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Iron Absorption from Bouillon Fortified with Iron-Enriched *Aspergillus oryzae* Is Higher Than That Fortified with Ferric Pyrophosphate in Young Women

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ABSTRACT

Background: Bouillon cubes are a potential vehicle for iron fortification. They are currently fortified with ferric pyrophosphate (FePP), which is known to be poorly absorbed. The objective of this study was to assess the iron absorption of *Aspergillus oryzae* grown in FePP (ASP-p) and compare it with FePP and ferrous sulfate ($FeSO_4$)–fortified bouillon cubes.

Methods: In 2 single-blinded, crossover studies, healthy women with serum ferritin concentrations <40 μg/L were randomly assigned to consume a rice-vegetable meal with iron-fortified chicken bouillon. Subjects in study I (n = 17, 18–26 y) consumed iron from both iron sources as ^{57}FePP and ^{58}ASP-p (intrinsically labeled with ^{58}FePP) with a meal containing 4.2 mg of total iron provided for 3 d. Study II (n = 18, 18–29 y) was similar except that subjects consumed $^{57}FeSO_4$ and ^{58}ASP-p. Whole-blood stable isotope enrichment after 14 d was used to measure fractional iron absorption. Hemoglobin, hematocrit, serum ferritin, hepcidin, and serum C-reactive protein were analyzed at baseline and at 14 d. A t test was used to compare the mean differences in fractional absorptions within each study and baseline characteristics between studies.

Results: Geometric mean (95% CI) fractional iron absorption of FePP [0.94% (0.63%, 1.40%)] was lower than ASP-p [2.20% (1.47%, 3.30%)] ($P < 0.0001$) in study I. In study II, ASP-p fractional absorption [2.98% (2.03%, 4.38%)] was lower than that of $FeSO_4$ [9.88% (6.70%, 14.59%)] ($P < 0.0001$). Both ferritin ($r = -0.41$, $P = 0.014$) and hepcidin ($r = -0.42$, $P = 0.01$) concentrations were inversely correlated with ASP-p iron absorption. Fractional absorption of ASP-p was also positively correlated with FePP ($r = 0.92$, $P < 0.0001$) and $FeSO_4$ ($r = 0.52$, $P < 0.02$) absorption.

Conclusions: ASP-p–fortified bouillon provided 2.3-fold higher absorbable iron than the currently used FePP. Bouillon fortified with ASP-p may contribute sufficient bioavailable iron to meet the daily iron requirements in young women only if consumed with other iron-fortified staple foods. This trial was registered at clinicaltrials.gov as NCT03586245. *J Nutr* 2020;150:1109–1115.

Keywords: iron absorption, stable iron isotope, iron fortification, ferrous sulfate, ferric pyrophosphate, *Aspergillus oryzae*, bouillon cubes

Introduction

Iron deficiency (ID) is estimated to be responsible for 50% of the global anemia burden that affects 1.62 billion people (1). Iron deficiency anemia (IDA) frequently coexists with a number of other anemias, such as those caused by malaria, parasitic infection, hemoglobinopathies, and other nutritional deficiencies (1). Anemia is most common in children, women of childbearing age, and pregnant women. Negative health consequences of IDA include the following: decreased cognitive function, decreased productivity, and poor pregnancy outcome and an increase in mortality (1).

Several strategies have been implemented to address IDA including supplementation, dietary modifications, consuming biofortified staple foods (such as rice, cereals, and beans), and fortification of foods (2). Fortification of staple foods and widely consumed condiments are the most sustainable and affordable strategies to improve iron status and can make a significant contribution in meeting daily iron requirements (3). There are numerous iron compounds available for food fortification, but

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not all are acceptable for sensory reasons and a relatively small number are recommended by the WHO (4). The challenges in fortification include finding a suitable iron compound with high iron bioavailability while remaining inert to sensory changes in the food matrix. Soluble iron compounds such as ferrous sulfate ($FeSO_4$) and ferrous gluconate are well absorbed; however, they are also highly reactive in food, causing color, flavor changes, and rancidity during storage of cereals (5). Insoluble iron sources like FePP are useful for fortification as they do not alter the organoleptic properties of the food; however, they have low absorption (5). The absorption of FePP was only 13–15% of $FeSO_4$ when consumed in a bouillon drink and rice (6, 7). Attempts have been made to improve iron status and absorption from FePP by reducing its particle size (8) and adding sodium pyrophosphate to bouillon cubes (6); the latter process has produced limited success.

Bouillon cubes are fortified with iron because they are regularly consumed in many West African countries where ID is highly prevalent. Although bouillon cube consumption as a condiment cannot provide all the iron daily requirements, together with iron-fortified staple foods, such as wheat or maize, they could provide all the iron lacking in a normal diet. Based on the amount of FePP added to bouillon, the mean daily consumption, and low absorption of iron from bouillon, FePP-fortified bouillon cubes have made little contribution to iron nutrition (6); therefore, there is a need for alternative iron-fortification compounds.

Aspergillus oryzae (Ao) is a filamentous fungus, also known as koji culture. It is reported to be safe to consume (9) and has been used for thousands of years to make fermented soy and rice products such as sake, soy sauce, amazake, and miso paste (10, 11). Cura Global Health, Inc., discovered in 2013 (12) that Ao had the ability to take up high amounts (≤10% of its biomass) of minerals, including iron. In a previous human study conducted by our team, iron-enriched Ao grown in $FeSO_4$-containing media (ASP-s) showed absorption similar to $FeSO_4$ when given with a semipurified liquid meal, suggesting its potential as a new, highly bioavailable, natural source of iron for food fortification (13). However, ASP-s caused slight color changes when added to bouillon. Subsequently, another iron-enriched Ao product (ASP-p), using insoluble FePP instead of $FeSO_4$ in the growth media of the Ao, was produced, which did not change the color of bouillon. The objective of this study was to investigate the iron absorption from iron-fortified chicken bouillon from ASP-p provided with a rice-vegetable meal to young women and to compare it with FePP and $FeSO_4$.

Methods

Subjects

Healthy, nonsmoking women aged 18–35 y, with ferritin <40 μg/L but who were not anemic, were recruited for both iron isotope studies by sending a mass e-mail to faculty and students at Iowa State University (ISU). These criteria were used because individuals in this category have a high risk of developing IDA and an increased response to food iron due to suboptimal iron stores (14). Exclusion criteria included history of chronic or gastrointestinal conditions, in addition to having known food allergies to the ingredients in our administered test meal. Participants were excluded from the study if they were vegetarian, pregnant or lactating, or clinically classified as underweight [BMI (in kg/m^2) <18.5] or overweight (BMI ≥25). Participants were ineligible if they were taking any medications other than oral contraceptives. Those consuming vitamin and/or mineral supplements were asked to discontinue their use ≥2 wk prior to and throughout the study period. Participants were not allowed to donate blood or plasma 1 mo prior to or while in the study. All subjects formally agreed to participate and signed consent forms. The study was approved by the Institutional Review Board of ISU.

In study I, comparing the absorption of FePP to ASP-p, a total of 58 subjects were screened, of whom only 18 were eligible based on inclusion criteria. One subject dropped out of the study halfway through the feeding trial after becoming ill with the flu; therefore, 17 subjects completed the study. In study II, evaluating $FeSO_4$ and ASP-p absorption, 77 individuals were screened and 19 were eligible. Similarly, 1 subject dropped out midway through the study due to not being able to finish eating the meal containing the iron treatment. Eighteen subjects participated in the final analyses for study II (**Figure 1**). Of the total 35 participants, 77% were white, 11.4% Hispanic/Latino, 5.7% Asian, and 2.9% African American and American Indian. Both iron-absorption studies required ≥16 participants to detect a 30% difference in iron absorption with 80% power at $P < 0.05$ using the previously reported SD of 0.2 (within-subject) for absorption following $\log_{10}$ transformation (15).

Stable isotope analysis

Stable isotopes (^{57}Fe and ^{58}Fe) were purchased from Chemgas (Boulogne, France) and shipped directly to Dr Paul Lohmann® (Emmerthal, Germany) for labeling ^{57}FePP, $^{57}FeSO_4$, and ^{58}FePP using laboratory methods similar to their commercial preparation of FePP and $FeSO_4$ with natural abundance iron. Iron content and enrichment of compounds were as follows: [^{57}Fe]-FePP (26.4% Fe with 95.8% enrichment), [^{57}Fe]-$FeSO_4$ (dried, 35.95% Fe with 95.3% enrichment) and unlabeled, and naturally abundant FePP containing 22.3% Fe and $FeSO_4$ with 37.0% Fe. ^{58}FePP was used to intrinsically label Ao to make ^{58}ASP-p. The methodology for intrinsically labeled ^{58}ASP was described in our previous study (13). The ^{58}ASP-p powder contained 5.0% iron with 99.5% ^{58}Fe enrichment. Isotope enrichments and iron content of all 3 iron sources were measured by magnetic sector thermal ionization MS and inductively coupled MS (16). Unenriched Ao and enriched ASP-p with natural abundance iron contained 0.5 mg Fe/g and 87.9 mg Fe/g, respectively. The Ao was used to match the fungal biomass content of ASP-p in all other test meals.

Test meal

The test meal composition used to feed the stable isotopes was modified based on a previous study (7). The rice with vegetable meal was formulated to contain low elemental iron and low amounts of iron-absorption inhibitors and enhancers. Meal composition and preparation are described in the **Supplemental Methods**. Chicken bouillon powder was weighed in individual 1-ounce cups and all iron treatments (tracers and natural abundance) were meticulously added on top of the bouillon powder to prevent any isotopic loss. On the day of the feeding, the rice and vegetable sauce were individually heated thoroughly in a microwave, combined, and mixed with the chicken bouillon containing the respective iron tracers. The cups were rinsed 3 times with a small amount of purified water to ensure all residual isotope was added to the meal. All of the ingredients were carefully mixed prior to serving.

Study design

The study design is described in **Figure 2**. In 2 controlled, single-blinded, crossover study designs, participants were randomized separately in

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Abbreviations used: Ao, *Aspergillus oryzae*; ASP-p, *Aspergillus oryzae* enriched with ferric pyrophosphate; ASP-s, *Aspergillus oryzae* enriched with ferrous sulfate; CRP, C-reactive protein; EAR, Estimated Average Requirement; FePP, ferric pyrophosphate; ID, iron deficiency; IDA, iron deficiency anemia; ISU, Iowa State University; RBV, relative biological value; SF, serum ferritin.

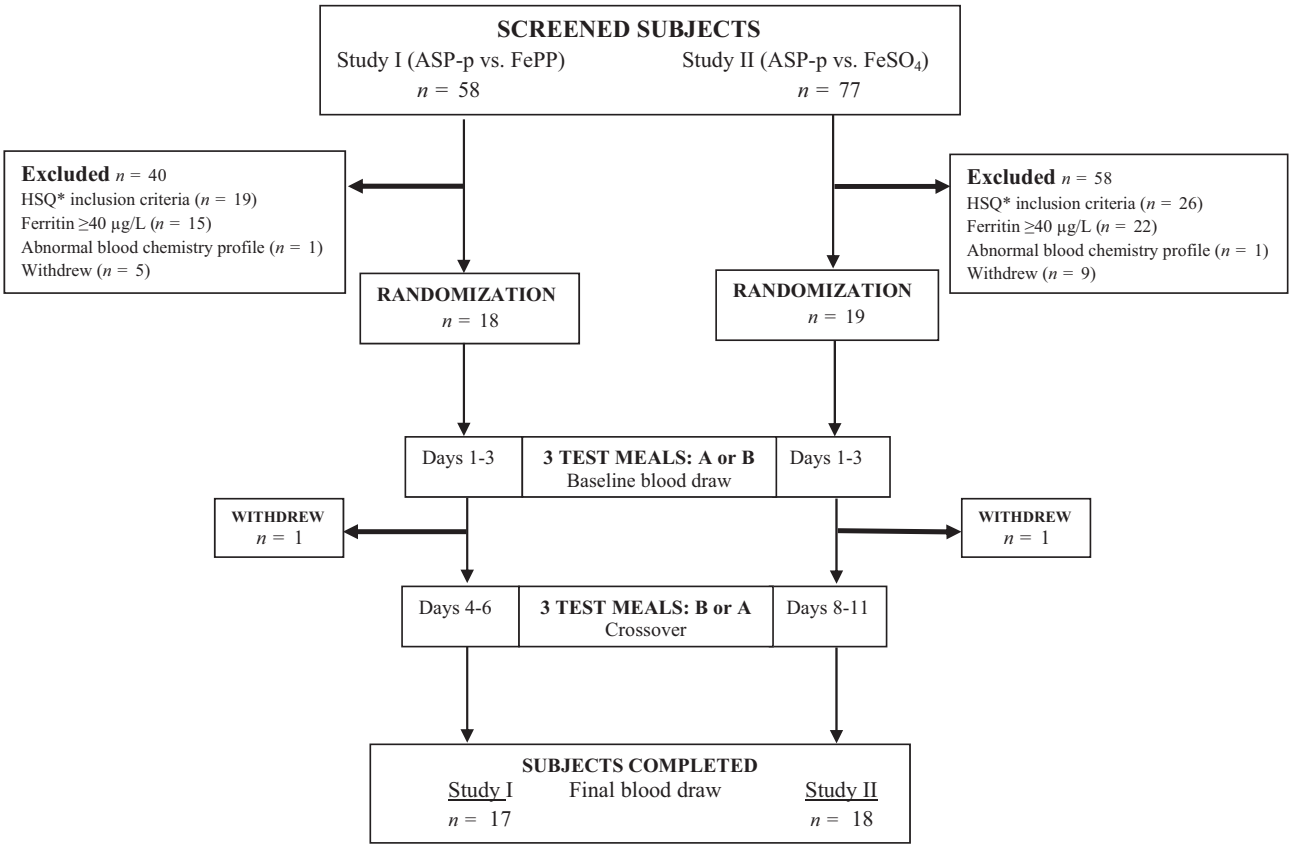

FIGURE 1 Study design and eligibility of women in studies I and II. *Participants were recruited based on eligibility from HSQ including BMI (kg/m^2) of 18.5 to 25.0 and age >18 y. Test meals AB and BA were fed in a crossover design for both studies. Isotopes with meals were given to each subject over 3 test meals. One study I subject withdrew due to flu; a study II subject withdrew from an unfinished test meal. ASP-p, *Aspergillus oryzae* grown in ferric pyrophosphate; FePP, ferric pyrophosphate; HSQ, health screening questionnaire.

each study (using the "RAND" function in Microsoft Excel). In study I, on days 1–3 subjects consumed meal A (^{57}FePP) or meal B1 (^{58}ASP-p) once per day, followed by meal B1 or A on days 4–6. In study II, subjects were randomly assigned similarly, except that they consumed meal B2 (same as B1, ^{58}ASP-p) or C ($^{57}FeSO_4$) on days 1–3 followed by meal C or B2 on days 8–11 to accommodate the students' 3-h availability during the weekday with class schedules. Doses of iron added to each meal are described in detail later in the fortification labeling section (Table 1).

On day 1 of the study, participants were required to complete a 10-h overnight fast prior to consuming the first test meal between the hours of 06:00 and 08:30 h at our Nutrition and Wellness Research Center at ISU. In both studies, baseline blood was drawn followed by administration of the isotopes in meal A, B, or C (described in the Table 1). Participants were instructed to consume the entire meal within 15 min, and bowls and spoons were rinsed with bottled water for a minimum of 3 times or until all food residue was gone and consumed by the subjects. Bowls and spoons were checked by research personnel to confirm all isotope was consumed. Subjects were not allowed to eat or drink for an additional 3 h, except for water. Fourteen days after the last test meal was eaten, participants' 10-h fasted blood was drawn for final hemoglobin and iron-enrichment analyses.

Fortification and labeling of test meals

All 4 meals were designed to contain equal amounts of 4.2 mg added Fe/meal per day (Table 1) plus 0.56 mg Fe contributed by the rice and bouillon, providing 4.76 mg total Fe/meal per day. The rationale for

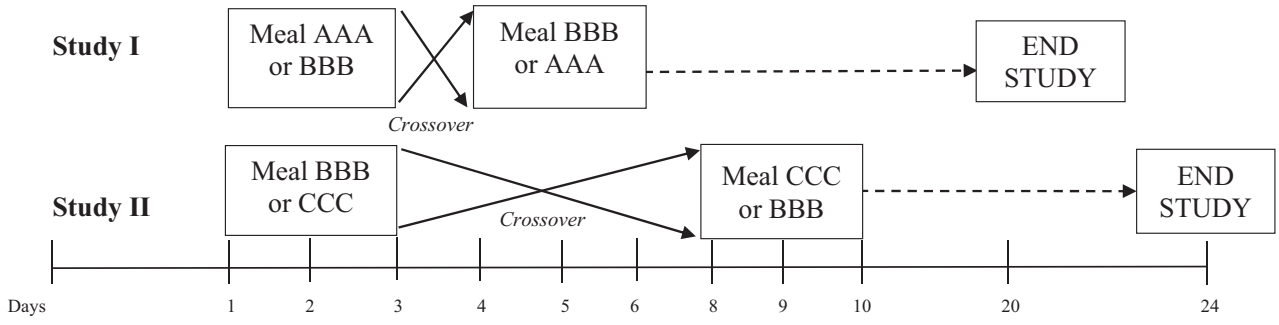

FIGURE 2 Study design for studies I and II in women who were provided meals A (^{57}FePP), B1/B2 (^{58}ASP-p), and C ($^{57}FeSO_4$) once a day for 3 consecutive days. ASP-p, *Aspergillus oryzae* grown in ferric pyrophosphate; FePP, ferric pyrophosphate.

TABLE 1 Iron content in test meals provided to women in study I (FePP and ASP-p) and study II (ASP-p and $FeSO_4$)[1]

Test meal composition	Iron enrichment (Fe %)	Study I: Fe, mg	Study II: Fe, mg	Total Fe added, mg
Meal A				4.2
Aspergillus oryzae[2]	Unenriched (0.05)	0.03	—	
^{57}FePP	95.8% enrichment (26.4)	3.34	—	
FePP	Natural abundance (22.3)	0.87	—	
Meal B1				4.2
ASP-p	Natural abundance (8.8)	3.52	—	
^{58}ASP-p	99.5% enrichment (5.0)	0.68	—	
Meal B2				4.2
ASP-p	Natural abundance (8.8)	—	3.2	
^{58}ASP-p	99.5% enrichment (5.0)	—	1	
Meal C				4.2
A. oryzae[2]	Unenriched (0.05)	—	0.03	
$^{57}FeSO_4$	95.4% enrichment (36.0)	—	4.15	

[1] 6.6 g chicken bouillon + 4.2 mg Fe (2.5 mg Fe/4g bouillon). Iron from rice and bouillon is 0.56 mg Fe and a negligible amount from vegetable puree. ASP-p, *Aspergillus oryzae* grown in ferric pyrophosphate; FePP, ferric pyrophosphate.
[2] Unenriched *A. oryzae* was added to the meals A and C to match fungal biomass that was in meals B1 and B2, respectively.

the addition of iron was based on current iron-fortified bouillon (3.3 g bouillon fortified with 2.1 mg Fe consumed twice a day or 6.6 g bouillon with 4.2 mg Fe/d) (6). Table 1 describes in detail the amount of iron (natural abundance and the enriched stable isotope iron) enriched in the meals in each of the 2 studies. To ensure enough enrichment because of the low absorption values in the first study (based on preliminary analysis), we provided a total of 12.5 mg ^{57}Fe and 3 mg ^{58}Fe as total (in 3 meals) enriched iron in the second study. Ao (grown without added iron) was added to meals A and C to match the fungal biomass contained in meals B1 and B2.

Biochemical and isotope enrichment analysis in blood

Serum and whole blood were collected at screening and stored (−20°C) until time of measurement. Serum ferritin (SF) concentrations (S-22 Spectro Ferritin kit; Ramco Laboratories, Inc.) were assessed to determine participant eligibility (<40 μg/L). Whole blood was sent to a certified diagnostic laboratory (Quest Diagnostics) for blood chemistry analysis, including hemoglobin and other iron status markers. Baseline and final serum samples were collected from participants and stored at −20°C until further analyses were performed for SF, hepcidin [Hepcidin 25 (Bioactive) ELISA; DRG International, Inc.] and serum C-reactive protein (CRP; ELISA; American Laboratory Products Company). Final whole-blood samples (frozen) were sent to ETH Zürich (Zurich, Switzerland) for stable isotope enrichment analysis to assess the fractional iron absorption of each isotope. Whole-blood samples were mineralized by microwave digestion, and iron was extracted by anion exchange chromatography (17). The amounts of ^{57}Fe and ^{58}Fe isotopic labels in blood 14 d after the second meal feeding were assessed on the basis of the shift in iron-isotope ratios and the estimated amount of iron circulating in the body (17). Circulating iron was calculated on the basis of blood volume that was estimated based on height, weight (18), and hemoglobin concentrations. Fractional iron absorption was calculated on the assumption that 80% of iron is incorporated into hemoglobin (19). Isotope measurements were performed using a negative thermal ionization–MS at ETH Zürich laboratory.

Statistical analysis

All statistical analyses were performed by using GraphPad Prism 6.07 software (GraphPad Software, Inc.). Normally distributed data are presented as means ± SDs and ranges. Non–normally distributed data were log transformed prior to statistical analysis. Non–log-transformed values are presented as geometric means and their 95% CI for iron absorption, ferritin, hepcidin, and CRP. The zero values for CRP were changed to 0.001 for analysis purposes. Student's *t* test was used to compare the baseline characteristics of the subjects between studies. Paired *t* test was used to compare the differences in fractional absorption between ASP-p and FePP (study I) and ASP-p and $FeSO_4$ (study II) within each study. One outlier was identified with ASP-p absorption; therefore, statistical analysis was performed with and without using the subject. Pearson's correlation analyses were performed among fractional absorptions and ferritin and hepcidin. All differences were considered significant at $P \leq 0.05$.

Results

Subject characteristics

General baseline anthropometric and biochemical characteristics of subjects in studies I and II are presented in Table 2. Mean ages of participants in study I and study II were 20 y and 21 y, respectively. Mean BMI was similar in both studies, 22.1, and was within the normal range (18.5–24.9) (20). At screening, all participants in both studies had hemoglobin concentrations within normal levels (>12 g/dL) according to the reference values provided by the diagnostic laboratory (Quest Diagnostics™). The mean ± SD hemoglobin concentration in study I (12.8 ± 1.4 g/dL) was similar to that in study II (12.9 ± 0.7 g/dL). Geometric mean SF concentrations of 18.2 μg/L and 15.4 μg/L in study I and II, respectively, were not significantly different between the 2 studies. One

TABLE 2 General baseline characteristics and iron status indicators of the women who consumed the meals in studies I (FePP and ASP-p) and II (ASP-p and $FeSO_4$)[1]

	Study I ($n = 17$)	Study II ($n = 18$)
Age, y	20.1 ± 2.4 (18–26)	21.3 ± 2.7 (18–29)
Weight, kg	61.5 ± 6.3 (50.2–70.8)	62.2 ± 5.3 (49.6–71.3)
BMI, kg/m^2	22.1 ± 1.7 (18.8–24.6)	22.2 ± 1.2 (19.6–24.1)
Hemoglobin, g/dL	12.8 ± 1.4 (10.4–15.4)	12.9 ± 0.7 (11.7–14.3)
Hematocrit, %	38.3 ± 3.6 (32.2–45.1)	38.0 ± 2.0 (35.5–42.5)
Serum hepcidin,[2] ng/mL	4.7 (3.3, 6.7)	2.4 (1.6, 3.5)
Serum CRP,[2] mg/L	0.41 (0.14, 1.2)	0.68 (0.28, 1.6)
Serum ferritin,[2] μg/L	18.2 (12.5, 26.5)	15.4 (11.3, 21.1)

[1] Values are means ± SDs (range) unless otherwise indicated. ASP-p, *Aspergillus oryzae* grown in ferric pyrophosphate; CRP, C-reactive protein; FePP, ferric pyrophosphate.
[2] Values are geometric means (95% CIs).

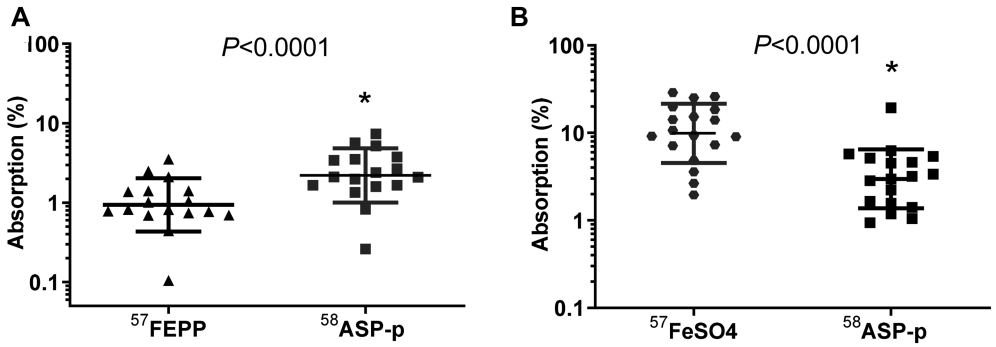

FIGURE 3 Fractional iron absorption in women provided meals with ^{57}FePP, ^{58}ASP-p in study I (A; $n = 17$) and ^{58}ASP-p and $^{57}FeSO_4$ in study II (B; $n = 18$). *Different from FePP and $FeSO_4$, $P < 0.0001$. ASP-p, *Aspergillus oryzae* grown in ferric pyrophosphate; FePP, ferric pyrophosphate.

subject in study I had elevated baseline (at the beginning of the study) SF (79.5 μg/L), but met the inclusion criteria at screening (33.1 μg/L). This subject's baseline CRP concentration was not elevated (<5 mg/L), but her ferritin increased during the study to a final SF of 97.5 μg/L. Similarly, in study II, 1 subject had high baseline SF (56.2 μg/L); however, her ferritin concentration at screening was 4.9 μg/L and the final value was 11.4 μg/L, with no elevated CRP values. Another study II subject did have an elevated baseline CRP concentration (8.1 mg/L), but SF concentrations at all time points remained within the normal range. No significant differences were found between the 2 study subjects for CRP concentrations (0.41 and 0.63 mg/L) and hepcidin concentrations (4.7 and 2.3 ng/mL). None of the other characteristics were significantly different between the studies.

Iron absorption

Fractional iron absorption values are presented as geometric means (95% CIs) in **Figure 3**. The fractional iron absorption of FePP was low [0.94% (0.63%, 1.40%)] and ASP-p absorption was significantly ($P < 0.0001$) higher by 2.3-fold [2.20% (1.47%, 3.30%)] (Figure 3A) in study I. The geometric means (95% CI) of $FeSO_4$ and ASP-p absorption were 9.88% (6.70%, 14.59%) and 2.98% (2.03%, 4.38%), respectively (Figure 3B). The 70% lower absorption of ASP-p compared with $FeSO_4$ was also significantly ($P < 0.0001$) different. The study outcome did not change with and without including the outlier in the ASP-p in the analysis, and the difference remained significant ($P < 0.001$). Correlations among hepcidin, ferritin, and ASP-p absorption are shown in **Figure 4**. Both ferritin ($r = -0.41$, $P = 0.014$; Figure 4A) and hepcidin ($r = -0.42$, $P = 0.01$; Figure 4B) concentrations showed significant negative correlations with combined ASP-p fractional iron absorption measured in both studies. As expected, hepcidin was positively correlated with ferritin ($r = 0.46$, $P = 0.005$; data not shown). Highly significant correlations with the fractional absorptions were found between FePP and ASP-p ($r = 0.92$, $P < 0.0001$) in

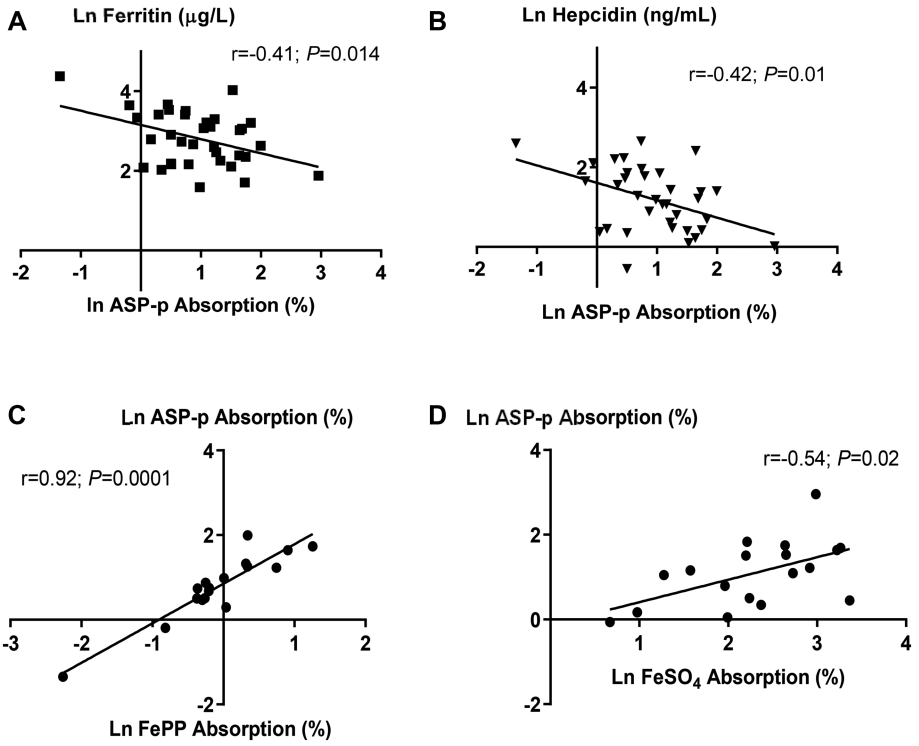

FIGURE 4 Pearson's correlations of ASP-p fractional absorption with ferritin (A) and hepcidin (B) concentrations and with FePP (C) and $FeSO_4$ (D) fractional absorptions in women provided test meals. Data from studies I and II were combined when the correlations were made with ASP-p absorption with hepcidin and ferritin. ASP-p, *Aspergillus oryzae* grown in ferric pyrophosphate; FePP, ferric pyrophosphate.

study I and $FeSO_4$ and ASP-p ($r = 0.52$, $P < 0.02$) in study II (Figure 4C).

Discussion

The female subjects in our study absorbed only 28% of $FeSO_4$ from the rice-vegetable meal fortified with the ASP-p bouillon (study 2; Figure 3B), a much lower absorption than we reported in our previous study. In our earlier study (13), iron absorption from the iron-enriched ASP-s (15%) was not significantly different from $FeSO_4$ (17%) when provided in a liquid-formula meal to women. It should be noted that the current study and the previous study used 2 different iron sources to grow the Ao. While ASP-s grown in $FeSO_4$ may be a better natural iron source for supplementation, it was incompatible with bouillon due to sensory problems limiting its use in fortification. Growing Ao in FePP overcame the sensory problems. Furthermore, Ao was able to absorb insoluble FePP efficiently, storing 8–10% of iron, similar to iron levels with ASP-s (12, 13). One possible explanation for the difference in relative iron absorption from ASP-s and ASP-p is that Ao absorbed and stored iron from the readily soluble $FeSO_4$ by a different mechanism compared with the water-insoluble FePP. Based on fungal iron metabolism, Ao grown with $FeSO_4$ and FePP probably takes up iron by using low- and high-affinity pathways and stores iron in vacuoles and siderophores, respectively (21). Another reason for the difference in relative iron absorption between ASP-s and ASP-p could be the different meals used in the 2 studies (semipurified liquid meals for ASP-s vs. a rice-vegetable meal for ASP-p) (13). The negative correlation of ASP-p absorption with ASP-p absorption with ferritin and hepcidin and a positive correlation with FePP and $FeSO_4$ absorption suggest that absorption of ASP-p is regulated similarly to $FeSO_4$ and FePP.

Most bouillon cubes are currently fortified with FePP because, unlike other widely used iron fortificants, it causes no sensory problems. The low absorption (<1%) we found with FePP was in agreement with a previous study (7) and ASP-p absorption was significantly higher than FePP, suggesting that fungal iron is protected from the food matrix. Although we did not measure the absorption of FePP and $FeSO_4$ in the same subjects, based on insignificant differences in ferritin values (18 and 15 μg/L in study I and II) and similar absorption of ASP-p in the 2 studies (<3%) the relative biological value (RBV) of FePP was 11% of $FeSO_4$, which is in the same order as the RBV reported in previous studies (6, 7), compared with an RBV of 28% with ASP-p. In human isotope-absorption studies the RBV of FePP varies with the composition of the meal and the iron status of the subjects and has been reported to be as low as 13–15% of $FeSO_4$ in liquid bouillon drink and fortified rice (6). The addition of trisodium citrate and citric acid during the rice extrusion process increased iron absorption from FePP due to the hot extrusion process that transformed the insoluble FePP into more soluble FePP citrate complexes (22). Unfortunately, no such heat treatment is used to manufacture bouillon cubes to be able to use citric acid or trisodium citrate. Not much success was found by adding tetra sodium pyrophosphate to FePP-fortifed bouillon cube broth since the RBV of FePP only increased from 13% to 19% (6). These studies suggest that it is difficult to significantly improve the absorption of FePP, the iron compound that is currently used for fortifying bouillon, which increases the need to find new strategies to improve iron fortification of bouillion.

The low absorption of FePP supports the conclusion of Cercamondi et al. (6) that the current iron-fortified bouillon cubes provide little additional bioavailable iron to a normal diet. These authors estimated that bouillon fortified with FePP contributes to 2–9% of the iron Estimated Average Requirement (EAR) (23) for women of childbearing age compared with 4–17% if cubes were fortified with $FeSO_4$. Our study suggests that women of childbearing age, with SF concentrations of 15 μg/L and consuming 6.6 g bouillon with 4.2 mg Fe/d, could meet 3.4% and 8.2%, respectively, of their EAR with FePP and ASP-p. Although the amount of iron absorbed from ASP-p is 2.3-fold higher than that from FePP, it is still much lower than that from $FeSO_4$, which, with ~10% iron absorption could provide >30% of the EAR. Unfortunately, $FeSO_4$ is well known to provoke unacceptable color or flavor changes in foods and is not suitable for bouillon fortification (5). With an iron absorption of ~3% from ASP-p, and a doubling of the iron-fortification level, it should be possible to provide 15–20% of the EAR for women of childbearing age.

Because of the relatively low consumption of bouillon cubes, they cannot alone provide all the iron needed for a fortification program. Nevertheless, iron-fortified bouillon cubes could make a useful contribution to a fortification program along with other fortified staple foods such as wheat, maize, or rice.

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