The effect of lipids, a lipid-rich ready-to-use therapeutic food, or a phytase on iron absorption from maize-based meals fortified with micronutrient powders[1,2]

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ABSTRACT

Background: Ready-to-use-therapeutic foods (RUTFs) high in lipid, protein, and iron are used to treat malnutrition. Lipids increase gastric residence time, which could increase iron absorption, particularly from poorly soluble iron compounds and in combination with phytase.
Objectives: The objectives were to *1*) assess the effect on iron absorption of a lipid emulsion given 20 min before or together with an iron-fortified maize meal and *2*) assess iron absorption from a micronutrient powder (MNP) given with a nutrient-dense RUTF and/or a microbial phytase.
Design: A total of 41 women participated in 3 studies. They consumed a maize meal fortified with isotopically labeled ferrous sulfate ($FeSO_4$; study 1) or ferric pyrophosphate (FePP; study 2). In studies 1 and 2, a lipid emulsion was given with or 20 min before the meal. In study 3, with the use of a 2×2 factorial design, subjects consumed a maize meal fortified with an MNP containing labeled $FeSO_4$ (MNP) given with an RUTF (MNP+RUTF), with a phytase (MNP+phytase), or both (MNP+RUTF+phytase). Iron absorption was assessed by isotope incorporation in erythrocytes 14 d after the test meals.
Results: The lipid emulsion given either before or with the meal significantly increased iron absorption from FePP by 2.55-fold (95% CI: 1.48-, 4.37-fold; $P = 0.001$) but not from $FeSO_4$. There was a trend to increase iron absorption with the MNP+RUTF meal, which did not reach significance (1.21-fold; 95% CI: 0.92-, 1.61-fold; $P = 0.060$). The addition of phytase to MNP and MNP+RUTF significantly increased iron absorption by 1.85-fold (95% CI: 1.49-, 2.29-fold; $P < 0.001$), with no interaction between phytase and RUTF.
Conclusions: In iron-fortified maize-based meals, the addition of lipids more than doubles iron absorption from FePP. Our results suggest the possibility of an enhancing effect on iron absorption of lipid-rich RUTFs, but more research is needed to determine this. This trial was registered at clinicaltrials.gov as NCT01991626. *Am J Clin Nutr* 2017;105:1521–7.

INTRODUCTION

Iron deficiency anemia is a major public health problem, particularly among infants, children, and women of childbearing age (1, 2). One of the major causes is low dietary iron bioavailability (3). Iron deficiency anemia and infant malnutrition can be reduced in infants and children through the consumption of iron-containing micronutrient powders (MNPs)[5] added to complementary foods (4) and ready-to-use therapeutic foods (RUTFs) (5–7).

Compared with MNPs, RUTFs are rich in lipid and protein and are calorie dense, a benefit compared with MNPs. However, complex food matrixes rich in lipid and protein may inhibit iron absorption (8). On the other hand, higher calorie loads and lipid ingestion delay gastric emptying and increase gastric residence time (GRT) (9, 10). Longer GRT could allow greater time for iron dissolution at a low pH and could increase iron absorption, particularly from iron compounds such as ferric pyrophosphate (FePP) and electrolytic iron, which require a low pH to release iron.

Iron absorption from MNPs can be increased by using exogenous phytase of microbial origin (11). This phytase has 2 activity maximums, at pH 2.0 and pH 5.0, and is active in the stomach (12). The combination of RUTFs and phytase could be particularly efficacious: delayed gastric emptying could extend the time that phytase could be active in the stomach before it is degraded, and further increase iron absorption. Such an effect would allow a reduction in the amount of iron added to these products. However, if the complex protein-rich matrix of RUTFs inhibits iron absorption (8), their iron content should be increased. Providing lower doses of highly bioavailable iron is of particular interest for regions with a high burden of infection (13), because high doses of oral iron in these regions may increase risk (14, 15).

Our study objectives were to assess, in iron-fortified maize-based meals, the following: *1*) the effect of a lipid bolus and the timing of its administration on iron absorption from ferrous sulfate ($FeSO_4$) compared with FePP and *2*) the effect of a nutrient-dense RUTF on iron absorption and if the enhancing effect of phytase would be more pronounced when co-administered with an RUTF. Our corresponding hypotheses were as follows: *1*) iron absorption would be increased by a lipid bolus given either with or before the meal, and this enhancing effect would be greater for FePP than for $FeSO_4$; and *2*) iron absorption would be greater from an MNP given with an RUTF than from an MNP alone, and the addition of phytase to an MNP given with an RUTF would increase iron absorption to a greater extent than the addition of phytase to an MNP alone.

METHODS

Subjects

Healthy young women (*n* = 54) were recruited from the student population of the ETH Zürich and the University of Zurich, Switzerland. Inclusion criteria were as follows: *1*) age 18–45 y, *2*) body weight <65 kg, *3*) BMI (in kg/m^2) of 17.5–25, *4*) not pregnant or lactating, *5*) no known gastrointestinal or chronic inflammation, *6*) no chronic medication use (except for oral contraceptives), *7*) no intake of vitamin and mineral supplements or the willingness to discontinue use 2 wk before the start of the study, and *8*) no blood donation or significant blood loss <4 mo before the start of the study. Written informed consent was obtained from all of the subjects. The study protocol was approved by the ethics committee at ETH Zürich and registered in the public clinical trial database clinicaltrials.gov as NCT01991626. The sample size of 14 women/study was sufficient to detect an intrasubject difference of 30% in iron absorption, with an α level of 0.05 and a β level of 0.20.

Study design

Subjects were enrolled in 3 separate studies (**Figure 1**, **Supplemental Figures 1–3**). Body weight and height were measured at enrollment. In all 3 studies on each day before a study day when isotopic labels were administered, subjects were asked to fast after 2000 and not to drink after 2400. A pilot MRI study was conducted (methods reported in **Supplemental Methods**) to assess the effect of consuming RUTFs on GRT (results reported in **Supplemental Figure 4** and **Supplemental Results**).

In study 1, after baseline venipuncture (day 1), 3 meals were served over 3 subsequent days with the use of 3 different stable iron isotopes administered as an aqueous solution of $^{54}FeSO_4$, $^{57}FeSO_4$, and $^{58}FeSO_4$. The meals consisted of the following: *1*) maize alone, *2*) maize consumed with a lipid emulsion (composition shown in **Table 1**), and *3*) maize consumed 20 min after the consumption of the lipid emulsion. After 14 d of incorporation (day 16), a final blood sample was collected for the determination of isotopic composition in red blood cells and subsequent calculation of iron absorption.

In study 2, meals were identical to study 1, but FePP ($^{57}FePP$) replaced $FeSO_4$ as the iron fortification compound. Isotopically labeled FePP ($^{57}FePP$) was manufactured with a downscaled procedure identical to the commercial manufacturing process in a single batch. Because we used only one iron isotope for FePP, test meals were administered with a 14-d interval, allowing a 14-d erythrocyte incorporation period. The isotopic composition assessed after the first incorporation period was used as a new baseline to measure subsequent iron incorporation from the following meals. Blood samples were drawn on each study day and at the end of the study (days 1, 15, 29, and 43).

In study 3, with the use of a 2 × 2 factorial design, subjects were served 4 different meals based on whole maize, with a 14-d interval (days 1, 15, 29, and 43), fortified with an MNP (ingredients shown in **Table 2**) given with the following: *1*) an RUTF (ingredients described below; Table 1; MNP+RUTF), *2*) phytase (full description below; MNP+phytase), *3*) both an RUTF and phytase (MNP+RUTF+phytase), or *4*) neither (MNP). Iron was administered as labeled ferrous sulfate (dried) powder ($^{57}FeSO_4$), which was, similarly to study 2, manufactured in a single batch with a downscaled process similar to that used in commercial manufacturing. The RUTF used in study 3

Study 1 (n=14), Maize-based test meals, FeSO₄ (solution)			
Day 1	Day 2	Day 3	Day 16
No lipid emulsion, $^{58}FeSO4$	Lipid emulsion with meal, $^{57}FeSO_4$	Lipid emulsion 20 min before meal, $^{54}FeSO_4$	
Venipuncture			Venipuncture

Study 2 (n=13), Maize-based test meals, ⁵⁷FePP (powder)			
Day 1	Day 15	Day 29	Day 43
No lipid emulsion, $^{57}FePP$	Lipid emulsion with meal, $^{57}FePP$	Lipid emulsion 20 min before meal, $^{57}FePP$	
Venipuncture	Venipuncture	Venipuncture	Venipuncture

Study 3 (n=14), Maize-based test meals, ⁵⁷FeSO₄ (powder)				
Day 1	Day 15	Day 29	Day 43	Day 57
MNP+RUTF	MNP+phytase	MNP+RUTF+phytase	MNP	
Venipuncture	Venipuncture	Venipuncture	Venipuncture	Venipuncture

FIGURE 1 Schematic representation of the study design of the 3 iron absorption studies. In study 1, iron absorption from a maize meal fortified with $FeSO_4$ provided with or without a lipid emulsion that was given concomitantly or 20 min before the maize meal is shown. In study 2, a similar design was used, but the maize meal was instead fortified with FePP. In study 3, MNPs or RUTFs were fortified with $FeSO_4$ and were given with and without the addition of microbial phytase by using a 2 × 2 design. In all 3 studies, test meal administrations were randomly assigned, and each subject acted as her own control. FePP, ferric pyrophosphate; $FeSO_4$, ferrous sulfate; MNP, micronutrient powder; RUTF, ready-to-use therapeutic food.

	Lipid emulsion (per 55-g portion)	RUTF (per 60-g portion)
Energy, kcal	277.3	334.8
Protein, g	3.2	9.1
Sugar, g	4.7	23.3
Lipids, g	26.9	22.5
Iron, mg	0.1	0.5
Zinc, mg	0.4	1
Sodium, mg	49.9	83.3
Calcium, mg	118.5	204.1
Magnesium, mg	11.1	44.3
Phosphorus, mg	90.3	206.3
Chlorine, mg	0	151.4
Retinol, μg	1.3	1.8
Vitamin E, mg	2.8	3.9
Thiamin, mg	0	0.2
Riboflavin, mg	0.2	0.3
Niacin, mg	0	2.4
Vitamin B-6, mg	0	0.1
Vitamin B-12, μg	0	0.6
Folic acid, μg	4.5	24
Vitamin C, mg	0.9	1.5
Fiber, g	0	1.2
Phytate, mg	—	122.85
Polyphenols, mg	—	51.75

[1] RUTF, ready-to-use-therapeutic food.

had comparable energy and phytic acid contents to the lipid emulsion given in studies 1 and 2. Blood samples were drawn on each study day and 14 d after the last meal (days 1, 15, 29, 43, and 57).

The order of the different test meals was randomized and balanced in all studies, and subjects were allocated by the principal investigator to the different administration schedules by using random numbers generated by a spreadsheet program (Microsoft Excel, RAND function). Test meals were served in the morning between 0700 and 0900 with 200 mL high-purity water under close supervision. No food or drink was allowed for 3 h after meal intake. Fasting venous blood was drawn into EDTA-treated tubes for study 1 on days 1 and 16; for study 2 on days 1, 15, 29, and 43; and for study 3 on days 1, 15, 29, 43, and 57. Iron absorption assessment was based on the shift in the isotopic ratio after a 14-d erythrocyte incorporation period (16, 17).

Test meals

The standardized base for all test meals was a 60-g portion of whole-grain maize flour cooked in 200 g high-purity water (18 MΩ · cm) sweetened with 5 g sugar. After cooking and cooling to room temperature, water was added to compensate for evaporation. Portion size was decreased to 45 g maize flour when 60 g RUTF was added to the maize meals in study 3. Single portions were weighed on plastic plates, stored at −20°C, and then heated to 34–38°C in a microwave oven before consumption. Four milligrams of isotopically labeled iron, 8 mg Fe with normal isotopic composition, and 40 mg ascorbic acid were added before serving directly to the maize (all meals in studies 1 and 2 and to MNP and maize in study 3) or incorporated into the RUTF with the MNP (to the other 2 fortified meals in study 3).

The compositions of the MNP and the RUTF are shown in Tables 1 and 2, respectively.

A single portion of 60 g RUTF (Table 1) consisted of 25% peanut oil (Erdnussöl, Florin), 25% roasted peanut paste, 25% icing sugar (Coop), and 25% skim milk powder (Magermilchpulver, Coop). Ingredients were blended at ETH Zürich by using a food mixer (HM6121, Moulinex). RUTF was produced in bulk for the entire study and stored at −20°C in portions of 600 g, which were transferred to a refrigerator at 4°C the evening before each study day. A single portion of 55 g lipid emulsion contained the following: 27 g peanut oil, 1 g lecithin (Soja-Lecithin, Morga), 9 g skim milk powder, and 18 g high-purity water. Emulsions were prepared on the day before each study day of studies 2 and 3 with the use of a mixer (HB714, Kenwood) and stored at 4°C. Meals were consumed under close supervision, and plates were rinsed twice with 10 mL high-purity water after meal completion to ensure full intake of the iron.

Stable isotope labels

Isotopically labeled compounds for study 1 (^{54}FeSO$_4$, ^{57}FeSO$_4$, and ^{58}FeSO$_4$) were prepared as solutions from isotopically enriched elemental iron (99.8% ^{54}Fe, 96.8% ^{57}Fe, and 99.8% ^{58}Fe, respectively; Chemgas) by dissolution in dilute sulfuric acid, and kept under argon atmosphere to maintain the iron in its +II oxidation state (18). Isotopically labeled FePP (study 2, ^{57}FePP) and dried FeSO$_4$ (study 3, ^{57}FeSO$_4$) were prepared as powders by Dr. Paul Lohmann GmbH from isotopically enriched elemental iron (99.8% ^{57}Fe; Chemgas) with the use of a downscaled procedure similar to their industrial-scale process.

Phytase

We used a purified recombinant phytase from *Aspergillus niger* (DSM Phytase, 20.000G; DSM Nutritional Products). Phytase activity is measured as the amount of enzyme that liberates 1 μmol inorganic phosphorus/min and is called a phytase

Ingredient	Quantity, g/kg
Dry vitamin A palmitate, >250,000 IU/g	3.466
Dry vitamin D$_3$, >100,000 IU/g	5.200
Dry vitamin E, >500 IU/g	32.780
Dry phylloquinone, >5%	1.560
Thiamine mononitrate	1.542
Riboflavin	1.260
Pyridoxine hydrochloride	1.532
Folic acid, 10% potato malt	2.250
Niacinamide	13.200
Trisodium citrate	20.000
Vitamin B-12, >0.1%	2.250
Copper gluconate	5.346
Potassium iodide, 10% on potato maltodextrin	0.968
Sodium selenite, 1% on cornstarch	8.200
Zinc gluconate	44.140
Iron	0

[1] MNP, micronutrient powder.

unit (FTU). Assuming an activity of $\sim 60\%$ at gastric pH and a GRT of ~ 60 min, 10 FTUs were necessary to degrade 1 μmol phytate. Therefore, a quantity of ~ 130 FTUs was necessary to adequately degrade 0.5 g phytic acid (see Results); and to ensure full degradation of the phytic acid present in the test meals, 190 FTUs were used.

Test meal analysis

The iron concentration in maize was measured by graphite-furnace atomic absorption spectrophotometry (AA240Z; Agilent) after mineralization by microwave-assisted digestion (MLS ETHOSplus; MLS). Phytate concentration was measured by using a modification of the Makower method (19) in which cerium replaced iron in the precipitation step. Inorganic phosphate concentration was determined after mineralization according to Van Veldhoven and Mannaerts (20) and converted into phytate concentration. A modified Folin-Ciocalteu method (21) was used to determine total polyphenol concentration in the peanut paste, expressed as gallic acid equivalents. It was assumed that refined peanut oil, skimmed milk powder, and icing sugar contain negligible amounts of iron and phytic acid and polyphenols. Thus, iron, phytic acid, and polyphenol concentrations in RUTFs were calculated on the basis of the analyzed values obtained from the peanut paste.

Blood analysis and iron isotope measurements

Hemoglobin was measured in whole blood on the day of collection with a Coulter counter (AcT8 Counter; Beckman Coulter) with 3-level control materials provided by the manufacturer. Plasma ferritin (PF) and C-reactive protein (CRP) were measured with an Immulite 2000 automatic system (Siemens Healthcare). Anemia was defined as hemoglobin <11.7 g/dL (consistent with the reference range of the University Hospital Zurich), iron deficiency was defined as PF <15 μg/L, and inflammation was defined as CRP >5 mg/L.

Whole-blood samples were mineralized by using concentrated nitric acid by microwave-assisted digestion (MLS UltraWAVE; MLS), and iron was separated by anion-exchange chromatography (22). Iron isotope ratios were determined with a multi-collector double-focusing inductively coupled mass spectrometer (MC-ICP-MS Neptune; Thermo Finnigan) after a precipitation step with ammonium hydroxide for samples from study 2 (22) or after extraction into diethyl ether followed by negative thermal ionization mass spectrometry (16) for samples from studies 1 and 3 (MAT 262; Finnigan MAT) at ETH Zürich.

Calculation of iron absorption

Height and weight were used to estimate blood volume, which was used to calculate circulating iron (23). The calculation of the fractional absorption of iron was conducted assuming an 80% incorporation into red blood cells (16).

Statistical methods

Statistical analysis was performed with IBM SPSS Statistics (version 22). All of the data were log-transformed before analysis. Assuming a 30% difference in iron absorption to be a nutritionally relevant effect, and based on the SD in past studies

conducted at the Human Nutrition Laboratory, a sample size of 14 subjects was required; with consideration of attrition, 16 subjects were recruited. Each study was analyzed separately because subjects acted as their own controls. Iron absorption was the dependent variable in all models, and data were fit by using linear mixed models with the presence of phytase, RUTF, and the interaction term phytase $\times$ RUTF as fixed factors and subjects as random factors (intercept). If an effect was found, post hoc analyses were carried out to test differences between individual test meals (Fisher's least significant difference post hoc tests). No correction for multiple comparisons was performed. Data from studies 1 and 2 were also analyzed together with a linear mixed model by using compound, presence of lipid emulsion, and the interaction term compound $\times$ lipid emulsion as fixed factors with subjects as the random factor. In all of the models, iron status expressed as PF was tested as a covariate. To allow comparison between studies, absorption data were adjusted to the geometric mean PF value for all 3 studies (27.1 μg/L) by using the formula proposed by Cook et al. (24). Parameter estimates are listed together with the 95% CIs and corresponding P values.

RESULTS

Fifty-five subjects were assessed for eligibility. Six subjects decided not to participate. Forty-eight subjects (16 subjects/study) were assigned to the meal administration schedules. One subject could not consume the lipid emulsion, and 6 dropped out because they lost interest (2 dropouts/study); thus, 41 subjects completed the 3 studies (Supplemental Figure 1). Subject characteristics are shown in **Table 3**. No subject had anemia or inflammation. The prevalence of iron deficiency was 22% (9 of 41).

Mean $\pm$ SD iron contents of unfortified maize flour and peanut paste were 1.77 $\pm$ 0.10 and 1.81 $\pm$ 0.06 mg/100 g, respectively; thus, native iron content was 1.06 mg for all test meals. The mean $\pm$ SD phytate content was 883 $\pm$ 59 mg/100 g for maize flour and 819 $\pm$ 59 mg/100 g for peanut paste, which provided between 520 and 530 mg phytate/meal in meals containing maize and RUTF and maize only, respectively. The molar ratio of phytic acid to iron in the fortified test meals was 3.5:1. The mean $\pm$ SD content of total polyphenols in the peanut paste was 345 $\pm$ 23 mg gallic acid equivalents/100 g.

In studies 1 and 2, when data were pooled for analysis, the presence of a lipid emulsion ($P = 0.014$) and iron compound ($P < 0.001$) significantly affected iron absorption and there was a significant interaction between the iron compound and the presence of a lipid emulsion ($P = 0.012$). However, the lipid emulsion did not significantly affect iron absorption from FeSO$_4$ (**Table 4**), but increased fractional iron absorption from FePP by 2.55-fold (95% CI: 1.48-, 4.37-fold; $P < 0.001$; Table 4), which was reflected in a change from 0.19% to 0.50% in fractional iron absorption. The timing of intake of the emulsion (20 min before or with the test meal) did not influence iron absorption from FeSO$_4$ or FePP. Overall iron absorption from the maize meal fortified with FeSO$_4$ was 3.51-fold higher (95% CI: 2.17-, 9.31-fold; $P = 0.003$) than that from FePP.

In study 3, phytase increased iron absorption by 1.85-fold (95% CI: 1.49-, 2.29-fold; $P < 0.001$) from MNP and MNP +RUTF. Although there was a trend to increase iron absorption with the MNP+RUTF meal, this did not reach significance

TABLE 3
Characteristics of the young women in the absorption studies[1]

Study	n	Age, y	BMI, kg/m²	Hemoglobin, g/dL	PF,[2] μg/L	Iron deficiency,[3] n	Plasma CRP,[2] mg/L
1	14	20.9 ± 0.51	20.92 ± 0.47	13.72 ± 0.15	34.2 (21.7, 58.3)	2/14	0.86 (0.3, 2.3)
2	13	21.8 ± 0.73	20.10 ± 0.18	13.53 ± 0.09	24.04 (12.4, 42.3)	4/13	0.84 (0.26, 3.05)
3	14	24 ± 1.2	20.65 ± 0.43	13.4 ± 0.15	29.6 (14.9, 40.8)	3/14	0.76 (0.32, 1.6)

[1] Values are means ± SEs unless otherwise indicated. CRP, C-reactive protein; PF, plasma ferritin.
[2] Values are geometric means (IQRs).
[3] Defined with a PF <15 μg/L.

(1.21-fold; 95% CI: 0.92-, 1.61-fold; $P = 0.060$; **Table 5**). There was no significant interaction between the RUTF and phytase on iron absorption ($P = 0.623$).

There was a significant correlation of PF with iron absorption for all 4 study meals in study 3: MNP, MNP+phytase, MNP+RUTF, and MNP+RUTF+phytase showed Spearman ρ values of -0.644 ($P = 0.013$), -0.789 ($P = 0.01$), -0.886 ($P < 0.001$), and -0.754 ($P < 0.001$), respectively. In study 3, PF was also a significant predictor ($P = 0.012$) of iron absorption in the linear mixed model. In contrast, in studies 1 and 2, the following was observed: *1*) PF was not correlated with iron absorption from FePP, *2*) PF was correlated with iron absorption from FeSO₄ only when the fat emulsion was given 20 min beforehand ($\rho = -0.552$, $P = 0.041$), and *3*) PF was not a significant predictor of iron absorption in the linear mixed models.

DISCUSSION

The main findings of this study in iron-fortified, maize-based meals were as follows: *1*) the addition of a lipid emulsion more than doubles iron absorption from FePP but has no effect on iron absorption from FeSO₄; *2*) the addition of a microbial phytase increases iron absorption from FeSO₄, with and without the addition of RUTF; *3*) the combination of an RUTF and phytase has no additional effect on iron absorption; and *4*) the addition of a nutrient-dense RUTF results in a tendency toward an increase in iron absorption from FeSO₄, which requires confirmation in further studies. A possible explanation for why the addition of nutrient-dense lipids enhanced iron absorption more strongly from FePP is that absorption from FePP is dependent on its dissolution in the low pH of gastric juice (25), and on the basis of in vitro solubility experiments, the dissolution of FePP

is incomplete even after 30 min at a pH of 1 (25, 26). In contrast, FeSO₄ is water soluble and rapidly dissolves in the stomach.

The addition of a dietary lipid to a meal has generally been shown to increase iron absorption in animal models (27–30). However, a human study that investigated the effect of removing or replacing macronutrients in a semisynthetic meal reported no significant effects of changing lipid and calorie content on iron bioavailability (8). In the latter study, semisynthetic meals contained only low amounts of phytic acid, and the presence of an inhibitory matrix may be necessary for the effect of increased GRT and/or duodenal residence time on iron absorption to become apparent.

Several potential mechanisms may explain the effect of dietary lipids on iron absorption. First, the increased time at low stomach pH during longer GRT would generally favor the solubilization of ferrous and ferric iron moieties and diminish the inhibitory effect of phytic acid on iron solubility, because protonated forms of phytic acid prevail at lower pH ranges (31, 32). Second, a lipid emulsion that slows gastric emptying and increases GRT (33) might increase the ability of the divalent metal transporter 1 (DMT1) to take up luminal iron. It is also possible that lipid microdroplets and free fatty acids may noncovalently bind iron, protecting it from chelation by phytic acid and thus facilitating its absorption (34). Longer GRT could possibly also increase iron absorption from other iron compounds commonly used as fortificants that require a low pH to release iron, such as electrolytic iron and ferrous fumarate.

In human studies, the entry of nutrients, including lipids, into the ileum slows gastrointestinal transit of the more proximal luminal contents via an "ileal" or "duodenal brake" mechanism (10). Thus, we hypothesized that administering the lipid emulsion 20 min before the fortified maize meal would result in a

TABLE 4
Fractional iron absorption from the test meals fortified with FeSO₄ (study 1) or FePP (study 2) measuring the effect of consumption of a maize meal with or without a lipid emulsion given either at or 20 min before consumption in Swiss women[1]

Test meal					Iron absorption,[2] %	
Maize, g	Lipid emulsion, g	Phytic acid, mg	Lipid, g	kcal	13 mg Fe as FeSO₄[3]	13 mg Fe as FePP[4]
60	—	530	1.6	212	1.59 (0.48, 5.23)ᵃ	0.19 (0.06, 0.56)ᵇ
60	55 (consumed 20 min before maize)	530	27.7	486	1.42 (0.36, 5.66)ᵃ	0.45 (0.17, 1.23)ᶜ
60	55 (consumed with maize)	530	27.7	486	1.73 (0.67, 4.43)ᵃ	0.50 (0.15, 1. 62)ᶜ

[1] Study 1, $n = 14$; study 2, $n = 13$. FePP, ferric pyrophosphate; FeSO₄, ferrous sulfate.
[2] As geometric means (geometric mean − SD, geometric mean + SD). Values were adjusted to the geometric mean plasma ferritin concentration obtained across all participants of studies 1–3 (27.1 μg/L) (24). For iron absorption, values in the same column or in the same row with different superscript letters differ ($P < 0.01$).
[3] There was no effect of meal by linear mixed models ($P > 0.05$).
[4] There was a significant effect of test meal by linear mixed models ($P < 0.05$).

TABLE 5
Fractional iron absorption in Swiss women from the test meals in study 3 assessing the effect of consumption of a maize meal either fortified with MNP or together with an RUTF[1]

Test meal[2]					Iron bioavailability			
Maize, g	RUTF, g	Additives	Phytic acid, mg	kcal	Iron absorption,[3] %	P^4	P^5	P^6
60	—	—	530	204	5.3 (2.0, 13.8)[a]	<0.001	0.060	0.623
60	—	Phytase, 190 FTUs	530	204	10.2 (5.06, 20.4)[b]			
45	60	—	520	488	6.67 (2.83, 15.7)[a]			
45	60	Phytase, 190 FTUs	520	488	11.9 (6.10, 23.3)[b]			

[1] $n = 14$. The calorie and phytic acid contents of the meals were matched. $FeSO_4$, ferrous sulfate; FTU, phytase unit; MNP, micronutrient powder; RUTF, ready-to-use therapeutic food.

[2] All test meals contained 1 g MNP, 30 mg ascorbic acid, and 13 mg Fe as $FeSO_4$ per meal.

[3] Values are geometric means (geometric mean − SD, geometric mean + SD). Values were adjusted to the geometric mean plasma ferritin concentration obtained across participants of studies 1–3 (27.1 μg/L) (24). Individual study results were compared by using Student's paired t test; different superscripts in the column differ significantly ($P < 0.001$).

[4] Overall effect of phytase.

[5] Overall effect of consuming an RUTF.

[6] Interaction term between phytase and an RUTF.

slower gastrointestinal transit, a more pronounced increase in GRT, and enhanced iron absorption. However, in the MRI pilot study, postprandial changes in gastric content volumes were comparable whether the RUTF was given with or 20 min before the meal (Supplemental Figure 4, Supplemental Results). In study 1, there was no difference in iron absorption whether the lipid emulsion was given with or before the maize meal. Although it is possible that the effect of the previous bolus may have been greater if there had been a longer time between the lipid bolus and meal administration, our data do not support previous lipid administration as an approach to increase iron absorption.

Our data show that phytase increases iron absorption by a factor of 1.8 from an RUTF and an MNP, which confirms findings from previous studies on phytase in MNPs (11). We speculated that a calorie-rich meal as provided by an RUTF would increase GRT and allow more time for degradation of phytic acid before the phytase is inactivated by pancreatic enzymes. However, our findings do not support this hypothesis. This is despite the fact that the ∼80% increase in iron absorption in the current study likely reflects only partial dephytinization, because complete dephytinization would likely result in a more pronounced increase in iron absorption (35). We tested the interaction between calorie-rich meals and phytase on iron absorption from $FeSO_4$, but not from FePP; thus, we cannot exclude an interaction between RUTF and phytase on poorly water-soluble iron compounds. We chose to use $FeSO_4$ in study 3 because RUTFs are generally fortified with water-soluble iron compounds, such as $FeSO_4$, or compounds that are soluble in diluted acid, such as ferrous fumarate, due to their generally higher bioavailability.

In study 3, iron absorption from the MNP+RUTF meal tended to be higher than with MNP alone, although this was not significant ($P = 0.060$). The upper limit of the 95% CI suggests the possibility of a 1.61-factor increase in iron absorption on consumption of an RUTF. Such an effect would be of wide public health relevance and calls for the conduct of further studies, in particular in target population groups such as young children. However, although our data are consistent with a possible enhancing effect of RUTFs on iron absorption, more studies are required to prove (or disprove) this.

Our study has several strengths. We measured iron absorption with high precision by using isotopically labeled iron fortificants (FePP in study 2 and $FeSO_4$ in study 3) manufactured specifically for this use by a downscaling of the commercial manufacturing process. We matched phytic acid contents between the different meals to be able to compare solely the effect of the addition of RUTFs and phytase. We tested the effect of lipid on iron absorption both mechanistically with a lipid emulsion and with a commonly used product (RUTF). The limitations of the current study include the following: 1) we did not test FePP in study 3 (for the reasons described above), 2) the sample size was relatively small, and 3) we studied young adults, whereas RUTFs and MNPs are products typically provided to infants and children.

Our findings suggest that the addition of lipids substantially enhances iron absorption from FePP. This could be relevant for foods in which FePP is the fortificant of choice, such as bouillon powders (36) and rice (26); the consumption of fat with these fortified foods may increase iron absorption. The use of iron-containing RUTFs and other lipid-based nutritional supplements is increasing worldwide. Our data indicate that the addition of a phytase would likely increase iron absorption from these products. Our data also show a tendency for the addition of RUTFs to an MNP-fortified meal to result in higher iron absorption than from the MNP alone. This needs to be confirmed in future studies.

REFERENCES

1. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. Public Health Nutr 2009;12:444–54.

2. Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, Regan M, Weatherall D, Chou DP. Eisele TP, et al. A systematic analysis of global anemia burden from 1990 to 2010. Blood 2014;123:615–24.
3. Zimmermann MB, Hurrell RF. Nutritional iron deficiency. Lancet 2007;370:511–20.
4. Zlotkin SH, Schauer C, Christofides A, Sharieff W, Tondeur MC, Hyder SM. Micronutrient sprinkles to control childhood anaemia. PLoS Med 2005;2:e1.
5. Patel MP, Sandige HL, Ndekha MJ, Briend A, Ashorn P, Manary MJ. Supplemental feeding with ready-to-use therapeutic food in Malawian children at risk of malnutrition. J Health Popul Nutr 2005;23:351–7.
6. Manary MJ. Local production and provision of ready-to-use therapeutic food (RUTF) spread for the treatment of severe childhood malnutrition. Food Nutr Bull 2006;27(3 Suppl):S83–9.
7. Adu-Afarwuah S, Lartey A, Brown KH, Zlotkin S, Briend A, Dewey KG. Home fortification of complementary foods with micronutrient supplements is well accepted and has positive effects on infant iron status in Ghana. Am J Clin Nutr 2008;87:929–38.
8. Monsen ER, Cook JD. Food iron absorption in human subjects. V. Effects of the major dietary constituents of semisynthetic meal. Am J Clin Nutr 1979;32:804–8.
9. Kwiatek MA, Menne D, Steingoetter A, Goetze O, Forras-Kaufman Z, Kaufman E, Fruehauf H, Boesiger P, Fried M. Schwizer W, et al. Effect of meal volume and calorie load on postprandial gastric function and emptying: studies under physiological conditions by combined fiberoptic pressure measurement and MRI. Am J Physiol Gastrointest Liver Physiol 2009;297:G894–901.
10. Maljaars PW, Peters HP, Mela DJ, Masclee AA. Ileal brake: a sensible food target for appetite control—a review. Physiol Behav 2008;95:271–81.
11. Troesch B, Egli I, Zeder C, Hurrell RF, de Pee S, Zimmermann MB. Optimization of a phytase-containing micronutrient powder with low amounts of highly bioavailable iron for in-home fortification of complementary foods. Am J Clin Nutr 2009;89:539–44.
12. Sandberg AS, Hulthen LR, Turk M. Dietary Aspergillus niger phytase increases iron absorption in humans. J Nutr 1996;126:476–80.
13. UNICEF Supply Division. Ready to use therapeutic food: current outlook [Internet]. May 2014 [cited 2015 Dec 22]. Available from: http://www.unicef.org/supply/files/RUTF_Supply_Update__May_2014.pdf.
14. Zlotkin S, Newton S, Aimone AM, Azindow I, Amenga-Etego S, Tchum K, Mahama E, Thorpe KE, Owusu-Agyei S. Effect of iron fortification on malaria incidence in infants and young children in Ghana: a randomized trial. JAMA 2013;310:938–47.
15. Jaeggi T, Kortman GA, Moretti D, Chassard C, Holding P, Dostal A, Boekhorst J, Timmerman HM, Swinkels DW, Tjalsma H, et al. Iron fortification adversely affects the gut microbiome, increases pathogen abundance and induces intestinal inflammation in Kenyan infants. Gut 2015;64:731–42.
16. Walczyk T, Davidsson L, Zavaleta N, Hurrell RF. Stable isotope labels as a tool to determine the iron absorption by Peruvian school children from a breakfast meal. Fresenius J Anal Chem 1997;359:445–9.
17. 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.
18. Moretti D, Zimmermann M, Wegmuller R, Walczyk T, Zeder C, Hurrell R. Iron status and food matrix strongly affect the relative bioavailability of ferric pyrophosphate in humans. Am J Clin Nutr 2006;83:632–8.
19. Makover R. Exctraction and determination of phytic acid in beans. Cereal Chem 1970;47:288–95.
20. Van Veldhoven PP, Mannaerts GP. Inorganic and organic phosphate measurements in the nanomolar range. Anal Biochem 1987;161:45–8.
21. Singleton VL, Rossi JA. Colorimetry of total phenolics [in grapes and wine] with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 1965;16:144–58.
22. Hotz K, Krayenbuehl PA, Walczyk T. Mobilization of storage iron is reflected in the iron isotopic composition of blood in humans. J Biol Inorg Chem 2012;17:301–9.
23. Brown E, Hopper J Jr., Hodges JL Jr., Bradley B, Wennesland R, Yamauchi H. Red cell, plasma and blood volume in healthy women measured by radiochromium cell-labelling and hematocrit. J Clin Invest 1962;41:2182–90.
24. 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.
25. Hurrell R. Linking the bioavailability of iron compounds to the efficacy of iron-fortified foods. Int J Vitam Nutr Res 2007;77:166–73.
26. Hackl L, Cercamondi C, Zeder C, Wild D, Adelmann H, Zimmermann M, Moretti D. Co-fortification 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.
27. Bowering J, Masch GA, Lewis AR. Enhancement of iron absorption in iron depleted rats by increasing dietary fat. J Nutr 1977;107:1687–93.
28. Hirooka M, Ikeda S, Kubota N. Effect of dietary fat (soybean oil) on intestinal iron absorption in rats. Tohoku J Exp Med 1968;95:243–52.
29. Johnson PE, Lukaski HC, Bowman TD. Effects of level and saturation of fat and iron level and type in the diet on iron absorption and utilization by the rat. J Nutr 1987;117:501–7.
30. Mazzanti R, Srai KS, Debnam ES, Boss AM, Gentilini P. The effect of chronic ethanol consumption on iron absorption in rats. Alcohol Alcohol 1987;22:47–52.
31. Bretti C, Cigala RM, Crea F, De Stefano C, Vianelli G. Solubility and modeling acid-base properties of adrenaline in NaCl aqueous solutions at different ionic strengths and temperatures. Eur J Pharm Sci 2015;78:37–46.
32. Oberleas D. In vitro properties of ferrous and ferric ions with phytate. In: Roussel AM, Anderson RA, Favrier AE, editors. Proceedings of the 10th International Symposium on Trace Elements in Man and Animals (TEMA 10); 1999 May 2–7, Evian, France. Boston: Springer; 2000. p. 823–4.
33. Haenni A, Sundberg B, Yazdanpandah N, Viberg A, Olsson J. Effect of fat emulsion (Fabuless) on orocecal transit time in healthy men. Scand J Gastroenterol 2009;44:1186–90.
34. Simpson RJ, Peters TJ. pH-Sensitive transport of Fe2+ across purified brush-border membrane from mouse intestine. Biochim Biophys Acta 1986;856:109–14.
35. Hurrell RF, Reddy MB, Juillerat MA, Cook JD. Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. Am J Clin Nutr 2003;77:1213–9.
36. Cercamondi CI, Duchateau GS, Harika RK, van den Berg R, Murray P, Koppenol WP, Zeder C, Zimmermann MB, Moretti D. Sodium pyrophosphate enhances iron bioavailability from bouillon cubes fortified with ferric pyrophosphate. Br J Nutr 2016;116:496–503.