± 13.19 (7)	48.13 ± 5.83 (7)	—
	−Fe 14 days	45.44 ± 5.99 (7)	47.19 ± 3.10 (7)	—
3	Normal†	56.18 ± 9.03 (5)	47.19 ± 7.82 (5)	5.62 ± 0.72 (5)
	−Fe 21 days	47.20 ± 4.54 (5)	51.30 ± 2.79 (5)	5.70 ± 0.60 (5)

Units of uptake: $pmol \cdot \mu g\ protein^{-1} \cdot 10\ min^{-1}$. Values are mean ± SEM. ( ) = number of preparations; each preparation was made from the mucosa of two rats. Uptakes by −Fe BBMV were not significantly different from normal (*t* test).
*The normal and −Fe diets contained 35 to 40 mg Fe and 7 to 8 mg Fe per kg diet, respectively.
†The normal and −Fe diets contained 98 and 7 mg Fe per kg diet, respectively.

ure 1). Equimolar Mn^{+2} was less inhibitory to Fe^{+2} uptake than Zn^{+2}, but in most comparisons the inhibition was statistically significant *(Figure 1)*. Calcium had no effect on Fe^{+2} uptake in experiment 1 and was not measured in the other experiments.

Inhibition of Zn^{+2} and Ca^{+2} uptake by Fe^{+2}

Ferrous iron added to the media depressed Zn^{+2} uptake by 14 to 22%, or about half as much as Zn^{+2} depressed Fe^{+2} uptake *(Table 4)*. The Fe^{+2} depressed Ca^{+2} uptake by only 5 to 11% and was not statistically significant in the individual experiments *(Table 4)*.

Discussion

The report of Topham et al.[6] suggested that in rats the Fe^{+2} uptake step is regulated in response to iron status. We expected to confirm this result using BBMV in the work reported here. In addition, we intended to determine whether the membrane response was specific for enhancing Fe^{+2} uptake, or if the uptake of a closely related mineral such as Zn^{+2} was also enhanced.

In contrast to Topham et al., we did not observe differences in Fe^{+2} uptake between −Fe and normal BBMV. Furthermore, we were unable to induce a difference in Fe^{+2} uptake, even after enhancing the contrast in iron status by increasing the iron nutriture in the normal rats and increasing the period of iron deprivation in the −Fe rats (experiment 3). These results are puzzling because the BBMV preparation and uptake conditions of Topham et al. appear to be similar to ours. One possible explanation is that Topham et al. used a different strain of rats. Also, they state that they used "mucosal scrapings," implying that they may have combined different segments of the small intestine, whereas we used only the duodenum. We selected the duodenum because iron absorption from the duodenum is much more rapid and is increased to a greater extent in iron deficiency than in the more distal sections of the small intestine.[31] However, in a study that compared iron uptake in duodenal and ileal "brush borders" prepared from Wistar rats, the Fe^{+2} uptake by duodenal brush borders was only marginally enhanced by iron depletion; the uptake by normal ileal brush borders was half as high, but increased by 80% in iron deficiency.[9] Thus, if Topham et al.[6] used mucosal scrapings from the

distal sections of the small intestine, they may have observed an adaptation to iron deficiency that occurs only in the lower small intestine.

It is a valid concern that the vesicles may have been nonfunctional. However, our enzyme enrichment values for alkaline phosphatase and sucrase are similar to those reported in BBMV prepared from rabbits[27] and apparently higher than the values for mouse and rat BBMV utilized in other studies of the iron-uptake mechanism.[5,32] The low enrichment of ouabain-sensitive Na^+-K^+-ATPase indicated that basolateral membrane contamination should not have distorted our results. Electron microscopy confirmed that the vesicles were intact. BBMV prepared by the Mg precipitation method have been shown repeatedly to be oriented right side out.[15] Topham et al.[6] did not report data on membrane purity. It is possible that some non-membrane component of their BBMV preparations accounted for the increased Fe^{+2} uptake in −Fe vesicles. In any case, if one believes that our results are not an artifact of a technical error, the data indicate that the Fe^{+2} uptake by duodenal brush border membrane vesicles of rats does not reflect iron status of the animal. In apparent agreement with our results, Simpson and Peters also found that Fe^{+2} uptake was not increased in BBMV prepared from the hypoxic mouse, their experimental model for increased iron absorption.[8]

If one assumes that BBMV are a useful model to study trace element interactions at the level of the mucosal tissue,[33] our results indicate that manganese and zinc may share an uptake pathway with Fe^{+2}. In our study, equimolar Zn^{+2} effectively inhibited Fe^{+2} uptake by 29 to 50% *(Figure 1)*. Conversely, equimolar Fe^{+2} inhibited Zn^{+2} uptake by 13 to 22% *(Table 4)*. These results suggest that the simultaneous presence of Fe^{+2} and Zn^{+2} at the brush border membrane may significantly decrease uptake of each of these minerals. Perhaps the uptake pathways of these minerals are not entirely shared, as Fe^{+2} uptake was affected to a much greater extent by Zn^{+2} than vice versa.

An equimolar amount of Mn^{+2} inhibited Fe^{+2} uptake by 11 to 39%, indicating that the presence of manganese in the intestinal lumen would significantly decrease iron uptake. We were unable to measure Mn^{+2} uptake, as no radioisotope for Mn was available at the time of the experiments. In related studies, it has been shown in perfused rat duodenum that Mn was taken up less rapidly than iron.[34] Other investi-

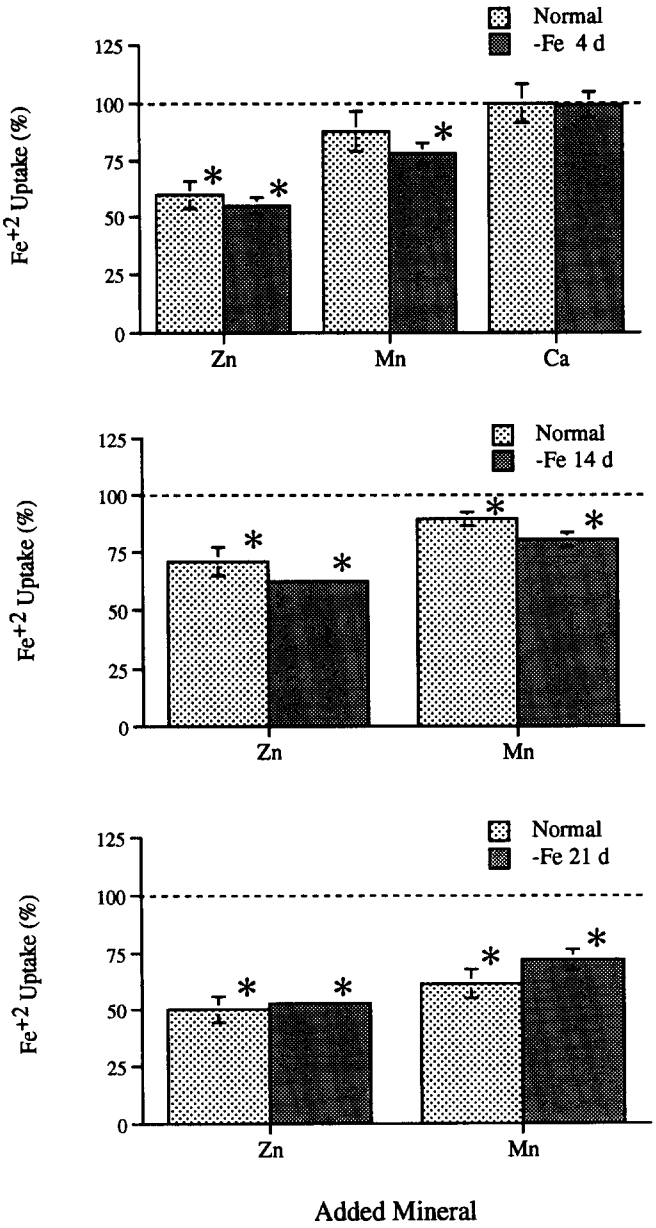

Figure 1 Ferrous iron uptake in the presence of equimolar Zn^{+2}, Mn^{+2}, or Ca^{+2}. Values are expressed as a percentage of the Fe^{+2} uptake in the absence of those ions, in normal duodenal brush-border membrane vesicles (BBMV) and vesicles prepared from rats fed an iron-deficient (−Fe) diet for 4, 14, or 21 days. Asterisks indicate significant ($P < 0.05$) difference versus Fe^{+2} uptake in the absence of added mineral.

Table 4 Percentage change in Zn^{+2} and Ca^{+2} uptakes into duodenal BBMV prepared from normal rats and rats that had been fed an iron-deficient diet for 4, 14, or 21 days, when equimolar Fe^{+2} was added to the uptake media†‡

Expt. No.	Fe Status	% change in Zn^{+2} uptake	% change in Ca^{+2} uptake
1	Normal§	−22.2 ± 3.3** (7)	−8.3 ± 3.7 (6)
	−Fe 4 days	−20.3 ± 3.0** (7)	−10.7 ± 4.8 (6)
2	Normal§	−17.7 ± 3.9* (5)	—
	−Fe 14 days	−13.5 ± 2.0** (5)	—
3	Normal‖	−13.8 ± 4.6* (5)	−10.0 ± 3.6 (5)
	−Fe 21 days	−16.8 ± 2.0** (5)	−5.4 ± 5.0 (5)

Uptake values for Zn^{+2} and Ca^{+2} without added Fe^{+2} are presented in *Table 3*.

†The % change was $\frac{(\text{uptake with Fe} - \text{uptake without Fe})}{\text{uptake without Fe}} \times 100$

‡Values are mean ± SEM. ( ) = no. of preparations. Uptake was significantly lowered by adding equimolar Fe^{+2}, $^*P < 0.05$; $^{**}P < 0.01$.
§The normal and −Fe diets contained 35 to 40 mg Fe and 7 to 8 mg Fe per kg diet, respectively.
‖The normal and −Fe diets contained 98 and 7 mg Fe per kg diet, respectively.

gators observed that in BBMV prepared from normal mice, a 10-fold excess of Mn^{+2} was necessary to decrease Fe^{+2} uptake by 43%.[8] Taken together these observations suggest that Mn^{+2} and Fe^{+2} uptake pathways are less closely related than Zn^{+2} and Fe^{+2} pathways.

Calcium is chemically and biologically different from iron. We measured Ca^{+2} uptake and its interaction with Fe^{+2} to serve as a negative control to ascertain that there was some specificity to the mineral uptake. This was confirmed in that equimolar calcium did not interfere with iron uptake *(Figure 1)*, and iron had a minimal effect on calcium uptake *(Table 4)*. It has been shown previously in rats that equimolar calcium does not inhibit iron absorption, although it does interfere when included as a 10-fold or 100-fold excess relative to iron.[35] In two human studies, Hallberg et al. concluded that inhibition of dietary iron absorption by calcium (present in relatively high amounts and in a high molar ratio to iron) is not due to competition for uptake into the mucosa, but rather, for transfer after the iron has entered the cell.[36,37]

In conclusion, we were unable to confirm that BBMV uptake of iron is increased in iron deficiency. It should be determined why we and Topham's group, using ostensibly similar conditions, got contradictory results. Different species and experimental models are used to understand the regulation of iron absorption on the assumption that this regulation is highly conserved in all animal species. If there are true differences in the regulation of uptake by different strains of rats or different mammalian species, this should be clarified so that only relevant models are chosen to study the ultimate objective, which is to understand the regulation of iron absorption in humans. Alternatively, if technical problems have led to the contradictory results, it is important to elucidate the critical features of the experimental model.

It is clear that there are iron-zinc and iron-manganese interactions in the brush border membranes, but the mechanisms are not known. Clarification of the mechanisms may improve our understanding of the regulation of iron absorption as well as provide better information on which to determine optimum ranges for dietary mineral content.

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