

# Iron supplements inhibit zinc but not copper absorption in vivo in ileostomy subjects<sup>1-3</sup>

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## ABSTRACT

**Background:** Iron supplements may inhibit intestinal zinc and copper uptake because these elements may compete for binding to a transporter molecule (divalent metal transporter 1) that is located on the apical side of the small intestinal epithelium.

**Objective:** We quantified the interaction between different amounts of administered iron and the absorption of zinc and copper in humans.

**Design:** Eleven subjects with an ileostomy [mean ( $\pm$  SD) age:  $55 \pm 9$  y] ingested a stable-isotope-labeled zinc and copper solution containing 12 mg Zn ( $^{66}\text{Zn}$  and  $^{67}\text{Zn}$ ) and 3 mg Cu ( $^{65}\text{Cu}$ ) in the presence of 0, 100, or 400 mg Fe as ferrous gluconate on 3 respective test days. Subsequently, 1 mg  $^{70}\text{Zn}$  was injected intravenously. Subjects collected ileostomy effluent and urine for 24 h and 7 d, respectively. Zinc status and true zinc absorption were calculated from the urinary excretion of the zinc isotopes. Apparent copper absorption was calculated from ileostomy effluent excretion of the orally administered copper isotopes.

**Results:** Zinc status did not differ significantly between the 3 iron doses. Mean ( $\pm$  SEM) zinc absorption was significantly higher in the absence of iron than with the concomitant ingestion of 100 or 400 mg Fe ( $44 \pm 22\%$  compared with  $26 \pm 14\%$  and  $23 \pm 6\%$ , respectively;  $P < 0.05$ ), whereas zinc absorption did not differ significantly between the 100- and 400-mg Fe doses. Apparent copper absorption was  $48 \pm 14\%$ ,  $54 \pm 26\%$ , and  $53 \pm 7\%$  in the presence of 0, 100, and 400 mg Fe, respectively, and did not differ significantly between the 3 iron doses.

**Conclusion:** Oral iron therapy may impair zinc absorption and thus zinc status in a dose-independent fashion but does not affect copper absorption. *Am J Clin Nutr* 2003;78:1018–23.

**KEY WORDS** Ileostomy, zinc, copper, iron, zinc absorption, copper absorption, small intestine

## INTRODUCTION

Approximately 2 billion persons worldwide suffer from iron deficiency (1). Iron deficiency is often accompanied by other nutrient deficiencies such as zinc and copper deficiencies, especially when the iron deficiency is caused by insufficient dietary intakes of micronutrients, as is often the case in developing countries (2, 3). As a result, supplements containing iron and multiple trace elements and minerals are used by millions of people worldwide. It is important to provide detailed information regarding safe upper intake limits for supplements to minimize adverse effects on mineral absorption caused by mineral-mineral interactions. It has long been recognized that iron metabolism interacts with the

metabolism of several other micronutrients, such as zinc and copper. The discovery of divalent metal transporter 1 (DMT1) (4) provided an explanation of previous indications of the competitive absorption of iron, zinc, and copper from the small intestine. Iron was shown to decrease zinc absorption in humans in a dose-dependent way when given in a water solution but not when given with a meal (5, 6). Iron supplements have been reported to decrease zinc absorption in pregnant women (7), and lower serum zinc concentrations are observed in teenage pregnant women taking daily multivitamin supplements containing 18 mg Fe than in those taking multivitamin supplements without any iron (8). However, other human studies showed that iron supplementation did not alter markers of zinc status or zinc absorption (9–11).

The iron-copper interaction may pose a health risk because marginal copper deficiency greatly increases the effect of iron deficiency (12, 13). Animal experiments have shown that iron supplementation diminishes copper status in rats and sheep (14).

In the present study of interactions between iron, zinc, and copper, iron and isotopically enriched zinc and copper were administered orally in an aqueous solution after the subjects fasted overnight, as is generally advised to ensure maximal micronutrient absorption. Zinc and copper absorption were calculated from the appearance of the isotopically enriched stable isotopes in ileostomy effluent and urine. Subjects with an ileostomy were recruited to ensure complete recovery of test drinks. This study aimed to investigate the acute effects of moderate (100 mg) and high (400 mg) doses of iron routinely used in a clinical setting on the absorption of copper and zinc in adults.

## SUBJECTS AND METHODS

### Subjects

Eleven subjects [9 men, 2 women; mean ( $\pm$  SD) age:  $55 \pm 9$  y] with an ileostomy were recruited for participation.

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When the indication for proctocolectomy was Crohn disease, subjects were admitted into the study only if they had been in remission for  $\geq 3$  y and  $< 10$  cm of the terminal ileum was removed. None of the participants took medication that affected the normal functioning of the upper gastrointestinal tract, and none had symptoms of an absorptive disorder or nutrient deficiencies. In addition, all subjects were free of inflammation associated with an acute phase response of the immune system, as determined by measurement of serum C-reactive protein concentration. Detailed information about the study was given orally and in writing. All subjects signed a written informed consent form before participation. The Ethics Committee of the University Hospital Maastricht, Maastricht, Netherlands, approved the study.

### Preparation of isotopes

The zinc isotopes  $^{66}\text{Zn}$  (enriched to 99% abundance),  $^{67}\text{Zn}$  (enriched to 90.6% abundance), and  $^{70}\text{Zn}$  (enriched to 95% abundance) were obtained as zinc metal (ChemGas, Boulogne, France). Both oral and intravenous zinc doses were prepared in the Dose Preparation Unit (DPU) at the Institute of Food Research. Elemental zinc was dissolved in concentrated hydrochloric acid and evaporated to dryness. The precipitate was dissolved in trisodium citrate, and the pH was adjusted to 7 by using sodium bicarbonate. The final solution was filtered through a 0.2- $\mu\text{m}$  filter to remove pyrogens. The oral doses were divided into aliquots in the DPU, and the individual intravenous doses were subsampled, autoclaved, and subjected to sterility testing at the Ipswich Hospital Pharmacy, Ipswich, United Kingdom.

The copper isotope ( $^{65}\text{Cu}$ , enriched to 99.8% abundance) was obtained as elemental copper foil (ChemGas), and individual oral doses were prepared in the DPU. Elemental copper was dissolved in concentrated nitric acid (Aristar grade; BDH, Poole, Dorset, United Kingdom) on a hot plate at 150 °C. The solution was evaporated to virtual dryness and then redissolved in concentrated hydrochloric acid (Aristar grade; BDH). The solution was subboiled to dryness, and the residue was redissolved in a 6-mol hydrochloric acid/L solution and removed from the heat. Sterile water was added, and the pH was adjusted to 5 with 1 mol NaOH/L. The solution was made up to the appropriate volume with sterile water and filtered through a 0.22- $\mu\text{m}$  filter. However, filtering proved to be progressively difficult, and hydrochloric acid was added to adjust the pH to 3.5, which allowed the remaining solution to be filtered. The final solution was subsampled into individual doses in the DPU and stored at  $-20$  °C until required.

### Protocol

For 3 d before the first test day and during the first test day, subjects kept a weighed food intake diary to record their habitual diet. The food diary was used to ensure that the subjects consumed an identical diet before all 3 experimental days. The study was a randomized crossover design.

After the subjects had fasted overnight, baseline ileostomy effluent and urine samples were collected in acid-washed containers when the subjects woke up at 0800. A venous blood sample was taken from an antecubital vein in the forearm for measurement of serum ferritin and blood hemoglobin and hematocrit as indicators of iron status. Serum C-reactive pro-

tein was measured to confirm the absence of inflammation associated with an acute phase response. After blood sampling, a test drink, which is described below, was consumed, and all ileostomy effluent was subsequently collected for the following 24 h. Effluent samples were immediately frozen on dry ice and stored in plastic airtight boxes at  $-20$  °C until analyzed. Complete 24-h urine collections were obtained for 7 d after administration of the isotopic dose, and 100-mL fractions were also stored at  $-20$  °C until analyzed. All sample containers were acid-washed before sample collection.

### Test solutions

The test beverages contained 300 mL of an isotonic maltodextrin solution. Isotopic solutions containing 7 mg  $^{67}\text{Zn}$ , 5 mg  $^{66}\text{Zn}$ , and 3 mg  $^{65}\text{Cu}$  were added to this test beverage. The test beverage was ingested on 3 occasions: once without addition of an iron supplement, once with synchronous ingestion of a pill containing 100 mg Fe as ferrous gluconate (Numico, Wageningen, Netherlands), and once with synchronous ingestion of 400 mg Fe as ferrous gluconate.

Zinc was abundantly present as  $^{67}\text{Zn}$  and  $^{66}\text{Zn}$ . Ingestion of 7 mg  $^{67}\text{Zn}$  was needed to ensure sufficient urinary  $^{67}\text{Zn}$  enrichment. The less expensive  $^{66}\text{Zn}$  was added to the test beverages to increase the total amount of zinc to 12 mg.

Immediately after oral administration of the test beverage, 1 mg  $^{70}\text{Zn}$  was administered intravenously into an antecubital vein in the forearm. The exact amounts of oral and intravenous isotopes were determined for each individual subject. The subjects then refrained from eating for 4 h but were allowed water ad libitum after 1 h after administration of the dose. After 4 h after administration of the dose, the subjects consumed their habitual diet as described above.

### Sample processing

All glassware, crucibles, and other equipment or containers used during sample processing were acid-washed before use. Ileostomy effluent was freeze-dried, autoclaved, and ground by using a grinder with no metal parts in the grinding basket. All 24-h effluent samples were pooled and homogenized, and subsamples were dried to ashes at 450 °C for 48 h. Aliquots and an internal standard of  $^{68}\text{Zn}$  were accurately weighed into crucibles and dissolved in a 4-mol HCl/L solution while being heated for 2 h on hot plates.

Urine aliquots were accurately weighed into 150-mL beakers, concentrated on hot plates, transferred to silica crucibles, heated to dryness, and then heated to ashes at 450 °C for 48 h. The ash was subsequently dissolved in a 0.5-mol HCl/L solution while being heated for 2 h on hot plates.

Zinc was then extracted from urine samples by using anion-exchange chromatography. An anion-exchange resin (Type AG1X-8; BioRad Laboratories, Hercules, CA) was soaked in deionized water for  $\geq 24$  h before use. Two milliliters of presoaked resin was packed into acid-washed glass columns (1-mL pipette tips; Sarstedt, Nümbrecht, Germany). The columns were connected to a peristaltic pump (Watson Marlow, Falmouth, United Kingdom) with a flow rate of 1 mL/min and were washed with a 2-mol  $\text{HNO}_3$ /L solution for 1 h to remove any minerals. The resin was reconstituted to the chloride form by using a 0.5-mol HCl/L solution, which was pumped through the column for 60 min. The sample solutions were loaded onto

the columns, the columns were washed with a 0.5-mol HCl/L solution for 30 min, and then zinc was eluted with a 1-mol HNO<sub>3</sub>/L solution. This fraction was collected, and the total zinc concentration was adjusted to 1 part per million for determination of zinc isotopic enrichment with a Micromass "Isoprobe" multicollector inductively coupled plasma mass spectrometer (MC-ICP-MS; Micromass, Wythenshawe, Manchester, United Kingdom) as described below. For copper extraction from the ileostomy fluid samples, presoaked resins were washed with a 2-mol HNO<sub>3</sub>/L solution for 1 h and regenerated into the chloride form by pumping a 6-mol HCl/L solution through the column for 1 h. Samples were subsequently loaded, and the columns were washed with a 6-mol HCl/L solution for 30 min. Copper was eluted with a 2.5-mol HCl/L solution, dried down under a hot lamp, and reconstituted for MC-ICP-MS analysis in 2% (by vol) ultrapure HNO<sub>3</sub>.

Stable copper and zinc isotopic ratios were determined by using an MC-ICP-MS combined with a Cetac "Aridus" desolvator (Cetac, Omaha) at the Institute of Food Research (Norwich, United Kingdom). The samples were analyzed with bracketing standards and instrument blanks. For correction of instrumental mass bias, a simple linear correction between samples and bracketing standards was applied. Standard Reference Material 976 (certified for isotopic composition; National Institutes of Standards and Technology, Gaithersburg, MD) was used as a reference in the copper analyses. For zinc, Spectrosol (BDH, Poole, United Kingdom) was used as a reference in the absence of a certified isotopic standard, and the zinc isotopic composition was assumed to be the average natural composition as defined elsewhere (15).

The external reproducibility of the calculated zinc isotopic enrichment, which was based on the isotopic variation in the baseline urine samples, was 0.6%, 4.9%, and 10.1% for <sup>66</sup>Zn:<sup>64</sup>Zn, <sup>67</sup>Zn:<sup>64</sup>Zn, and <sup>70</sup>Zn:<sup>64</sup>Zn, respectively. The external reproducibility of the calculated copper isotopic enrichment, which was based on the isotopic variation in the baseline ileostomy effluent samples, was 4.0%.

## Calculations

### Zinc absorption from urinary monitoring

The mass spectrometric ratios measured by the MC-ICP-MS were converted into mole fractions of recovered dose (oral and intravenous) by using the equations set out in Lowe et al (16). It was assumed that 48 h after dose administration, the zinc from the oral and intravenous doses would have equilibrated and that thereafter any 24-h pooled sample would be adequate to calculate the true zinc absorption of the oral dose. Thus, for the second 24-h sample ( $t = 48$  h), true zinc absorption is given by the following equation:

True absorption ( $t = 48$  h) =

$$\left( \frac{\text{mole fraction}_{\text{oral}}}{\text{mole fraction}_{\text{IV}}} \right) \times \left( \frac{\text{dose}_{\text{IV}}}{\text{dose}_{\text{oral}}} \right) \quad (1)$$

where mole fraction<sub>oral</sub> and mole fraction<sub>IV</sub> are the mole fractions of the oral and intravenous doses, respectively, from a single 24-h pooled urine sample collected  $\geq 48$  h after the dose, and dose<sub>oral</sub> and dose<sub>IV</sub> are the quantities of labeled zinc given as oral and intravenous doses, respectively.

This can obviously be repeated for all subsequent 24-h samples ( $t = 72, 96, 120, 144,$  and  $168$  h). The final true absorption value can then be calculated as the mean of these 6 values.

### Copper absorption

Because copper has only 2 stable isotopes, fecal monitoring is the method used to calculate the apparent absorption. The details of this are contained in a separate publication (17). Briefly, the following formula was used:

$$\text{Apparent absorption} = (\text{dose}_{\text{oral}} - \text{recovered}_{\text{oral}}) / \text{dose}_{\text{oral}} \quad (2)$$

where recovered<sub>oral</sub> is the quantity of copper from the oral dose that was found in the feces and dose<sub>oral</sub> is the quantity of copper in the oral dose.

### Exchangeable zinc pool

This method is based on the technique developed by Miller et al (18). The endogenous zinc contained in the pool is assumed to exchange with any newly absorbed zinc in the plasma within 2 d. As mentioned previously, mass spectrometric ratios can be used to calculate the mole fractions of the individual sources of zinc in the study. These sources are the oral and intravenous tracers and the naturally abundant (NA) tracee. Enrichment in any particular urine sample is defined as the ratio of the mole fraction of zinc from the intravenous source (mole fraction<sub>IV</sub>) to the mole fraction of zinc from the NA source (mole fraction<sub>NA</sub>).

$$\text{Enrichment} = \text{mole fraction}_{\text{IV}} / \text{mole fraction}_{\text{NA}} \quad (3)$$

By plotting the natural logarithm of this ratio against time (for  $t > 48$  h), fitting a straight line through the data, and extrapolating back to  $t = 0$ , the size of the exchangeable zinc pool (EZP) can be obtained. In this method, the entire intravenous dose is assumed to equilibrate in the EZP instantaneously, and extrapolation back to  $t = 0$  is assumed to compensate for any loss of intravenous dose from the EZP.

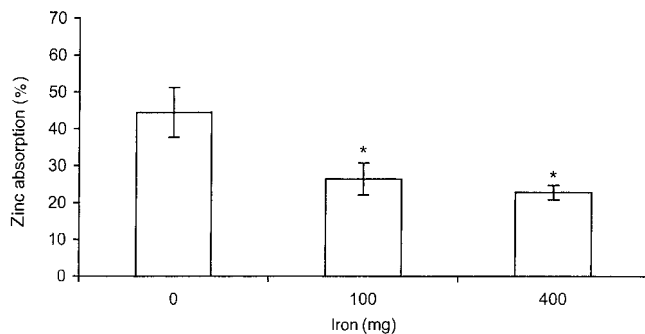
## Statistics

EZPs and zinc and copper absorption during the 3 different interventions were compared with the use of repeated-measures analysis of variance. Differences were regarded as statistically significant if  $P < 0.05$ . Significant results were further analyzed by using Scheffe post hoc tests. Statistical results were computed by using the STATVIEW 5.0 software package for MACINTOSH (SAS Institute Inc, Cary, NC).

## RESULTS

All the subjects had normal iron status: the mean ( $\pm$  SD) serum ferritin and blood hemoglobin concentrations were  $117.1 \pm 85.4$   $\mu\text{g/L}$  and  $9.5 \pm 0.7$  mmol/L, respectively, and the mean blood hematocrit was  $44.2 \pm 3.3$  L/L. C-reactive protein concentrations were  $< 9$  mg/L in all the subjects.

The sizes of the total EZPs on the days in which 0, 100, and 400 mg Fe were ingested were  $126 \pm 28$ ,  $120 \pm 27$ , and  $119 \pm 35$  mg, respectively. No significant difference in EZP size between the 3 iron doses was observed.



**FIGURE 1.** Mean ( $\pm$  SEM) zinc absorption from a water solution containing 12 mg Zn and 0, 100, or 400 mg Fe as ferrous gluconate ( $n = 11$ ). \*Significantly different from 0,  $P < 0.05$  (ANOVA and Scheffe post hoc test).

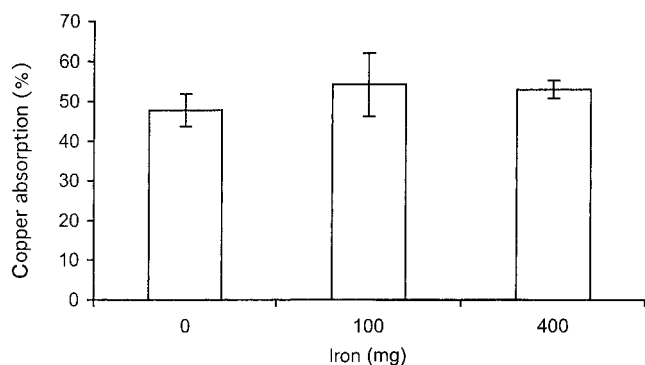
All the subjects completed the intervention study. The data from one subject on one experimental day were excluded from further analyses because of contamination of the samples.

Mean ( $\pm$  SEM) true zinc absorption, which was calculated from urinary zinc isotope excretion, was significantly lower in the presence of 100 or 400 mg Fe than in the absence of iron ( $26.4 \pm 14.4\%$  and  $22.9 \pm 6.4\%$ , respectively, compared with  $44.5 \pm 22.5\%$  of the administered dose;  $P < 0.05$ ). Zinc absorption did not differ significantly between the 100- and 400-mg Fe interventions (**Figure 1**).

Copper absorption in the presence of 0, 100, and 400 mg Fe as ferrous gluconate was  $47.8 \pm 13.6\%$ ,  $54.1 \pm 26.1\%$ , and  $52.8 \pm 7.3\%$  of the administered dose, respectively. No significant differences were observed between any of the interventions (**Figure 2**).

## DISCUSSION

The present study clearly shows that iron supplements decrease zinc absorption when they are given in an aqueous solution. The marked decrease in zinc absorption in the presence of 100 and 400 mg Fe as ferrous gluconate shows that chronic oral iron supplementation increases the risk of developing or maintaining zinc deficiency. Previous studies showed that iron, zinc, and copper compete for absorption when given in aqueous solution (5, 6). However, this was not the case when iron and zinc were given with a meal, although data from different studies are equivocal (5, 6, 10, 11).



**FIGURE 2.** Mean ( $\pm$  SEM) copper absorption from a water solution containing 3 mg Cu and 0, 100, or 400 mg Fe as ferrous gluconate ( $n = 11$ ). There were no significant differences between the 3 iron doses.

The earlier supplementation studies warrant further investigation of in vivo interactions between iron, zinc, and copper absorption in humans (19). In the present study, we used a validated and accurate in vivo model to assess zinc and copper absorption and at the same time measured the EZP as a meaningful indicator of zinc status.

During the 24-h ileostomy effluent collection period, zinc isotopes were excreted into the ileostomy effluent. This zinc contained not only the unabsorbed fraction of stable isotope-labeled zinc that was administered via the test meal but also absorbed zinc isotopes, which were rapidly reexcreted in the intestinal lumen. Calculation of zinc absorption from zinc isotope enrichment in ileostomy effluent would therefore have caused underestimation of zinc absorption. To avoid this problem, zinc absorption was determined by using urinary excretion of stable zinc isotopes.

The observed effect of iron on zinc absorption may deteriorate zinc status. Zinc deficiency is associated with impaired immune function, growth, appetite, and wound healing and mucosal dystrophy. The absence of a dose-response relation between the 2 iron doses suggests that the mechanism by which iron and zinc compete for absorption is saturated by ingestion of 100 mg Fe in the ferrous form.

The low, 100-mg Fe dosage is medically used to treat iron deficiency anemia by oral supplementation, whereas the amount of zinc present in the test drink equals the average habitual daily intake of zinc and is also present in comparable quantities in food supplements. The high, 400-mg Fe dosage is occasionally prescribed for rapid oral iron replenishment therapy.

The interaction between iron and zinc absorption may be explained by competitive binding to the transporter protein DMT1 (formerly called DCT1 or Nramp2), which is located at the apical membrane in the small intestine, or by an iron-induced decrease in the expression of DMT1. Although the exact mechanism of iron absorption is still under investigation, it is clear that DMT1-mediated iron transfer accounts for most of the iron absorption from the intestine (4, 20). After reduction by ferric reductase, ferrous iron crosses the apical membrane of the gut by binding to DMT1. Furthermore, DMT1 also transports other divalent cations such as zinc and copper (4, 20). Its expression is regulated by the iron concentration in epithelial cells of the small intestine (21, 22). A decrease in DMT1 expression may have caused the observed decrease in zinc absorption in the present study. However, a single dose of iron is unlikely to significantly affect DMT1 expression in the proximal small intestine, where most iron absorption takes place, especially given the relatively short transit period of this segment of the intestine. Furthermore, recent findings suggest that the shared absorption pathway for iron and zinc is distinct from binding to DMT1, although the actual absorption mechanism remains to be elucidated (23). However, in that study, which used Caco-2 cells, the effects of 50 and 100  $\mu\text{mol Fe/L}$  on zinc absorption were investigated, whereas millimolar iron concentrations were used in the present study. Because different absorption routes may be involved in the presence of higher iron concentrations, the data from the in vitro study may not fully apply to the present in vivo investigation.

We showed competitive absorption of iron and zinc. The data show that zinc uptake in the human small intestine is not solely dependent on an absorption route in which zinc com-

petes with iron. This confirms data from previous *in vitro* studies that identified several specific zinc transporters, which may serve an important role in mediating zinc absorption from the gut lumen (24, 25). Future research is needed to identify functional zinc transporters in brush border membranes in humans.


The presence of a feedback mechanism in which zinc status mediates zinc absorption, as is the case for iron, is speculative but may indeed be present. Zinc status may change within days as a result of the diet consumed. Therefore, to minimize the confounding effects of zinc status on zinc absorption, all subjects in the present study were instructed to maintain and record their habitual diet during the 3 d before the first test day and during the first test day and to repeat the same diet during the following 2 test periods. Zinc status was assessed by measuring the size of the EZP, which provides an accurate, validated indicator of zinc status (18). This indicator of zinc status was the same at each test period. Thus, the results of the present study were not affected by changes in zinc status. Iron status was assessed at the start of the first experiment. Iron status was assumed not to change within a few weeks provided that the same habitual diet was consumed.

Zinc absorption may be impaired by both the low and the high dosage of iron. The absorbed zinc fraction in the iron intervention experiments was transported from the gut lumen by mechanisms in which zinc does not compete with iron.

Previous human and animal studies showed that increased iron intake is associated with decreased serum copper concentrations and decreased activity of corresponding copper enzymes. Animal studies provide indications of competitive absorptive mechanisms for iron and copper. Moderate iron supplementation decreases copper concentrations and copper absorption in rats (14). In humans, iron-folate supplements decrease copper status (26), whereas zinc supplementation lowers iron and copper status in adult females (27). These studies do not provide direct proof for nutrient absorption interactions in the intestine. In the present study, we showed that iron administration does not inhibit copper absorption. This finding is supported by a previous study in Caco-2 cells, in which copper uptake was shown to be mediated not merely by DMT1 but also by another mechanism (28). Recently, a newly discovered human copper transporter (hCTR1) was identified and characterized (29). This protein serves an important role in cellular copper uptake from the plasma and may also prove to be important in copper absorption from the intestine. However, the exact mechanism of copper absorption in humans remains to be elucidated.

Copper absorption was measured in the present study by determining intake/output for copper isotopes. The use of urinary isotope excretion to measure copper absorption, as was used for calculation of zinc absorption, was not possible because copper has only 2 stable isotopic forms, which meant that intravenous administration of a third stable copper isotope could not be performed. Furthermore, a relation between urinary copper excretion and copper absorption or copper status has not been established. The results of the present study show that, despite previous findings, the DMT1 route is not a rate-limiting factor in copper absorption *in vivo* in humans. This confirms an important role for other copper transporters such as hCTR1 in the small intestine.

The ileostomy patients who were recruited for participation in the present study had small intestines that functioned normally, as shown by normal serum C-reactive protein concentrations and medical interviews. Absorption of iron, zinc, and copper takes place mainly in the small intestine. The ileostomy model provides an accurate model for determining the true absorption of nutrients (30, 31). The model guarantees full test meal recovery, whereas complete fecal collection is often problematic in healthy subjects. Unfortunately, the model cannot be applied in estimating zinc absorption because of the unknown rate of reexcretion of absorbed stable zinc isotopes, as mentioned above.

We conclude that therapeutic amounts of iron inhibit zinc absorption but not copper absorption in the human small intestine. Iron supplements may impair zinc status, especially in populations with inadequate dietary micronutrient intakes. This should be taken into careful consideration when micronutrient supplements are indicated. 

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FJT, R-JMB, and WHMS were involved in designing the study. FJT was responsible for the practical phase of the study, including sample collection. FJT, VJB, and JAH were responsible for data analysis, and JRD was responsible for mathematical calculations. FJT prepared the first draft of the manuscript, and RJ-MB, JRD, JAH, and WHMS were involved in revisions and final approval. None of the authors had any financial or personal interest in the organization that sponsored the research.

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