Zinc Absorption from Milk Is Affected by Dilution but Not by Thermal Processing, and Milk Enhances Absorption of Zinc from High-Phytate Rice in Young Dutch Women

Elise F Talsma, Diego Moretti, Sou Chheng Ly, Renske Dekkers, Ellen GHM van den Heuvel, Aditia Fitri, Esther Boelsma, Tjeerd Jan Stomph, Christophe Zeder, and Alida Melse-Boonstra

Abstract

Background: Milk has been suggested to increase zinc absorption. The effect of processing and the ability of milk to enhance zinc absorption from other foods has not been measured directly in humans.

Objective: We aimed to assess zinc absorption from milk undergoing various processing and preparatory steps and from intrinsically labeled high-phytate rice consumed with milk or water.

Methods: Two randomized crossover studies were conducted in healthy young women [age: 18–25 y; body mass index (in kg/m²): 20–25]: 1) a milk study (n = 19) comparing the consumption of 800 mL full-fat ultra-high temperature (UHT) milk [heat-treated milk (HTM)], full-fat UHT milk diluted 1:1 with water [heat-treated milk and water (MW)], water, or unprocessed (raw) milk (UM), each extrinsically labeled with 67Zn, and 2) a rice study (n = 18) comparing the consumption of 90 g intrinsically 67Zn-labeled rice with 600 mL of water [rice and water (RW)] or full-fat UHT milk [rice and milk (RM)]. The fractional absorption of zinc (FAZ) was measured with the double-isotope tracer ratio method. In vitro, we assessed zinc extraction from rice blended into water, UM, or HTM with or without phytate.

Results: FAZ from HTM was 25.5% (95% CI: 21.6%, 29.4%) and was not different from UM (27.8%; 95% CI: 24.2%, 31.4%). FAZ from water was higher (72.3%; 95% CI: 68.7%, 75.9%), whereas FAZ from MW was lower (19.7%; 95% CI: 17.5%, 21.9%) than HTM and UM (both P < 0.01). FAZ from RM (20.7%; 95% CI: 18.8%, 22.7%) was significantly higher than from RW (12.8%; 95% CI: 10.8%, 14.6%; P < 0.01). In vitro, HTM and UM showed several orders of magnitude higher extraction of zinc from rice with HTM than from rice with water at various phytate concentrations.

Conclusions: Milk enhanced human FAZ from high-phytate rice by 62% compared with water. Diluting milk with water decreases its absorption-enhancing proprieties, whereas UHT processing does not. This trial was registered at the Dutch trial registry as NTR4267 (http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=4267).

Keywords: zinc, absorption, milk, rice, phytate, food processing, thermal processing, human, isotope dilution

Introduction

Zinc deficiency is widespread globally (1), with estimates indicating that 20% of the total world population has some degree of zinc deficiency (2). Zinc deficiency increases the risk of infections and restricts physical growth in children (3). Increased requirements, malabsorption, increased losses, and impaired utilization of zinc can all lead to inadequate zinc status, but the primary cause of zinc deficiency is low dietary intake of absorbable zinc (4). Zinc absorption is hampered by phytate, which is present in large amounts in plant-based foods, such as whole-meal cereals, legumes, and vegetables (5). Increasing the amount of absorbable zinc intake from the food supply is needed to combat zinc deficiency.
Rosado et al. (6) found that the addition of ultra-high temperature (UHT)10-treated skimmed milk or yogurt increased zinc absorption by 50% and 68%, respectively, from a typical plant-based rural Mexican diet rich in phytate by using an isotopic dilution protocol with extrinsic labeling of meals (6). Extrinsic zinc labels have been reported to exchange with native zinc in milk, which suggests that this is a valid method to determine zinc absorption from milk-based diets (7). However, extrinsically added zinc isotopes may not fully exchange with endogenous zinc in many other foods (8). Therefore, it cannot readily be concluded from the Mexican study if the increased amount of absorbed zinc was derived from the solid food or from the dairy source provided in the study.

Human studies on zinc absorption from regular dairy products are scarce. The enhancing factors in milk on zinc absorption have not been fully elucidated (9), and the effect of food-processing methods such as UHT processing as well as dilution and concentration of milk solids have not been investigated. It has been suggested that digestive products of casein, such as caseinophosphopeptides, enhance zinc absorption, but their addition when derived from pasteurized skim milk did not lead to higher zinc bioavailability (10, 11). Unheated casein, however, was found to lead to higher zinc uptake in a Caco-2 cell model compared with heated casein (12), which suggests increased zinc absorption from raw milk. Furthermore, heuristically, if milk constituents have an enhancing effect on zinc absorption then diluting milk with water would reduce it.

Therefore, our aims were as follows: 1) to assess zinc absorption from milk in humans compared with water, 2) to evaluate the effects of the dose of milk solids and thermal processing on zinc absorption from the milk matrix, and 3) to assess the potential enhancing properties of milk on zinc absorption from intrinsically labeled high-phytate rice compared with water.

Methods

Human studies

Study design. We conducted 2 separate open-label, single-blinded human absorption studies, both with a randomized crossover design including a 1-mo washout period: a rice study and a milk study. The milk study consisted of 4 different treatments with 800 mL of 1) full-cream UHT milk (HTM; LangLekker; Friesland-Campina), 2) UHT milk diluted 1:1 with water (MW), 3) demineralized water, or 4) raw milk (unprocessed milk (UM)). All UHT milk used in both studies was obtained from the same batch. The rice study comprised 2 treatments: 90 g steamed rice that was intrinsically labeled with 67Zn (75% diluted 1:1 with water (MW), with water).

Intrinsically labeled rice. Intrinsically labeled rice (cv 90B290) was grown hydroponically as previously described (14, 18) in a climate-controlled greenhouse in Wageningen between June and October 2013. The carbon dioxide level was ~370 mmol/mol, and the relative humidity was 60–80%. Seeds were germinated and grown to the second leaf stage on quartz sand and transplanted to water containers (14). Until 36 d after transplanting (DAT) the rice received a slightly amended half Hoagland nutrition solution (14) and zinc was given as 0.05 ppm ZnSO4 with natural isotope abundance (12.6 mL 0.01-M ZnSO4.7H2O per container). At 36 DAT (just before panicle initiation) the Hoagland solution was fully replaced and zinc was given as 67Zn (isotope enrichment: 89.6%; Isolux), 0.1 ppm 67Zn (24.6 mL 0.01-M 67Zn SO4.7H2O per container). At 54 DAT and 68 DAT (~50% flowering) extra 67Zn was added (24.6 mL 0.01-M 67ZnSO4.7H2O per container). Extra Hoagland solution was added and 54 DAT and 72 DAT when electrical conductivity was reduced by ~33% to bring the nutrient concentration back to half the Hoagland strength (electrical conductivity = 1.3 S/m). The rice was harvested at full maturity at 117 DAT. After harvesting, the rice was hulled

potential bias introduced by differences in raw-milk composition between batches, this treatment was assigned to participants as the last of the 4 treatments in the milk study and only to those who had fully completed the randomized scheme of the other 3 treatments.

Study procedures. Participants were instructed to consume a similar evening meal low in zinc before each test day. They arrived fasted at the test facilities at the Division of Human Nutrition, Wageningen University, with a baseline spot urine sample collected that morning to be able to account for any residual enrichment from the previous administration (except on the first exposure day when they had not been previously exposed to labeled zinc). An intravenous catheter was inserted, and a blood sample was drawn into a trace element–free tube with a clot activator (article no. 368380; BD Diagnostics). Blood samples were centrifuged at 840 × g for 30 min (1200 × g; 10 min; 4°C), and serum aliquots were stored at −80°C until analysis. Participants consumed the experimental meals containing the 67Zn label in 2 portions 1 h apart. All drinks were served in coded nontransparent beakers with a closed lid and consumed through a straw, thereby blinding the allocated experimental meals. Within 10 min after the first portion of the meal was consumed, participants received an intravenous infusion dose of 0.2 mg 70Zn in saline, as described elsewhere (13, 14). Subsequently, participants were monitored for 3 h to make sure that no foods or liquids (except for demineralized water) were consumed. Four days after exposure to each of the experimental meals, a spot urine sample was collected for isotopic ratio analysis (15, 16). Participants of the rice study had 2 test days and participants of the milk study had 4 test days.

On each test day, participants filled out an electronically based FFQ based on an existing FFQ (17), extended with questions to assess zinc and phytate intake. The Estimated Average Requirement of zinc is 6 mg/d for women ≥19 y of age with a mixed or refined vegetarian diet, assuming a physiologic daily requirement of 1.86 mg absorbed zinc and 34% absorption from the diet (4). Body weight and height were measured on the first day to the nearest 0.1 kg and 0.1 cm by using a mechanical floor scale (Seca 703) and a portable stadiometer, and BMI was calculated as kg/m2. A pregnancy test was performed before participants were exposed to the raw-milk treatment.

Participants. Thirty-seven female participants (nonpregnant, nonlactating, and apparently healthy) aged between 18 and 25 y with a normal BMI (20–25) were recruited in the vicinity of Wageningen, Netherlands, to participate in 1 of the 2 absorption studies. All of the participants had no history of illnesses, were not taking any long-term medication (except for contraceptives), and reported to tolerate milk and rice well. None of the participants were taking nutritional supplements or had donated blood from 2 wk before the start of the study until the end of the study. Data were collected from April to September 2014. A written informed consent was obtained from all participants before the start of the study.

The study protocol was approved by the medical ethics committee of Wageningen University and was registered with the Dutch trial registry (NTR4267).

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manually and polished by using a Pearlest laboratory scale grain polisher (Kett Electric Laboratory). Grains were polished in portions of 20 g at a time for 90 s in order to artificially keep the phytate-to-zinc molar ratio high. Rice was harvested, polished, and stored dry and cool until the experiment was conducted in May and June 2014.

Experimental meals. All of the rice was cooked in portions of 45 g (raw weight) by steaming it with distilled water (Aqua B Braun) in a rice cooker, stored at 18°C, kept frozen until the day before consumption, and was then thawed overnight in the refrigerator. Samples were reheated by microwave just before consumption. For the milk study, 67ZnO was purchased (isotope enrichment: 90.59%; Chemgas) and dissolved in stoichiometric amounts of 2-M H2SO4, and the resulting 67ZnSO4 solution was diluted to a zinc concentration of 0.5 mg 67Zn/g. All of the meals in the milk study were extrinsically labeled by adding 2 g of the 67ZnSO4 solution (1.0 mg 67Zn/g) on the day before exposure to the ZnSO4 solution (1.2 mg Zn/g; zinc sulfate monohydrate FCC 8; Dr. Paul Lohmann GmbH). Similarly, total zinc contents of meals in the milk study were adjusted by adding 2 g ZnSO4 solution (0.8 mg Zn/g) to the MW treatment and 2 g ZnSO4 solution (1.6 mg Zn/g) to the water treatment. After consuming any of the meals, empty bowls with spoon and lid and cups (with lid) were rinsed twice with 10 ml demineralized water and the water was also consumed. Raw milk was collected 3 d before exposure from 1 farmer and stored at 4°C until use. This milk adhered to all production and quality-control parameters of untreated milk as marketed in Germany (Vorzugsmilk) before use in the intervention. Energy, protein, and calcium contents of the experimental meals were calculated on the basis of the Dutch food-composition table (NEVO 2013/4.0).

Preparation of intravenous doses. Sterile 70ZnCl2 doses for intravenous administration were prepared by dissolving 70ZnO (isotope enrichment: 98.5%; Chemgas) in diluted HCl (4 M). NaHCO3 (8.4%) was added until a pH of 5.7 (5.5–6.0) was reached, and the solution was further diluted by adding NaCl (0.9%) to a final concentration of 22 μg 70Zn/mL. Individual doses of 9.5 mL were transferred to sealed injection vials, and the sterility of the batch was assessed by determining the colony count. Intravenous doses were prepared and released by the pharmacy of the Erasmus University according to Good Manufacturing Practice.

The amount of intravenously injected 70Zn was calculated for each individual by exactly weighing the syringe on an analytical balance before and after administration. The zinc concentration of the intravenous solution was measured by atomic absorption spectroscopy in unused vials (n = 6); moreover, the zinc concentration of the solution remaining in the vials after administration was also measured to assess any possible change in zinc concentration over the duration of the study.

Analytical methods. Total zinc concentrations and the 67Zn enrichment of raw rice were measured by isotope dilution MS [MC–inductively coupled plasma (ICP)–multicollector, Neptune; Thermo-Finnigan] at ETH Zurich, Switzerland, and total zinc in cooked rice was measured by flame atomic absorption spectroscopy after microwave digestion at Wageningen University, Netherlands. The phytic acid concentration in raw rice was measured by the modified Makower method (14, 21). Milk samples were analyzed for zinc concentration by ICP–atomic emission spectroscopy at the laboratory of Friesland-Campina, Leeuwarden, Netherlands. The phytate content of the rice was analyzed with a colorimetric method as described previously (25).

Statistical analysis. The fractional absorption of the 67Zn dose from either rice or milk was calculated by using the oral-to-intravenous tracer ratio method as described by Friel et al. (15). Baseline urine samples for the second, third, and fourth exposures were used in the analysis as the new natural abundance concentrations. FAZ values are presented as means and SDs. A linear mixed-effects model (LMM) was fitted to each study separately with log-transformed FAZ as the dependent variable, meals as the fixed factor, and participants as the random factor (intercept). In the milk study, when an overall effect of meal was found, post hoc tests were conducted and P values reported with the Bonferroni correction. A pooled LMM analysis with data from both studies was performed with log-transformed FAZ as a dependent variable and meal, CRP, AGR, and plasma zinc as fixed factors and participants as the random factor (intercept).

Results are presented as fold changes in FAZ with 95% CIs estimated by the LMM model. Pearson and Spearman’s correlation coefficients, where appropriate, were calculated between FAZ, serum zinc, and inflammatory markers on pooled data in each study and with dietary zinc intake on the same day. Results were considered significant when P ≤ 0.05. Data were analyzed by using SPSS software (version 20; IBM SPSS Statistics) and GraphPad Prism version 5.04 for Windows (GraphPad Software).

For the in vitro experiments, the amount of zinc extracted from the rice samples was calculated as the percentage extracted from white rice, after correcting the zinc in the liquid phase for the amount of zinc present in water and/or milk. Data were analyzed within each phytate-to-zinc molar ratio by using 1-factor ANOVA with Bonferroni post hoc test. In case of unequal variances (identified with Levene’s test) the Tamhane’s T2 post hoc test was applied. All values are presented as means ± SEs, with P < 0.05 indicating significance for comparisons with water as the control.
Results

In total, from the rice study, samples from 17 participants were available for data analysis, whereas from the milk study there were 16 participants with complete data over the full randomization scheme. Only 10 of the participants received the UM exposure. Subclinical inflammation had a low prevalence in this population; in the milk study, it affected 4 distinct participants at 3 time points (n = 1, n = 2, and n = 1 at HTM, MW, and UM administration, respectively), resulting in an incidence of subclinical inflammation of 5.8%. Furthermore, 1 participant had low plasma zinc (<10.7 μmol/L) and 1 participant had a low zinc intake (less than the Estimated Average Requirement of 6 mg/d).

In the rice study, 4 distinct participants were classified with clinical inflammation over 2 time points (n = 2 and n = 2 at RM and at RW administrations, respectively; incidence: 8.3%). Two participants had low plasma zinc and 2 had low zinc intakes (Table 1).

Experimental meals differed in protein and calcium contents (Table 2). Meals in the rice study had a somewhat lower total zinc content (3.6–3.8 mg) than did the meals in the milk study (4.0–4.2 mg). Intrinsically labeled rice contained 3 mg Zn/100 g zinc content (3.6–3.8 mg) than did the meals in the milk study.

When pooling the data from both studies, the meal factor was a significant predictor of FAZ, but CPR, AGP, and plasma zinc were not significant predictors in the linear mixed models. FAZ from water containing similar zinc amounts was 2.90-fold (95% CI: 2.58-, 3.25-fold) higher than from HTM and different from all other treatments (P < 0.01). In contrast, zinc from the MW treatment (1:1) was only 0.77 times (95% CI: 0.69, 0.86 times; P < 0.01) as well absorbed as zinc from HTM or UM treatments (both P < 0.01) (Figure 1, Table 3).

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In the in vitro experiments (Figure 3), HTM and UM showed a significantly higher in vitro accessibility of zinc from white rice in comparison to water at a phytate-to-zinc molar ratio of 15 (P = 0.008 and 0.002, respectively). This was also the case for HTM at a phytate-to-zinc molar ratio of 30 (P = 0.007) but not for UM (P = 0.123). There were no differences at a phytate-to-zinc molar ratio of 4.

### Discussion

Our study shows that zinc absorption from intrinsically labeled rice with a high phytate concentration (phytate-to-zinc molar ratio of 22) was 62% higher when consumed with full-fat UHT milk than with water. Our study also suggests that thermal (UHT) processing likely has a limited effect on zinc absorption from milk, because we did not detect a difference in zinc absorption between raw milk and UHT milk. In contrast, zinc absorption from water was 2.8-fold higher than from milk. Interestingly, when milk was diluted with water at the same zinc load, zinc absorption was lower, suggesting that milk properties affect zinc absorption in a dose-dependent manner. Together with our in vitro studies, which show a substantial increase in solubility and extractability of zinc from rice with milk, these findings suggest that milk possesses concentration-dependent, absorption-enhancing properties that facilitate zinc release from otherwise inhibitory food matrices.

Our findings from the rice study compare well with those by Rosado et al. (6), who reported a 50% and 68% increase in zinc absorption when adding 250 mL low-fat UHT milk or flavored yogurt, respectively, to a meal of tortillas and black beans (phytate-to-zinc molar ratio not reported). The consistency in results between our study (with the use of intrinsic labeling) and the study by Rosado et al. (6) (with the use of extrinsic labeling) is a further indication of the validity of the extrinsic tag approach to assess zinc absorption in humans in general (14), and from milk-containing foods in particular. An earlier report also showed that the combination of milk and cheese had a positive effect on zinc absorption from whole-meal bread with a high phytic acid content (phytate-to-zinc molar ratio of 9.4) (26).

When evaluating the constituents of dairy products that might cause the described concentration-dependent enhancing effect of milk, we can speculate that this may be due to the presence of proteins that are known to increase zinc absorption (27). Our findings are of interest for applications of milk solids in food manufacturing, where the addition of milk solids is common and which would enhance zinc absorption. Although we have not tested this hypothesis directly in our study, our results support a concentration-dependent positive effect of milk solids on zinc absorption. Yogurt or strained milks are enriched with milk solids, and in a previous study in which the addition of 150 g yogurt was compared with 250 mL milk with similar zinc contents, zinc absorption was ~10% higher (not significantly different) from yogurt meals despite the lower quantity of yogurt consumed (6). Further studies should be undertaken to quantify the effect of adding milk solids on total zinc absorbed.

Digestive products of milk protein, caseinophosphopeptides, have been suggested to loosely bind zinc, thereby potentially preventing it from binding to phytate and increasing intestinal zinc uptake (9, 11, 27, 28), although this was not confirmed in experimental studies (28–30). Alternatively, specific amino acids such as histidine, which is abundantly present in casein, may have functioned as a chelator of zinc, thereby preventing binding to phytate (31). Calcium has previously been suggested to reduce zinc bioavailability in the presence of phytate by the formation of strong and insoluble zinc-calcium-phytate complexes (32, 33). However, the high amount of calcium present in the RM meal did not reduce zinc bioavailability in our study. Earlier experiments have suggested that a phytate × Ca:Zn molar ratio >200 is required for hampering zinc absorption (32, 34). In contrast, an algorithm of zinc-absorption studies has shown that dietary calcium may have a modest enhancing effect on zinc absorption (35). In our experiment, the phytate × Ca:Zn molar ratio was ~145 for the RM meal. If any inhibition by calcium occurred in our experiment, this apparently was counteracted by other components, such as protein.

Our study shows that zinc absorption from milk was substantially lower than that from water, but is comparable to the 28% absorption from pasteurized cow milk (3% fat) reported in an earlier human study (36). Consistent with previous reports, we found that the bioavailability of zinc was very high (72.3%) from an aqueous solution (13, 37–40). Zinc absorption from a food matrix is known to be much lower than

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<th>TABLE 3</th>
<th>FAZ and TAZ for healthy young women in the milk and rice studies¹</th>
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¹ Values are n or means ± SDs. Within each study, women received all test meals and acted as their own controls. In the milk study, means in a row without a common superscript letter differ (P < 0.05). * In the rice study, different from RM. FAZ, fractional absorption of zinc; HTM, heat-treated (ultra-high temperature) milk; MW, heat-treated (ultra-high temperature) milk and water (1:1); RM, rice and milk meal; RW, rice and water meal; TAZ, totally absorbed zinc; UM, unprocessed (raw) milk; W, water.

**FIGURE 2** Fractional absorption of zinc from high-phytate rice consumed with either milk or water in 18 healthy young women (rice study). Participants received both meals in random order and acted as their own controls. Values are means.
Zinc absorption from milk and rice

from an aqueous solution, even if the fluid is a milk (9). This may be attributed to the food matrix itself as well as to differences in digestive processes, such as the release of gastric acid, intragastric pH, and transit time (41–43). Interestingly, diluting the milk with water resulted in lower zinc bioavailability than with undiluted milk, which may be explained by the lower amount of protein present in the meal.

In a study in Bangladeshi preschool children, an FAZ of 20% was found from a rice-based meal with a total zinc content of 3.8 mg and a phytate-to-zinc molar ratio of 20 (44), which is higher than the 12.8% that we found for the rice meal with water but similar to the 20.8% for the rice meal consumed with milk. The rice meal in the Bangladeshi study was provided with lentil soup, papaya, and banana; had a somewhat lower phytate-to-zinc ratio; contained a different variety of rice; was extrinsically labeled; and was conducted in growing children, which limits a direct comparison with our study in women (45). In a recent study that used the same rice variety as was used in the present study, we found the FAZ from an intrinsically labeled agronomically biofortified rice meal containing 1.1 mg Zn and with a phytate-to-zinc ratio of 12.3 to be 25% among Swiss adults aged 18–45 y (46). The higher FAZ in that study can be explained by the lower amount of zinc and phytate-to-zinc molar ratio in the meal.

In agreement with the human study, our in vitro experiments suggest that milk increases zinc extraction from rice. Because no digestive enzymes or gastric acid was added in the experiments, the overall amphiphilic nature of milk or amphiphilic compounds or amino acids present in milk may have facilitated this increased solubility of zinc from the cereal matrix. It is interesting that specifically raw milk showed a strong effect on zinc extraction with increasing phytate-to-zinc molar ratios, but these results were not confirmed in our human study.

Our studies have several strengths. First, intrinsic labeling of zinc in the rice allowed us to state that the increase in FAZ with milk can be attributed to higher zinc absorption from the rice matrix. In addition, absolute absorption values are better predicted by studies in which foods are labeled intrinsically (47, 48). For milk, extrinsic labeling has previously been shown to be a valid approach for determining zinc absorption from milk-based foods (7). Second, careful balancing of the amount of zinc in the meals allowed us to make true comparisons of FAZ between treatments. A drawback of this may be that the absorption of added zinc from the water fraction in the rice study was so high (as shown in the milk study) that it reduced zinc absorption from the rice fraction. However, this is unlikely because the rice and water were consumed together, and zinc from different foods typically readily exchanges in the gastrointestinal tract (39, 49). In addition, a recent study showed that zinc absorption from fortified water consumed with maize porridge was similarly low compared with zinc absorption from maize porridge with unfortified water (40). Third, the randomization of treatments over time points accounted for differences in effect over time, with the exception of the UM treatment. Only 10 of the 19 individuals participating in the milk study received the UM treatment. Therefore, the small nonsignificant difference in zinc bioavailability from raw milk compared with UHT milk can only be regarded as indicative.

Zinc is one of the many essential nutrients found in milk. With a concentration of ~0.4 mg Zn/100 g semiskimmed milk, it forms an important source of zinc in dairy-consuming populations such as in The Netherlands (50). Milk consumption is on the increase in Asian countries where rice is the main staple food. In some of those countries, zinc deficiency is of public health concern (51). The combined consumption of rice with milk can hypothetically increase the total amount of zinc that is absorbed from rice. For example, Chinese women ingest, on average, 4.1 mg Zn from rice (52). When consumed with milk, the total absorbed zinc from rice would increase from 0.53 to 0.85 mg when applying the FAZ values found in the present study. This amount does not yet include the absorbed zinc from the milk itself and it does not take into account that FAZ could be lower due to higher inflammation rates in a different population, as our results suggest. Therefore, it remains to be explored whether this could be an acceptable and effective strategy to improve the supply of absorbable zinc in the diet.

In conclusion, we have shown that the combination of milk with a phytate-rich rice meal enhances zinc solubility in vitro and increases the FAZ from rice by 62% in humans. Furthermore, we found that dilution of UHT milk with water decreases the FAZ, indicating that the enhancement of zinc absorption by milk components is concentration dependent and suggests that the addition of milk solids may enhance zinc absorption. Furthermore, zinc absorption from milk seems unaffected by UHT thermal processing, and the consumption of raw milk instead of UHT milk for the enhancement of zinc absorption does not seem justified.

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and calculated FAZ values; AM-B: performed the data analysis and had primary responsibility for the final content of the manuscript; EFT: wrote the first draft of the manuscript; EF, DM, SC, RD, EGHvMvdH, AF, EB, TJS, CZ, and AM-B: contributed to follow-up versions of the manuscript, in particular DM; and all authors: read and approved the final manuscript.

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