The Journal of Nutrition
Community and International Nutrition

Cold Extrusion but Not Coating Affects Iron Bioavailability from Fortified Rice in Young Women and Is Associated with Modifications in Starch Microstructure and Mineral Retention during Cooking

Laura Hackl,[1] Cornelia Speich,[1] Christophe Zeder,[1] Antoni Sánchez-Ferrer,[2] Horst Adelmann,[2] Saskia de Pee,[3] Fabian Tay,[4] Michael B Zimmermann,[1] and Diego Moretti[1]

[1]Laboratory of Human Nutrition and [2]Laboratory of Food and Soft Materials, Institute of Food, Nutrition and Health, Department of Health Sciences and Technology, ETH Zurich, Zurich, Switzerland; [3]World Food Programme, Rome, Italy; and [4]Clinical Trials Center, University Hospital Zurich, Zurich, Switzerland

Abstract

Background: Rice can be fortified with the use of hot or cold extrusion or coating, but the nutritional qualities of the resulting rice grains have never been directly compared.

Objective: Using fortified rice produced by coating or hot or cold extrusion, we compared *1*) iron and zinc absorption with the use of stable isotopes, *2*) iron and zinc retention during cooking, and *3*) starch microstructure.

Methods: We conducted 2 studies in young women: in study 1 [n = 19; mean ± SD age: 26.2 ± 3.4 y; body mass index (BMI; in kg/m^2): 21.3 ± 1.6], we compared the fractional iron absorption (FAFe) from rice meals containing isotopically labeled ferric prophosphate (^{57}FePP), zinc oxide (ZnO), citric acid, and micronutrients fortified through hot extrusion (HER1) with rice meals fortified through cold extrusion containing ^{57}FePP, ZnO, citric acid, and micronutrients (CER); in study 2 (n = 22; age: 24 ± 4 y; BMI: 21.2 ± 1.3), we compared FAFe and fractional zinc absorption (FAZn) from rice meals fortified through hot extrusion (HER2) compared with rice meals fortified through coating containing ^{57}FePP, ZnO, a citric acid and trisodium cirate mixture (CA/TSC), and micronutrients (COR) relative to rice meals extrinsically fortified with ferrous sulfate (reference). Rice types HER1 and CER contained citric acid, whereas types HER2 and COR contained CA/TSC. We assessed retention during standardized cooking experiments and characterized the rice starch microstructure.

Results: FAFe (95% CI) was greater from CER [2.2% (1.4%, 3.4%)] than from HER1 [1.2% (0.7%, 2.0%)] (P = 0.036). There was no difference in FAFe between HER2 [5.1% (3.7%, 7.1%)] and COR [4.0% (2.9%, 5.4%)] (P = 0.14), but FAFe from COR was lower than that from the reference meal [6.6% (4.9%, 9.0%)] (P = 0.003), and the geometric mean FAZn (95% CI) did not differ between HER2 [9.5% (7.9%, 11.6%)] and COR [9.6% (8.7%, 10.7%)] (P = 0.92). Cooking in a rice-to-water ratio of 1:2 resulted in iron and zinc retentions >80%, and cooking in excess water did not affect iron retention from hot-extruded rice but caused iron losses of 25% from CER and COR. Distinct variations in starch microstructure were found in CER and HER1.

Conclusions: Iron absorption was 64% higher from CER than from hot-extruded rice, with no difference between COR compared with hot-extruded rice. Lower extrusion temperatures may generate a more readily digestible starch structure, allowing for greater iron release in vivo but lower mineral retention during cooking. This trial was registered at clinicaltrials.gov as NCT02176759. *J Nutr* 2017;147:2319–25.

Keywords: iron absorption, rice, zinc absorption, extrusion, coating

Introduction

Iron and zinc deficiency remain major public health problems (1), affecting many individuals in both developing and industrialized countries (2–4). Rice is a staple food for >3 billion people (5), and its fortification could be an important strategy to combat micronutrient deficiencies (6). However, iron fortification of rice is challenging because rice is mainly consumed as intact grains, and sensory properties, particularly its white color, are important for its marketability and consumer acceptance (7, 8).

Extrusion and coating techniques are currently the 2 main technologies available for large-scale fortification; they are typically used to fortify a small fraction (1–2%) of the rice kernels (9, 10). In coating, a suspension of nutrients with waxes and polymers is applied to the surface of intact natural rice kernels (9, 10),

Manuscript received August 9, 2017. Initial review completed August 18, 2017. Revision accepted September 19, 2017.
First published online October 18, 2017; doi: https://doi.org/10.3945/jn.117.259085.

maintaining the grain's inner starch structure. In contrast, during extrusion, artificial rice kernels are generated by forcing moistened, fortified rice flour through a restricted opening, exposing it to high shear and pressure (9). Cold extrusion is conducted at 30–40°C and results in marginal or no starch gelatinization (9, 10) in contrast with hot extrusion, conducted at 80–110°C, where starch is gelatinized (9, 11, 12). Starch consists of amylopectin and amylose in a 3-dimensional network characterized by semicrystalline domains embedded into an amorphous glassy matrix, which is composed of alternating crystalline and amorphous lamellae at a fixed repeating distance. The microstructure of starch may predict the release of minerals and bioavailability in hot-extruded rice and cold-extruded rice.

Although both hot and cold extrusion have been shown to be feasible (13) and efficacious (14–16), iron absorption from rice produced with these 2 extrusion techniques has not been directly compared. Moreover, there are few data on human mineral bioavailability or efficacy from coated rice (17, 18) and no published data on zinc absorption from rice fortified through extrusion or coating (19).

To provide data for rice fortification programs on the choice of fortification technique and mineral fortification amounts, our study objectives were: *1*) to measure and compare iron absorption from hot-extruded compared with cold-extruded rice, *2*) to measure and compare iron and zinc absorption from hot-extruded compared with coated rice, and *3*) to assess iron and zinc retention from rice produced with these different technologies after varying pretreatments and cooking procedures. Our hypotheses were as follows: there would be *1*) no significant difference in iron absorption from hot-extruded compared with cold-extruded fortified rice, and *2*) no significant difference in iron or zinc absorption from hot-extruded compared with coated fortified rice.

Methods

Subjects. We enrolled women from the student and staff population of ETH Zurich and the University of Zurich. The inclusion criteria were as follows: *1*) women aged between 18 and 40 y, *2*) BMI (in kg/m^2) between 19 and 26 and body weight <69 kg, *3*) apparently healthy with no chronic diseases or intake of medication (except for oral contraceptives), *4*) nonsmoker, *5*) no blood donation or substantial blood loss within 4 mo before the study's start, *6*) not pregnant or lactating, *7*) no intake of mineral or vitamin supplements from ≥2 wk before or during the study, and *8*) no prior participation in a study where iron or zinc stable isotopes were administered. Informed written consent was obtained from all participants. The ethics committee of the canton of Zurich reviewed and approved the studies (KEK-ZH-Nr. 2015–021); the trial was registered at clinicaltrials.gov as NCT02176759.

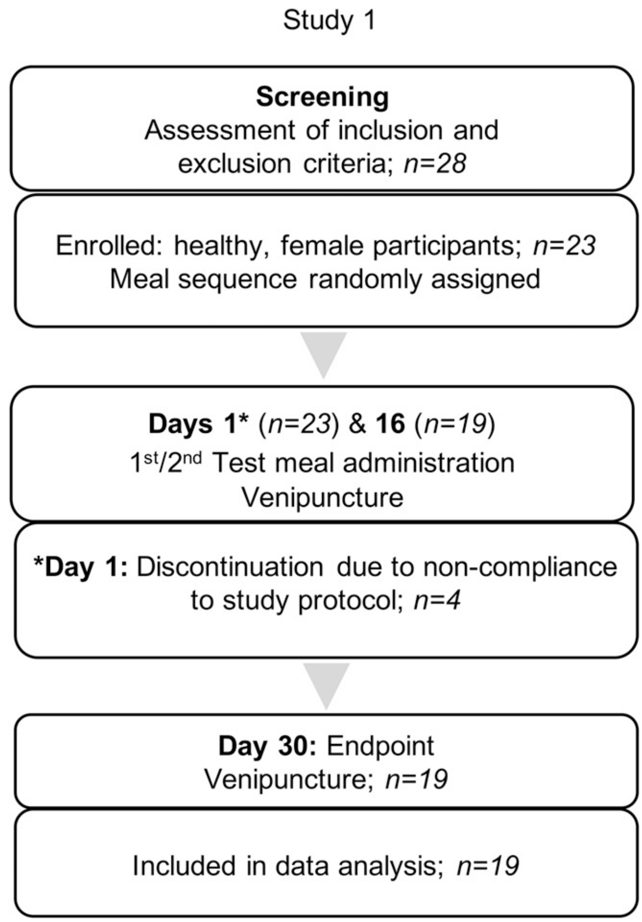

FIGURE 1 Schematic diagram of study 1. Two different study meals were administered to healthy Swiss women (*n* = 19). Meals were given with a 2-wk delay.

Human absorption studies. We performed 2 studies using a single-blind, randomized crossover design, where different rice meals were administered with each woman serving as her own control. Different participants were included in each study. Participants were allocated to the different groups after enrollment, and they were assigned to a predefined schedule of all possible test meal combinations (20). In study 1 (**Figure 1**), each woman consumed 2 different isotopically labeled test meals containing isotopically labeled ferric pyrophosphate (^{57}FePP)-fortified rice meals produced through *1*) rice meals containing ^{57}FePP, zinc oxide (ZnO), citric acid, and micronutrients fortified through hot extrusion (HER1) or *2*) rice meals fortified through cold extrusion containing ^{57}FePP, ZnO, citric acid, and micronutrients (CER). Meals were served with a 2-wk period between test meals. The total study duration was 28 d.

In study 2 (**Figure 2**), each woman consumed 3 different isotopically labeled meals containing ^{57}FePP and isotopically labeled zinc oxide (^{67}ZnO) cofortified rice, produced through *1*) rice meals fortified through hot extrusion containing ^{57}FePP, ^{67}ZnO, CA/TSC, and micronutrients (HER2) or *2*) rice meals fortified through coating containing ^{57}FePP, ^{67}ZnO, citric acid and trisodium citrate (CA/TSC), and micronutrients (COR), and *3*) 1 reference meal consisting of hot-extruded rice fortified with an isotopically labeled ferrous sulfate ($^{58}FeSO_4$) solution added before consumption (reference). There was a 4-wk period between test meals to ensure washout of the stable zinc isotopes administered on

Supported by ETH Zürich, the World Food Programme, the USDA, and the Global Alliance for Improved Nutrition. Vitamins for the different rice types were provided by DSM Nutritional Products free of charge. Coated rice kernels were provided by The Wright Group, Inc.

Author disclosures: LH, CS, CZ, AS-F, HA, SdP, FT, MBZ, and DM, no conflicts of interest.

Supplemental Figures 1–4, Supplemental Tables 1–4, and Supplemental Methods are available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

Address correspondence to LH (e-mail: laura.hackl@hest.ethz.ch).

Abbreviations used: CA/TSC, citric acid and trisodium citrate mixture; CER, rice meals fortified through cold extrusion containing isotopically labeled ferric pyrophosphate, zinc oxide, citric acid, and micronutrients; COR, rice meals fortified through coating containing isotopically labeled ferric pyrophosphate, isotopically labeled zinc oxide, citric acid and trisodium citrate mixture, and micronutrients; CRP, C-reactive protein; FAFe, fractional iron absorption; $FAFe_{corr}$, corrected fractional iron absorption; FAZn, fractional zinc absorption; FePP, ferric pyrophosphate; HER1, rice meals containing isotopically labeled ferric pyrophosphate, zinc oxide, citric acid, and micronutrients fortified through hot extrusion; HER2, rice meals fortified through hot extrusion containing isotopically labeled ferric pyrophosphate, isotopically labeled zinc oxide, citric acid and trisodium citrate mixture, and micronutrients; ID, iron deficiency; PA, phytic acid; PZn, plasma zinc; ZnO, zinc oxide; ^{57}FePP, isotopically labeled ferric pyrophosphate; $^{58}FeSO_4$, isotopically labeled ferrous sulfate; ^{67}ZnO and ^{70}ZnO, isotopically labeled ZnO.

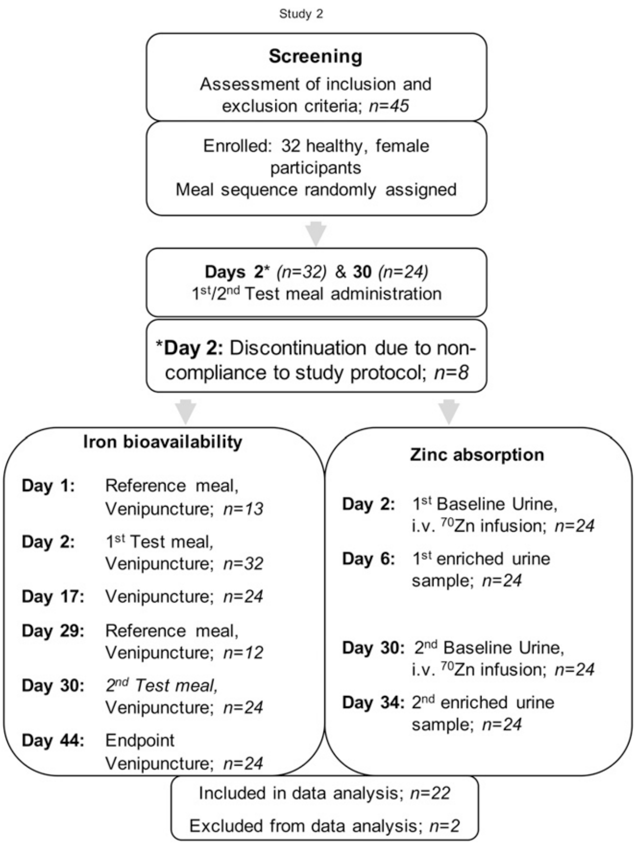

FIGURE 2 Schematic diagram of study 2. Two different study meals were administered to healthy Swiss women (n = 22). Meals were given with a 4-wk delay. One reference meal was administered 1 d before either of the test meals. ^{70}Zn, isotopically labeled zinc.

day 2 (21). The reference meal was given 1 d before one of the test meals (day 1 or 29). The total study duration was 44 d (Figure 2).

In both studies, body weight and height were measured during screening (1–2 wk before administration of the first meal), and a pregnancy test was conducted. All meals were administered in the morning after an overnight fast. The participants completely consumed the meals under direct supervision of the investigators. The empty bowls were then rinsed twice with a total of 20 mL of 18 MΩ · cm water, which was also consumed by the participants. Participants were not allowed to eat or drink for ≥3 h after complete test meal consumption.

Study 1. The study was conducted between August and September 2015 at the Institute of Food, Nutrition and Health. All participants underwent baseline venipuncture for the measurement of hemoglobin, plasma ferritin, and plasma C-reactive protein (CRP) on day 1 before receiving their first test meal (CER or HER1). On day 15, participants received their second test meal (CER or HER1) after undergoing venipuncture for measurements of hemoglobin, plasma ferritin, plasma CRP, and isotopic iron composition after the same procedure as on day 1. On day 29, 14 d after the last test meal administration, whole blood samples were collected to measure hemoglobin, plasma ferritin, plasma CRP, and isotopic iron composition (Figure 1).

Study 2. The study was conducted between September and December 2015 at the Institute of Food, Nutrition and Health and the Clinical Trials Center. Participants were divided into 3 cohorts, which only differed in the study dates and the sequence for the reference meal administrations. Depending on their assignment to a cohort, participants received their reference meals on day 1 or 29 at the Institute of Food, Nutrition and Health. On days 2 and 30 either COR or HER2 were administered at the Clinical Trials Center, and the administration sequence of the meals was randomized. Baseline spot urine samples were collected in the morning of days 2 and 30 before each test meal administration, enriched spot urine samples were collected on the morning of days 6 and 34 after an overnight fast to determine the fractional zinc absorption (FAZn) via the double isotopic tracer ratio technique (22). Additionally, all participants underwent baseline venipuncture for the measurement of hemoglobin, plasma ferritin, plasma CRP, and plasma zinc (PZn) before receiving the first test meal. After complete consumption of their test meals on days 2 and 30, participants received intravenous doses of isotopically labeled zinc oxide (^{70}ZnO) as previously described (21). On day 17, 15 d after the first test meal administration, a blood sample was collected to measure hemoglobin, plasma ferritin, plasma CRP, and isotopic iron composition in the blood. On day 30, after a 4-wk washout period for zinc, participants received their second test meal (COR or HER2) after undergoing venipuncture for measurements of hemoglobin, plasma ferritin, plasma CRP, and isotopic iron composition following a similar procedure as on days 1 or 2. On day 44, 14 d after the last test meal, whole blood samples were collected to measure hemoglobin, plasma ferritin, plasma CRP, and isotopic iron composition (Figure 2).

Preparation of isotopically labeled extruded rice grains and intravenous doses. Isotopically labeled extruded rice grains for all meals were extruded on a Brabender single-screw extruder (DSE 20/24 Do-Corder). Grains for meals HER1, HER2, and the reference were produced under hot extrusion conditions as previously described (20). The only difference was that regular ZnO was used in study 1, whereas in study 2, ^{67}ZnO was used. CER grains were of the same composition as those for the HER1 meal, however, the barrel temperatures at extrusion differed (**Table 1**).

Coated rice was produced by Wright Enrichment, Inc., following a down-scaled coating process comparable with their large-scale production technique that uses the same premix ingredients and labeled compounds of ^{57}FePP and ^{67}ZnO as for HER2. ^{57}FePP was produced by Dr. Paul Lohmann GmbH KG, mimicking ferric pyrophosphate (FePP) powder (study 1: Fe-metal: 94.8% enriched; study 2: Fe-metal: 96.0% enriched). ^{67}ZnO was obtained from Chemgas (Zn-metal: 90.6% enriched).

The HER1 and CER mixtures consisted of rice flour, ^{57}FePP, ZnO (Jungbunzlauer Suisse AG), citric acid (Sigma-Aldrich Chemie GmbH) and a vitamin premix, which was similar to a recently described one (23). The HER2 and COR mixtures contained rice flour, ^{57}FePP, ^{67}ZnO, a vitamin premix, and CA/TSC (Sigma-Aldrich Chemie GmbH). The reference mix contained rice flour, CA/TSC, and the same vitamin premix as HER2 and COR. Doses for intravenous administration were prepared from ^{70}ZnO (^{70}ZnO-metal: 95.5% enriched, Chemgas) at the Cantonal Pharmacy of the University Hospital Zurich according to Good Manufacturing Practice guidelines as previously described (21).

Test meal preparation. All test meals and the reference meal consisted of 48 g basmati rice and 30 g vegetable sauce and were administered with 300 mL 18 MΩ · cm water. To reach an iron fortification amount of 80 parts per million, 1.7 ± 0.004 g isotopically labeled extruded rice was added to CER and 1.7 ± 0.006 g to HER1 test meals; 1.7 ± 0.003 g isotopically labeled extruded and 2.1 ± 0.003 g isotopically labeled coated rice was added to the HER2 and COR meals, respectively. The reference meals contained 1.9 ± 0.005 g extruded rice. Rice was served with a standardized vegetable sauce as previously reported (23).

Before consumption, 4.0 ± 0.038 mg Fe in the form of a $^{58}FeSO_4$ solution, which was produced from enriched elemental iron (99.90% ^{58}Fe enrichment, Chemgas) as previously described (24), was added to the reference meal (study 2) only.

Test meal analysis. The iron and zinc contents in the basmati rice and vegetable sauce were analyzed by atomic absorption spectrophotometry after mineralization by microwave digestion (MLS TurboWave; MLS GmbH) with the use of nitric acid. The iron and zinc contents and isotopic composition were determined by isotopic dilution inductively coupled plasma mass spectrometry. Phytic acid (PA) content was determined as described (25, 26). The ascorbic acid content in the vegetable sauce was analyzed by HPLC (Acquity H-class UPLC system, 4824949; Waters AG) after stabilization and extraction with metaphosphoric acid and reduction by DTT (27). Iron solubility from HER1 and CER rice was determined after a modification of the method of Miller et al. (28)

TABLE 1 Test meal mineral composition and mineral absorption by healthy young women from various rice meals in studies 1 and 2[1]

Study	Meal	Barrel temperature, °C	Total Fe, mg/meal	Total Zn, mg/meal	FAFe, %	RBV iron, %	$FAFe_{corr}$, %	Total absorbed Fe, mg/meal	FAZn, %	Total absorbed Zn, mg/meal
1	CER	30–40	4.4 ± 0.12	4.0 ± 0.70	2.17[a] (1.39, 3.40)	NA	1.81 (1.36, 2.41)	0.08 (0.03, 0.12)	NA	NA
	HER1	80–92	4.4 ± 0.73	4.0 ± 0.37	1.19[b] (0.71, 2.00)	NA	1.07 (0.68, 1.69)	0.05 (0.02, 0.08)	NA	NA
2	Reference	NA	4.5 ± 0.01	1.2 ± 0.03	6.62[a] (4.86, 9.01)	100[a]	4.58 (3.47, 6.05)	0.26 (0.15, 0.37)	NA	NA
	HER2	80–92	4.4 ± 0.21	6.9 ± 0.27	5.14[a,b] (3.70, 7.13)	79[a,b] (57.7, 108.0)	3.64 (2.50, 5.31)	0.17 (0.11, 0.24)	9.54 (7.87, 11.57)	0.49 (0.38, 0.60)
	COR	NA	4.4 ± 0.11	7.1 ± 0.18	3.95[b] (2.89, 5.42)	61[b] (46.9, 78.9)	2.82 (2.08, 3.81)	0.12 (0.06, 0.18)	9.63 (8.70, 10.66)	0.59 (0.53, 0.66)

[1] Values are means ± SDs (n = 3) or geometric means (95% CIs) [n = 19 (study 1) or n = 22 (study 2)]. Within a study, labeled values in a column without a common superscript letter differ, $P < 0.05$, paired samples Student's t test (study 1) or repeated measures ANOVA with Bonferroni-corrected multiple comparisons (study 2). CER, rice meals fortified through cold extrusion containing isotopically labeled ferric pyrophosphate, ZnO, citric acid, and micronutrients; COR, rice meals fortified through coating containing isotopically labeled ferric pyrophosphate, isotopically labeled zinc oxide, citric acid and trisodium citrate mixture, and micronutrients; FAFe, fractional iron absorption; $FAFe_{corr}$, corrected fractional iron absorption; FAZn, fractional zinc absorption; HER1, rice meals containing isotopically labeled ferric pyrophosphate, ZnO, citric acid, and micronutrients fortified through hot extrusion; HER2, rice meals fortified through hot extrusion containing isotopically labeled ferric pyrophosphate, isotopically labeled zinc oxide, citric acid and trisodium citrate mixture, and micronutrients; NA, not applicable; RBV, relative bioavailability.

with digestive enzymes (amylase and pepsin) as recently described (20). Relative solubility was expressed as the solubility of the compound divided by the solubility of the reference sample [ferrous sulfate ($FeSO_4$)] as recently described (23).

Rice structure analysis. We investigated the starch microstructure of HER and CER, determined the overall degree of starch crystallinity and the type of starch polymorphism, and measured the lamellar distance in the semicrystalline starch domain and the lattice parameter of the unit cell of the starch helices. Small- and wide-angle X-ray scattering experiments were performed, and data analysis and modeling are described in **Supplemental Methods.**

Blood analysis. Plasma ferritin, plasma CRP, and hemoglobin were measured on the day of collection as recently described (23); anemia was defined as hemoglobin <12 g/dL (29). Iron deficiency (ID) was defined as plasma ferritin <15 μg/L and ID anemia as hemoglobin <12 g/dL and plasma ferritin <15 μg/L (29). Reference CRP concentrations for healthy individuals were <5 mg/L (30). Plasma separation and subsequent PZn determination as well as spot urine collection and analysis were performed as previously described (26). The determination of iron and zinc isotopic composition in blood, respective urine samples, and subsequent calculation of fractional iron absorption (FAFe) and FAZn were performed as previously described (19, 31, 32). Absorption values from 2 participants in study 1 were below the detection limit of 0.4% fractional absorption, and therefore, random positive numbers below this threshold were generated in Microsoft Excel (Microsoft Corporation) and used for subsequent analysis. The corrected FAFe ($FAFe_{corr}$) was calculated from the FAFe and corrected to a plasma ferritin concentration of 40 μg/L as described (33).

Micronutrient retention. Fortified rice samples, which differed in their nutrient concentrations compared with those samples used in the iron absorption studies, were tested regarding their mineral (iron and zinc) contents after 6 different preparation procedures. The rice samples were *1*) medium whole-grain rice kernels coated with a mixture of nutrients and waxes produced by Wright Enrichment, Inc., and 2) HER or CER both produced at ETH Zurich and consisting of rice flour, water, and a mixture of nutrients and additives. All samples had a nutrient profile and production parameters similar to those described for the human absorption studies. Fortified rice and unfortified basmati rice were weighed and mixed at a 1:50 (wt:wt) ratio (hereafter referred to as rice meals).

Rice meals were cooked in separate glass bowls (20 min, 220°C) with a 1:2 (wt:wt) or a 1:6 (wt:wt; excess water method) rice-to-water ratio and were either rinsed or soaked before cooking or not. Each preparation condition was applied to each rice meal, and all experiments were conducted in triplicate. For rinsing, the rice meals were rinsed twice with a total of 300 mL of 18 MΩ · cm water, and the water was decanted between the rinsing steps. For soaking, rice meals were soaked in their bowls for 30 min in 300 mL of 18 MΩ · cm water, and then the water was decanted and water for cooking was added (see above). Rice meals prepared with the 1:2 or 1:6 rice-to-water ratio alone received no previous treatment. After cooking, all meals were cooled (30 min, 8°C), and excess water, if present, was decanted.

For the determination of mineral retention, cooled rice meals were dried in an oven (110°C). Then, meals were weighed again and homogenized in a rotor mill (Retsch ZM1 GmbH & Co.KG) with a titanium sieve (sieve openings: 250 μm). Milled samples were stored in Ziploc bags under ambient temperature until further analysis by atomic absorption spectrophotometry (GTA 120 or AA240FS; both Agilent Technologies AG). The mineral contents retrieved in the dried rice meals were expressed as a fraction of the amounts present in the uncooked meals. The results obtained from meals prepared with the 1:2 rice-to-water ratio were defined as 100% retention, and the results from other methods were expressed as a fraction thereof (relative retention).

Sample size calculation and statistical analysis. Data were analyzed with SPSS software (version 22.0, 2013; SPSS Inc) and Microsoft Excel (2013; Microsoft Corporation). The power calculation was performed with GraphPad StatMate software for Windows (version 2; GraphPad Software) with the anticipation that analyses would be performed on log-transformed data, because iron absorption data are typically highly skewed. Based on previous data from iron absorption studies from FePP-fortified rice conducted in our laboratory (20), we expected an SD of $\log_{10}$ FAFe of 0.23, and a correlation coefficient of fractional absorption from different fortified rice test meals within the same subjects of r = 0.73. We expected a FAFe (not log transformed) from FePP-fortified rice to be 3% of the administered dose. A 30% change in iron absorption would result in an increase in fractional absorption to 3.9%, which in a $\log_{10}$ scale would translate to an effect size of 0.11. To achieve this, a sample size of 16 participants was estimated to be necessary with the use of paired Student's t tests with a power of 0.8 and an α coefficient of 0.05. We anticipated dropout rates of 20% and 35% in studies 1 and 2, respectively, and aimed at recruiting 20 subjects for study 1 and 24 subjects for study 2. Subjects who discontinued participation on the first study day were replaced.

The FAFe from the different meals for the same participant was compared by paired-samples Student's t test (study 1) or repeated-measures ANOVA followed by Bonferroni-corrected pairwise comparisons (study 2). The FAZn was compared by paired-samples Student's t test. The results for iron solubility were compared by unpaired Student's t test. The results for age and anthropometric features, hemoglobin, plasma ferritin, plasma CRP, and PZn are presented as means ± SDs, if normally distributed, otherwise they are presented as geometric means with a 95% CI. Calculations for iron, zinc, and PA concentrations of composite meals were based on the means from the analysis of single components (regular basmati rice, extruded rice, and vegetable sauce; n = 5 or 3), and SDs were adapted by calculating the square root of the squared and summed SDs from each single component. The results for iron and zinc retention were compared with the univariate general linear model with "preparation method" and "rice fortification technique" and their interaction as fixed factors and the "relative mineral retention" as the dependent variable. We used multiple comparisons to distinguish specific effects between individual preparation methods, and P values were Bonferroni corrected. Differences were considered as significant at $P < 0.05$. Non-normally distributed data were logarithmically converted for statistical analysis and reconverted for reporting. $FAFe_{corr}$ (corrected to a plasma ferritin concentration of 40 μg/L) was calculated from FAFe as described earlier (33).

Results

Study 1. Twenty-three women were recruited and started the study. Four discontinued participation because they could not comply with the study protocol, and thus 19 participants completed the study and were included in the analysis (**Table 2**). At baseline (day 1), 2 participants were iron deficient (ID), 1 participant was ID anemic, and 1 participant had an elevated plasma CRP concentration (6.4 mg/L).

Iron and zinc concentrations in the isotopically labeled extruded rice grains in the CER and HER1 meals were 229 ± 6.8 and 234 ± 4.2 mg/100 g and 166 ± 3.5 and 163 ± 0.9 mg/100 g, respectively. The native iron and PA concentrations in the composite meals were 0.5 ± 0.13 mg and 0.1 ± 0.01 g, respectively, and the sauce given with each meal contained 0.8 ± 0.0 mg ascorbic acid/100 g.

The FAFe from CER was greater than that from HER1 (P = 0.036) (Table 1). In the in vitro–simulated digestion experiments, CER had a greater relative iron solubility than HER1 (19.4% compared with 13.6%; P = 0.026). The starch structure differed between CER and HER1; both CER and basmati rice were characterized by A-type starch polymorphism (parallel packing of double helices). In contrast, HER1 showed a V-type polymorphism (anti–parallel packing of single helices; **Supplemental Figures 1** and **2**, **Supplemental Tables 1 and 2**).

Study 2. Thirty-two women were recruited for the study. Eight women were excluded on study day 1 because they could not comply with the study protocol, and 24 women started the study (Table 2). Two women were excluded during the study because they received heparin treatment, which may influence hepcidin and iron metabolism (34). Therefore, 22 participants completed the study. At baseline (day 1 or 2), 4 participants were ID, and none suffered from ID anemia or had elevated plasma CRP concentrations. Two participants had elevated CRP concentrations (5–10 mg/L) during 1 study visit apart from baseline.

Iron and zinc concentrations in HER2 were 237 ± 12.3 and 344 ± 16.1 mg/100 g, respectively, COR contained 194 ± 5.4 mg Fe/100 g and 291 ± 8.6 mg Zn/100 g, and the extruded reference rice contained 7 ± 0.4 mg Fe/100 g and 3 ± 0.2 mg Zn/100 g. The native iron, PA, and ascorbic acid concentrations were comparable with study 1 (see above).

The FAFe from COR did not differ significantly from HER2 (4.0% compared with 5.1%; P = 0.14), but was lower than that of the reference meal (6.6%; P = 0.03, Table 2). The FAZn was 9.7% from both HER2 and COR and did not differ (P = 0.91). Relative bioavailability from HER2 (79%) did not differ from COR (61%) (P = 0.45) or the reference (P = 0.41). However, relative bioavailability from COR differed from the reference (P = 0.003).

TABLE 2 Baseline characteristics of the women in study 1, assessing the differences between iron-fortified rice produced with hot and cold extrusion, and study 2, comparing iron and zinc absorption from rice produced with coating or hot extrusion[1]

	Study 1 (n = 19)	Study 2 (n = 22)
Age, y	26 ± 3	24 ± 4
Weight, kg	59.4 ± 4.8	59.0 ± 4.6
Height, m	1.65 ± 0.06	1.66 ± 0.05
BMI, kg/m^2	21.3 ± 1.6	21.2 ± 1.3
Hemoglobin, g/L	134.7 ± 9.7	134.2 ± 9.4
Plasma C-reactive protein, mg/L	0.7 (0.4, 1.2)	1.1 ± 1.0
Plasma ferritin, μg/L	46.2 ± 30.5	41.2 ± 31.3
Plasma zinc, μg/dL	NA[2]	85.5 (79.06, 92.42)

[1] Values are means ± SDs or geometric means (95% CIs).
[2] NA, not assessed.

Mineral retention. Iron and zinc concentrations (per 100 g fortified kernels) were 511 ± 43.8 mg and 730 ± 24.7 mg, 392 ± 7.1 mg and 619 ± 6.0 mg, and 389 ± 5.4 mg and 591 ± 3.0 mg in the coated rice, hot-extruded, and cold-extruded rice respectively. Both the preparation method and the rice fortification technique affected iron and zinc retention in cooked rice (**Table 3**). We found a significant interaction between rice fortification technique and preparation method for iron retention ($P < 0.001$), but not for zinc retention (P = 0.052). Retention on the use of the 1:2 rice-to-water ratio was 90%, 88%, and 87% for iron and 82%, 108%, and 109% for zinc in the coated rice, the hot-extruded, and the cold-extruded rice, respectively (Table 3). Cooking the rice at a 1:2 rice-to-water ratio, regardless of pretreatment, showed overall relative iron and zinc retentions >80% for all different types, except for coated rice with previous soaking (~60% relative zinc retention). Cooking in excess water without pretreatment did not affect relative iron retention from the hot-extruded rice; however, the coated rice and the cold-extruded rice retained approximately three-quarters of iron, and all types retained approximately four-fifths of zinc. Excess water cooking with previous rinsing resulted in 87%, 72%, and 48% iron retention in hot-extruded, cold-extruded, and coated rice, respectively, whereas for zinc, retention from all 3 types of rice ranged from 60% to 75%. Cooking in excess water with previous soaking showed a similar pattern.

Discussion

Our results from study 1 show a higher iron absorption from CER than HER1. The in vitro digestion experiments suggest this may be due to higher iron solubility from CER meals. The higher solubility from CER may be explained by differences in starch microstructure and amorphous content: the V-type polymorphism found in HER1 restricts swelling and stabilizes the rice kernel structure similar to parboiled rice (35–37). These findings are in agreement with observed differences in differential scanning calorimetry (**Supplemental Figure 3** and **Supplemental Table 3**) and mechanical properties (**Supplemental Figure 4** and **Supplemental Table 4**). Thus, compared with hot extrusion, lower extrusion temperatures appear to generate a more readily digestible starch structure, allowing for greater iron release in the proximal gut for absorption (38). Further investigations should focus on identifying ideal extrusion conditions for duodenal mineral delivery from extruded rice, regarding kernel starch and nonstarch structure, porosity, and integrity after cooking and during in vitro and in vivo digestion.

In study 2, we found no significant differences in iron and zinc absorption from fortified rice produced with coating or hot extrusion. Although iron bioavailability from extruded fortified rice has been extensively investigated (14, 20, 23), no evidence on mineral absorption from coated rice in humans existed so far. Our findings suggest that coating is a viable rice fortification technique, allowing a widening of the technological portfolio for the implementation of rice fortification, because coating technology may provide advantages in settings where extrusion cannot be readily implemented. However, although we did

TABLE 3 Relative in vitro retention (percentage) with different cooking methods for iron and zinc from fortified rice produced with 3 distinct rice fortification techniques undergoing 6 different cooking and preparation methods[1]

Mineral and rice fortification technique	Cooked in 1:2 rice-to-water ratio (wt:wt)			Cooked in 1:6 rice-to-water ratio (wt:wt)			P^2		
	No pretreatment	Rinsing	Soaking	No pretreatment	Rinsing	Soaking	Preparation method[3]	Rice fortification	Interaction
Iron retention							<0.001	<0.001	<0.001
Hot extrusion	$100.0^{b} \pm 3.1$	$102.0^{b,+} \pm 4.0$	$109.5^{a,+} \pm 1.5$	$99.8^{b,*} \pm 2.3$	$87.5^{c,+} \pm 3.5$	$81.3^{c,*} \pm 1.8$			
Cold extrusion	$100.0^{a} \pm 2.3$	$97.8^{a,+} \pm 2.2$	$95.6^{a,*,+} \pm 1.3$	$76.4^{b,+} \pm 3.0$	$72.3^{b,+} \pm 9.0$	$68.7^{b,*} \pm 3.7$			
Coating	$100.0^{a} \pm 2.4$	$101.7^{a,*} \pm 6.2$	$80.6^{b,*} \pm 1.0$	$75.4^{b,+} \pm 13.1$	$47.5^{c,*} \pm 9.7$	$44.3^{c,+} \pm 5.3$			
Zinc retention							<0.001	0.001	0.052
Hot extrusion	$100.0^{a} \pm 2.4$	$95.3^{a} \pm 1.9$	$81.6^{b,+} \pm 1.9$	$81.3^{b} \pm 6.8$	$63.7^{c} \pm 10.3$	$58.7^{d} \pm 2.1$			
Cold extrusion	$100.0^{a} \pm 2.1$	$97.1^{a} \pm 2.8$	$82.2^{c,+} \pm 1.7$	$78.8^{c} \pm 2.7$	$64.6^{d} \pm 6.2$	$61.1^{d} \pm 4.0$			
Coating	$100.0^{a} \pm 1.8$	$82.9^{a} \pm 2.0$	$59.6^{b,*} \pm 2.1$	$81.3^{a} \pm 19.9$	$59.1^{b} \pm 4.8$	$53.6^{b} \pm 4.4$			

[1] Values are means ± SDs, $n = 3$. Univariate general linear model and multiple comparisons (Bonferroni corrected, $P < 0.05$) for each micronutrient. Different superscript letters within a row and symbols within a column indicate significant differences.
[2] Models were calculated for each micronutrient separately.
[3] Effects of cooking and pretreatment methods on mineral retention in rice meals. Relative retention was calculated as the fraction of mineral retention after preparation with the 1:2 rice-to-water ratio (column 2). Retention on cooking with a 1:2 rice-to-water ratio was 88%, 87%, and 90% for iron and 108%, 109%, and 82% for zinc from the hot-extruded, the cold-extruded, and the coated rice, respectively.

not detect a statistically significant difference in iron and zinc absorption between the techniques, we cannot exclude a smaller impact on iron absorption from CER, which may not be captured by the power of this study. This study further suggests that 200 g of raw fortified rice containing either HER2 or COR could meet the daily requirements of ~2–3 mg absorbed zinc in adults (39). With regard to iron, 200 g raw fortified rice would cover half or two-thirds of the daily iron requirements (40) of women of reproductive age with sufficient iron stores (serum ferritin: 40 μg/L). However, our study did not account for higher PA amounts in more diverse diets, whose inhibitory effect on FAZn may be higher (41).

Iron absorption from fortified HER depends on the presence of other ingredients in the fortified kernel. The difference in $FAFe_{corr}$ from HER2 (3.6%) compared with HER1 (1.1%) may be attributed to 2 factors as follows: *1*) the presence of the CA/TSC buffering system, because the relative bioavailability of HER2 was ~80% (NS compared with $FeSO_4$), which is consistent with our previous findings (20), and *2*) the slightly higher ZnO amount present in HER1 than in HER2. We have recently shown that ZnO per se can reduce FePP absorption from rice (23), but whether an incremental increase of ZnO would affect human iron absorption is unclear. Thus, although the difference in iron absorption from HER1 and HER2 is very likely due to the presence of CA/TSC, we cannot exclude an effect of ZnO. Whether CA/TSC addition improves iron absorption from CER should be investigated.

Relative iron and zinc retention in coated and both extruded rice meals cooked with the 1:2 rice-to-water ratio regardless of pretreatment was >80%, with the exception of coated rice, which was soaked before cooking. After cooking in excess water, regardless of pretreatment, iron retention was highest in hot-extruded meals (>80%), followed by cold-extruded (69–76% retention) and coated rice meals (44–75% retention). Previous rinsing or soaking had the most detrimental effect on mineral retention in all meals. After excess water cooking, zinc retention in all 3 types of meals was lowest when meals were rinsed or soaked before cooking with retention values ranging from 54% (coated rice) to 65% (cold-extruded rice). In extrusion, minerals are embedded in the rice kernel matrix, protecting the kernel from micronutrient losses caused by precooking treatments, whereas in coated rice, micronutrients are located on the grain surface (9). This likely explains the comparatively low retention from coated rice after excess water cooking, regardless of pretreatment. Because of the higher kernel solubility on cooking with the A-type starch polymorphism in cold-extruded rice, we expected higher nutrient losses in cold extruded than in hot extruded rice. Thus, hot-extruded rice seems advantageous over cold-extruded and coated rice regarding iron and zinc retention. Nevertheless, the chosen fortification technology should account for the prevailing local preparation method.

A strength of these studies is the precise measurement of human iron absorption with the use of isotopically labeled rice that closely matches commercially fortified rice; both in the manufacture of the labeled fortification compounds and the rice kernels, produced by down-scaled versions of the large-scale process. However, our studies also have limitations. The standardized meals consisting mostly of white rice contained negligible amounts of PA, which can inhibit iron (42) and zinc (41) absorption and may not be fully generalizable to settings where legumes and whole grains are consumed along with rice. Further, we tested the rice meals in generally iron- and zinc-replete subjects and cannot exclude a different absorption rate in a depleted population, which would be targeted for a mass fortification program. We considered visual differences between fortified and unfortified rice after cooking as being minimal, however, we did not scrutinize the sensory properties of the different rice types. Due to the expected unavoidable losses of iron and zinc stable isotopes during the down-scaled production, the kernels were manufactured for a 1:25 fortified-to-unfortified rice blending ratio, whereas typical fortification programs use ratios of 1:100–200.

In summary, both coating and extrusion, utilizing the processes that were used to produce the fortified kernels used in this study, appear to be viable rice fortification techniques. In both processes, we recommend the use of FePP combined with CA/TSC. Our data suggest that the structure of the rice-kernel matrix affects nutrient release and bioavailability, and therefore, further experiments to optimize the kernel microstructure are needed. Although coating is generally less expensive than extrusion (10), large-scale fortification renders the cost per metric ton of fortified rice for both techniques comparable (9), and regardless of the technology used, fortified grains contribute to <1% of the wholesale price for fortified rice (43).

Acknowledgments

We thank Richard Hurrell and Raffaele Mezzenga for their technical and scientific advice, Alexandra Schelbert, Pornpimol Scheuchzer and Pascal Wiesli for laboratory support, and Monica Spitaleri for her support at CTC, Zurich. The authors' responsibilities were as follows—DM, LH, SdP, and MBZ: designed the studies; LH, CS, AS-F, HA, FT, and CZ: conducted the experiments; LH and DM: analyzed the data; LH, AS-F, and DM: wrote the first draft of the manuscript; LH, MBZ, and DM: had primary responsibility for the final content; and all authors: read and approved the final version of the manuscript.

References

1. WHO. Guidelines on food fortification with micronutrients. Geneva (Switzerland); WHO Press; 2006.
2. Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, Regan M, Weatherall D, Chou DP, Eisele TP. A systematic analysis of global anemia burden from 1990 to 2010. Blood 2014;123:615–24.
3. Wessells KR, Brown KH. Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. PLoS One 2012;7:e50568.
4. Kassebaum NJ. The global burden of anemia. Hematol Oncol Clin North Am 2016;30(2):247–308.
5. Muthayya S, Sugimoto JD, Montgomery S, Maberly GF. An overview of global rice production, supply, trade, and consumption. Ann N Y Acad Sci 2014;1324:7–14.
6. Piccoli NB, Grede N, de Pee S, Singhkumarwong A, Roks E, Moench-Pfanner R, Martin WB. Rice fortification: its potential for improving micronutrient intake and steps required for implementation at scale. Food Nutr Bull 2012;33(4 Suppl):S360–72.
7. Mridula D, Pooja J. Preparation of iron-fortified rice using edible coating materials. Int J Food Sci Technol 2014;49:246–52.
8. de Pee S, Fabrizio C, Rosenzweig J. Overview of evidence and recommendations for effective large-scale rice fortification. Scaling up rice fortification in Asia. Basel (Switzerland): Sight & Life; 2014:20–25.
9. Steiger G, Müller-Fischer N, Cori H, Conde-Petit B. Fortification of rice: technologies and nutrients. Ann N Y Acad Sci 2014;1324:29–39.
10. Alavi S, Bugusu B, Cramer G, Dary O, Lee T, Martin L, McEntire J, Wailes E. Rice fortification in developing countries: a critical review of the technical and economic feasibility. Washington (DC): Institute of Food Technologists; 2008.
11. Zhuang H, An H, Chen H, Xie Z, Zhao J, Xu X, Jin Z. Effect of extrusion parameters on physicochemical properties of hybrid Indica rice (Type 9718) extrudates. J Food Process Preserv 2010;34:1080–102.
12. Shannon JC, Garwood DL, Boyer CD. Genetics and physiology of starch development. In: Starch. 3rd ed. San Diego (CA): Academic Press; 2009. p. 23–82.
13. Moretti D, Lee TC, Zimmermann MB, Nuessli J, Hurrell RF. Development and evaluation of iron-fortified extruded rice grains. J Food Sci 2005;70:S330–6.
14. Moretti D, Zimmermann MB, Muthayya S, Thankachan P, Lee T-C, Kurpad AV, Hurrell RF. Extruded rice fortified with micronized ground ferric pyrophosphate reduces iron deficiency in Indian schoolchildren: a double-blind randomized controlled trial. Am J Clin Nutr 2006;84:822–9.
15. Beinner MA, Velasquez-Meléndez G, Pessoa MC, Greiner T. Iron-fortified rice is as efficacious as supplemental iron drops in infants and young children. J Nutr 2010;140:49–53.
16. Hotz C, Porcayo M, Onofre G, García-Guerra A, Elliott T, Jankowski S, Greiner T. Efficacy of iron-fortified Ultra Rice in improving the iron status of women in Mexico. Food Nutr Bull 2008;29:140–9.
17. Furter M, Lauter W, Ritter ED, Rubin S. Enrichment of rice with synthetic vitamins and iron. Ind Eng Chem 1946;38:486–93.
18. Quiogue ES. The rice enrichment project in Bataan, Philippines*. Am J Public Health Nations Health 1952;42:1086–94.
19. Brnić M, Wegmüller R, Melse-Boonstra A, Stomph T, Zeder C, Tay FM, Hurrell RF. Zinc absorption by adults is similar from intrinsically labeled zinc-biofortified rice and from rice fortified with labeled zinc sulfate. J Nutr 2016;146:76–80.
20. Hackl L, Cercamondi CI, Zeder C, Wild D, Adelmann H, Zimmermann MB, Moretti D. Cofortification of ferric pyrophosphate and citric acid/trisodium citrate into extruded rice grains doubles iron bioavailability through in situ generation of soluble ferric pyrophosphate citrate complexes. Am J Clin Nutr 2016;103:1252–9.
21. Wegmüller R, Tay F, Zeder C, Brnić M, Hurrell RF. Zinc absorption by young adults from supplemental zinc citrate is comparable with that from zinc gluconate and higher than from zinc oxide. J Nutr 2014;144:132–6.
22. Friel JK, Naake VL, Miller L, Fennessey PV, Hambidge KM. The analysis of stable isotopes in urine to determine the fractional absorption of zinc. Am J Clin Nutr 1992;55:473–7.
23. Hackl L, Zimmermann MB, Zeder C, Parker M, Johns PW, Hurrell RF, Moretti D. Iron bioavailability from ferric pyrophosphate in extruded rice cofortified with zinc sulfate is greater than when cofortified with zinc oxide in a human stable isotope study. J Nutr 2017;147:377–83.
24. Cercamondi CI, Egli IM, Mitchikpe E, Tossou F, Zeder C, Hounhouigan JD, Hurrell RF. Total iron absorption by young women from iron-biofortified pearl millet composite meals is double that from regular millet meals but less than that from post-harvest iron-fortified millet meals. J Nutr 2013;143:1376–82.
25. Cercamondi CI, Egli IM, Ahouandjinou E, Dossa R, Zeder C, Salami L, Tjalsma H, Wiegerinck E, Tanno T, Hurrell RF. Afebrile Plasmodium falciparum parasitemia decreases absorption of fortification iron but does not affect systemic iron utilization: a double stable-isotope study in young Beninese women. Am J Clin Nutr 2010;92:1385–92.
26. Brnić M, Wegmüller R, Zeder C, Senti G, Hurrell RF. Influence of phytase, EDTA, and polyphenols on zinc absorption in adults from porridges fortified with zinc sulfate or zinc oxide. J Nutr 2014;144:1467–73.
27. Parviainen MT, Nyssönen K. Ascorbic acid. In: Modern chromatographic analysis of vitamins. New York: Marcel Dekker; 1992. p. 235–60.
28. Miller DD, Schricker BR, Rasmussen RR, Van Campen D. An in vitro method for estimation of iron availability from meals. Am J Clin Nutr 1981;34:2248–56.
29. WHO. Iron deficiency anemia. Geneva (Switzerland):WHO Press; 2001.
30. Dati F, Schumann G, Thomas L, Aguzzi F, Baudner S, Bienvenu J, Blaabjerg O, Blirup-Jensen S, Carlström A, Hyltoft-Petersen P. Consensus of a group of professional societies and diagnostic companies on guidelines for interim reference ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP reference material (CRM 470). Eur J Clin Chem Clin Biochem 1996;34:517–20.
31. Zimmermann MB, Troesch B, Biebinger R, Egli I, Zeder C, Hurrell RF. Plasma hepcidin is a modest predictor of dietary iron bioavailability in humans, whereas oral iron loading, measured by stable-isotope appearance curves, increases plasma hepcidin. Am J Clin Nutr 2009;90:1280–7.
32. Agency IAE. Assessment of iron bioavailability in humans using stable isotope techniques. Vienna (Austria): International Atomic Energy Agency; 2012.
33. Cook JD, Dassenko SA, Lynch SR. Assessment of the role of nonheme-iron availability in iron balance. Am J Clin Nutr 1991;54:717–22.
34. Poli M, Girelli D, Campostrini N, Maccarinelli F, Finazzi D, Luscieti S, Nai A, Arosio P. Heparin: a potent inhibitor of hepcidin expression in vitro and in vivo. Blood 2011;117:997–1004.
35. Priestley R. Studies on parboiled rice: part 1—comparison of the characteristics of raw and parboiled rice. Food Chem 1976;1:5–14.
36. Priestley R. Studies on parboiled rice—part 3: characteristics of parboiled rice on recooking. Food Chem 1977;2:43–50.
37. Ong MH, Blanshard J. The significance of starch polymorphism in commercially produced parboiled rice. Starke 1995;47:7–13.
38. Zimmermann MB, Hurrell RF. Nutritional iron deficiency. Lancet 2007;370:511–20.
39. Maret W, Sandstead HH. Zinc requirements and the risks and benefits of zinc supplementation. J Trace Elem Med Biol 2006;20:3–18.
40. WHO/FAO. Vitamin and mineral requirements in human nutrition Joint FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements. Geneva (Switzerland): WHO/FAO; 2004.
41. Armah SM. Fractional zinc absorption for men, women, and adolescents is overestimated in the current dietary reference intakes. J Nutr 2016;146:1276–80.
42. Hurrell RF. Phytic acid degradation as a means of improving iron absorption. Int J Vitam Nutr Res 2004;74:445–52.
43. Forsman C, Milani P, Schondebare JA, Matthias D, Guyondet C. Rice fortification: a comparative analysis in mandated settings. Ann N Y Acad Sci 2014;1324:67–81.