High Bioavailability from Ferric Pyrophosphate-Fortified Bouillon Cubes in Meals is Not Increased by Sodium Pyrophosphate: a Stable Iron Isotope Study in Young Nigerian Women

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ABSTRACT

Background: It is challenging to find an iron compound that combines good bioavailability with minimal sensory changes when added to seasonings or condiments. Ferric pyrophosphate (FePP) is currently used to fortify bouillon cubes, but its bioavailability is generally low. Previously, the addition of a stabilizer, sodium pyrophosphate (NaPP), improved iron bioavailability from a bouillon drink.

Objective: We assessed whether there is a dose-response effect of added NaPP on iron bioavailability from local meals prepared with intrinsically labeled FePP-fortified bouillon cubes in young Nigerian women using iron stable isotope techniques.

Methods: In a double-blind, randomized, cross-over trial, women ($n = 24$; aged 18–40 y; mean BMI 20.5 kg/m^2) consumed a Nigerian breakfast and lunch for 5 d prepared with bouillon cubes containing 2.5 mg ^{57}Fe (as FePP) and 3 different molar ratios of NaPP: ^{57}Fe (0:1, 3:1, and 6:1). Iron bioavailability was assessed by measuring ^{57}Fe incorporation into erythrocytes 16 d after each 5 d NaPP: ^{57}Fe feeding period. Data were analyzed using a linear regression model of log iron absorption on NaPP ratio, with body weight and baseline body iron stores as covariates and subject as a random intercept.

Results: Of the women included, 46% were anemic and 26% were iron deficient. Iron bioavailability was 10.8, 9.8, and 11.0% for the 0:1, 3:1, and 6:1 NaPP:^{57}Fe treatments, respectively. There was no dose-response effect of an increasing NaPP:^{57}Fe ratio ($\beta \pm$ SE: 0.003 ± 0.028, $P = 0.45$).

Conclusions: In this study, the addition of NaPP did not increase iron bioavailability from FePP-fortified bouillon cubes. However, iron bioavailability from the Nigerian meals prepared with FePP-fortified bouillon cubes was higher than expected. These results are encouraging for the potential of bouillon cubes as a fortification vehicle. Further studies are needed to assess the effect of FePP-fortified bouillon cubes on improving iron status in low-income populations. This trial was registered at clinicaltrials.gov as NCT02815449. *J Nutr* 2019;149:723–729.

Keywords: iron, bouillon cubes, bioavailability, isotopes, Nigeria, women

Introduction

Two billion people worldwide have vitamin and mineral deficiencies (1). In 2008, it was estimated that almost 25% of the world's population is suffering from anemia, of which iron deficiency (ID) is the most significant common cause (2). It is estimated that 35–65% of the anemia burden in low- and middle-income countries is attributable to ID. The prevalence of anemia is highest in the Central and West African region at 48% in nonpregnant women and 56% in pregnant women. In

Nigeria, nearly 50% of women of reproductive age are anemic (3).

Studies show that iron fortification can be an effective strategy against nutritional ID (1). Condiments, spices, seasonings, and bouillon cubes are increasingly recognized as suitable food vehicles for fortification as they are widely consumed on a regular basis and are accessible and affordable for low-income consumers including those who are living in remote areas (4, 5). Moreover, a recent systematic review has demonstrated that iron fortification of condiments is associated with increased

hemoglobin concentrations, improved iron status, and reduced anemia across targeted populations (6).

For iron fortification of bouillon cubes, ferric pyrophosphate (FePP) has been the proposed form of iron, but technical challenges, such as sensory changes and segregation were still expected (1). Previously, tetra sodium pyrophosphate (NaPP), a common food additive which can form soluble complexes with iron, has been found to overcome these technical challenges, resulting in a more stable product. The bioavailability of FePP tends to be low in humans (1), however, a recent study showed that NaPP can also help to increase the bioavailability of FePP. In that study, conducted in Swiss women with a low iron status, the addition of NaPP to FePP in a bouillon drink improved relative iron bioavailability by 46% compared with FePP alone (7). However, this study did not take habitual meal consumption into account and therefore the influence of dietary factors could not be assessed. In West Africa, bouillon cubes are most often used in stews that are consumed with staple foods, such as rice, sorghum, and yam and may therefore contain large amounts of phytate and polyphenols which are known to inhibit iron absorption. On the other hand, there may be dietary factors present in these meals such as vitamin C and muscle tissue that are known to enhance the uptake of iron (8, 9). Furthermore, it would be of interest to assess whether the bioavailability of FePP could be further improved by adding more NaPP in order to establish an optimized NaPP: Fe ratio for bioavailability. In the study by Cercamondi et al. (7), molar ratios of NaPP: Fe of 1:1 and 0:1 were assessed. However, in vitro data assessing the dissolution behavior of FePP in the presence of NaPP showed that ratios of NaPP: Fe above 1:1 improved the solubility of FePP, suggesting that iron absorption could be further improved because multiple pyrophosphate ligands per iron cation would be available to form soluble complexes (10).

Therefore, the current study was designed to assess a dose-response effect of NaPP on the bioavailability of iron from FePP-fortified bouillon cubes with 3 different molar ratios of NaPP: Fe (0:1, 3:1, and 6:1) in Nigerian women consuming a typical Nigerian diet, using stable isotope techniques. Secondly, the iron bioavailability of the 3 treatments was assessed.

Methods

Subjects

The study was conducted at Obafemi Awolowo University, Ile Ife, in Nigeria. Women were recruited by means of leaflets and posters distributed and posted in the surroundings of the research site, such as hostels for females, central market, students' union building area, parks, and gardens. In an information meeting by the study staff, women were informed about the screening and study procedures and were asked to provide consent before participation in the screening. Women ($n = 161$) were screened for eligibility using the following inclusion criteria: age 18–40 y, body weight $\leq$65 kg, serum ferritin concentration <15 μg/L, apparently healthy as judged by a physician and willingness to participate in the study. Women were excluded if they

did >10 h/wk of intensive sport, consumed >14 units of alcohol per wk, donated blood, had significant blood loss in the past 6 mo, or were severely anemic (hemoglobin <80 g/L), reported use of any medically or self-prescribed diet, used medication (except oral contraceptives), used vitamin or mineral supplements <2 wk before the study start or during the study, smoking $\leq$2 wk before the study start or during the study, were pregnant or planning pregnancy during the study period, were lactating $\leq$6 wk before the study start or during the study or had known gastrointestinal or metabolic disorders. Subjects who were found eligible were asked to give a second written informed consent for agreeing to participate in the study and subsequently received a deworming drug (albendazole [Zentel], 400 mg, single dose), 7 d before the start of the study. Out of the eligible and dewormed women, the first 24 subjects who came to the randomization visit of the study were finally included in the trial. The intervention period was May–July 2017.

The study was reviewed and approved by the Health Research Ethic Committee (HREC) Institute of Public Health, Obafemi Awolowo University, Ile-Ife, Nigeria. The study has been registered at the US National Library of Medicine (https://www.clinicaltrials.gov, NCT02815449; 28 June 2016). The study conduct was monitored at the study site by OnQ Research PTY LTD (Johannesburg, South Africa) throughout the screening and intervention phase.

Design

The study followed a double-blind, randomized, full cross-over design, in which each woman served as her own control and the 3 treatments were given in a random order. Women received a breakfast and lunch meal for 5 consecutive days that were prepared with a bouillon cube containing 1 of 3 treatments of ascending molar ratios of NaPP: Fe, including 0:1, 3:1, and 6:1. Subjects arrived having fasted in the morning to the study site to consume breakfast between 0900 and 0930 and stayed on site where they consumed lunch between 1300 and 1330. Meals were consumed under the full supervision of study staff. Leftover quantities were assessed by weighing plates before and after consumption of the meal. Subjects were allowed to drink water between and after meals and were instructed not to eat within 3 h after meal consumption. After the 5-d period of meal consumption, subjects had a period of rest for 16 d, followed by a second and third period of 5-d meal consumption and 16 d of rest, until all subjects had received all 3 treatments. Thus, subjects received the same series of 10 meals with each of the 3 treatments during each period, in total 30 meals. On the first day of each treatment period and at the end of the study, a blood sample was drawn and weight and height of the women were measured. Safety and tolerability of the study products and meals were assessed on the days that the subjects visited the study site by monitoring adverse events according to predefined criteria on severity and relatedness.

Preparation of meals

Typical Nigerian meals were prepared for breakfast and lunch following a menu for the 5-d period of consumption (see **Supplemental Table 1**). Both breakfast and lunch consisted of a staple dish (such as rice, corn, bread, or yam), a stew made of bouillon cubes, tomatoes, red peppers, and a piece of beef, fish, or egg. The lunches also included a vegetable dish with amaranth leaves. Preparation of the meals was strictly separated by treatment to avoid any cross-contamination among the different treatments, using physically separated kitchen facilities and color-coded pots, and utensils in line with the color codes for the treatments. Per treatment and meal occasion, a total of 9 portions were prepared, of which 8 had to be consumed by the study participants and one portion was left for food composition analyses. Thus, in total, 27 portions were prepared per meal occasion, of which 24 were for consumption by the study participants and 3 were for food composition analyses.

Production of isotopically labeled bouillon cubes

Isotopically labeled ^{57}FePP in powder form (particle size D[4,3]: 18.1 μm) was prepared by Paul Lohmann GmbH from isotopically enriched elemental iron (^{57}Fe-metal: 96.7% enriched; Chemgas).

Bouillon cubes were made in a food-grade kitchen at lab scale at Unilever's Research & Development laboratory in Vlaardingen, The Netherlands, from ingredients that were sourced from Nigeria to closely match the commercial Nigerian consumer product. Bouillon cubes were produced as in the equivalent commercial product (salt, sugars, starch, vegetable fats, herbs, and spices) in a dry-blending protocol at 300 g scale. Ingredients were added in order of quantity required, starting with the largest amount by weight (salt), to a small stainless-steel bowl blender with K-mixer (Kenwood). NaPP ($Na_4P_2O_7$; Innophos) at 0, 36, or 72 mg/cube and ^{57}Fe at 2.52 mg/cube in the form of FePP were added in the final blending step, resulting in a 0:1, 3:1, and 6:1 molar ratio for equivalents NaPP: ^{57}Fe. Blend homogeneity was monitored by visual inspection. From the blend, 4,000 mg was accurately weighed (± 10 mg at maximum) and transferred to a stainless-steel die for immediate pressing with a fixed end-pressure to result in bouillon cubes (14 mm). Dose and uniformity of dose were checked via the total cube weight variation and by multiple, randomly selected cube iron analysis before final release. Per treatment type, bouillon cubes were packed and coded blind for the investigators.

Measurements of iron in the bouillon cubes at the end of the intervention

To measure the iron concentration (^{57}Fe) in the bouillon cubes, remaining sachets with bouillon cubes from the study site in Nigeria, still coded, were shipped to Eidgenössische Technische Hochschule, Zürich, Switzerland to measure the amount of ^{57}Fe in the bouillon cubes. For each treatment, 9 bouillon cubes were weighed and ground. Triplicate 4 g samples of the ground bouillon cubes were taken for ^{57}Fe content analysis. Samples were boiled in 40 mL hydrochloric acid (16%) for 2 h, followed by evaporation to near dryness; 30 mL of nitric acid (65%) was then added to the residues, and the mixtures were boiled for 14 h, followed by the addition of 5 mL of hydrogen peroxide (30%) and further boiling for 2 h. The solutions were then diluted to 50 g with water. Iron concentration in aliquots of the mineralized samples was determined by flame atomic absorption spectrometry. To determine the iron isotopic composition, 3 g aliquots of the mineralized samples were evaporated to dryness, dissolved in 2 mL hydrochloric acid (6 M), and iron was isolated by anion-exchange chromatography and a subsequent precipitation step with ammonium hydroxide. Iron isotope ratios were measured by a multicollector-inductively coupled plasma-mass spectrometer (MC-ICP-MS). For each treatment, iron concentration and isotopic label abundance of the bouillon cubes were expressed as means $\pm$ SDs per 4 g bouillon cube and used for the calculation of iron bioavailability.

Blood sample analysis

During the screening, a venipuncture was taken using the Becton-Dickinson vacutainer device and blood was collected into K_2EDTA tubes (3 mL) for analysis of blood hemoglobin and in plain tubes (3 mL) for analyses of serum ferritin and C-reactive protein (CRP). Blood hemoglobin was measured within 2 h of collection using the SFRI H18 Light Automated Hematology analyser at the research site. The samples for serum ferritin and CRP analyses were centrifuged within 2 h of collection at 2,000 g for 15 min and 2 aliquots with serum of 500 μL each were labeled and stored at $-20\,°C$. Within 2 d of collection, serum ferritin and serum high-sensitive CRP (HS-CRP) were determined using ELISA kits (Monobind Inc.) at the research site. Anemia was defined as hemoglobin <120 g/L, ID was defined as serum ferritin <15 μg/L [11]. Expected HS-CRP concentrations for healthy individuals was <5 mg/L [12].

During the intervention, a venipuncture was taken using the Becton-Dickinson vacutainer device and blood was collected into K_2EDTA tubes (3 mL) for analysis of blood hemoglobin, in plain tubes (3 mL) for analyses of serum ferritin, soluble transferrin receptors, CRP and α1-acid glycoprotein (AGP), and in lithium heparin tubes (4 mL) for iron isotopes in blood. Blood hemoglobin was determined at the research site as described above. For analyses of the biomarkers in serum, samples were centrifuged within 2 h of collection at 2,000 g for 15 min and aliquoted into 4 samples of 200 μL each, which were labeled and stored

at $-20\,°C$. For analyses of iron isotopes, 2 aliquots of 200 μL each were labeled and stored at $-20\,°C$. At the end of the study, serum and whole blood aliquots were shipped on dry ice to ETH Switzerland for analyses. Serum ferritin, soluble transferrin receptors, AGP, and CRP were measured using a combined Sandwich ELISA technique [13]. ID was defined as serum ferritin <15 μg/L and/or serum soluble transferrin receptor concentrations >8.3 mg/L [11]. Expected AGP and CRP concentrations for healthy individuals were <1 g/L and <5 mg/L, respectively [12]. Body iron stores (BIS) were calculated from serum ferritin concentration, transferrin receptors, and body weight following the Cook formula [14].

Each isotopically enriched blood sample was analyzed in duplicate for its isotopic composition. Whole blood was mineralized by microwave digestion, and iron was separated by anion-exchange chromatography and a subsequent precipitation step with ammonium hydroxide [15]. Iron isotope ratios were determined by an MC-ICP-MS instrument (CV of independent blood samples was 0.008%) (NEPTUNE, Thermo Finnigan) [15].

Calculation of iron bioavailablity

The concentration of ^{57}Fe-labeled isotopes in the blood were calculated based on the shift in iron isotope ratios in erythrocytes and the estimated amount of iron circulating in the body. The latter was calculated based on the blood volume estimated from height and weight [16], the measured hemoglobin concentration, and the iron content of hemoglobin of 3.47 mg Fe/g [16–18].

Anthropometric measurements

Height was recorded to the nearest cm using a stadiometer fixed to a wall and weight was recorded to the nearest kg using a Omron Digital Personal Scale HN289 Model weighing scale.

Food composition analysis of the meals

After preparation and plating out of the meals, the ninth meal was kept for food composition analyses. Each meal component of this meal was weighed, and duplicate samples of 25% of the weight were taken and put in a plastic sampling bag. After samples of all meal components were added, bags were flattened to remove the air, hermetically sealed with tape, and labeled. Subsequently, the sample in the plastic bag was added into an aluminum bag. In total, there were 90 food samples of 100–150 g each in duplicate (for each meal, each treatment, and each period). Samples were stored in a freezer at $-30\,°C$ at the study site and after the end of the intervention shipped on dry ice to The Netherlands. Nine composite samples were made containing all 10 meals of 1 treatment per period. The 10 meal samples were therefore thawed and mixed and ground together. Subsequently, samples were freeze-dried and analyzed for the mineral content of iron, zinc, and calcium using Inductive Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (ISO-16,943) at SGS Nederland BV, Spijkenisse, The Netherlands. Vitamin C content was determined by measuring ascorbic acid and dehydroascorbic acid after treatment with DL-homocysteine by Liquid Chromatography With Diode Array Detection (LC-DAD) at Institut Kuhlmann GmbH Analytik-Zentrum Ludwigshaven, Germany. Phytate was measured by spectrophotometric analysis of inositol phosphorus using the Megazyme K-PHYT kit. A factor of 6 was applied to estimate phytate from inositol phosphorus. Mean energy and heme iron content of the meals were calculated using the Nigerian and West African Food Composition tables [19, 20] and the USDA nutrient database.

Statistics

The sample size was calculated based on data from a previous study with iron-fortified bouillon cubes [7] which showed a mean difference in iron bioavailability of 2% between NaPP: Fe ratios 0:1 and 1:1 with a residual SD of 0.242 and a correlation between log iron bioavailability and NaPP ratio of 0.85. Data from this study were used to simulate linear dose-response effects on log transformed iron bioavailability of a range of NaPP: Fe ratios from 0 to 6, using an SD inflated by 10% over the observed SD. The simulation showed that 8 subjects would be needed in order to have a 0.9 chance of detecting a significant result
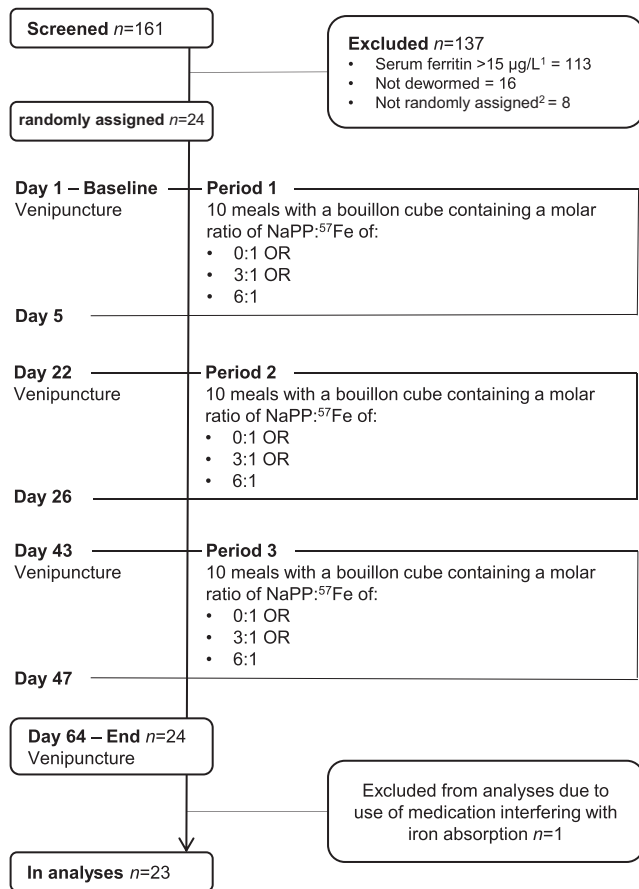
FIGURE 1 Flow chart and study design. [1]Based on serum ferritin analyses measured during the screening in Nigeria. [2]The first 24 women that were eligible were included in the study, women who showed up later were therefore not randomly assigned. NaPP, sodium pyrophosphate.

using a 0.05 significance level. In order to balance for ratio order (which needs 9 subjects) and first-order carry over effects (which needs 18 subjects) and to allow for possible drop outs, a sample of 24 women was recommended following Cook et al. (14).

The outcomes on iron bioavailability were log transformed and the primary outcome on the dose-response effect was analyzed using a mixed model analysis of variance, where the ratio of NaPP: ^{57}Fe was a continuous linear effect with subject as a random effect and day of treatment and ratio of stabilizer to FePP as fixed effects. Subject body weight and baseline BIS on day 1 were used as covariates. The use of CRP and AGP as covariates was also investigated but these were not retained in the model because they did not have a significance level <0.05. For secondary analyses, the same model was used except that the ratio of NaPP: ^{57}Fe was entered as a categorical rather than continuous effect. Because the outcomes of the per protocol and intention-to-treat analyses were similar, we decided to present only the outcomes of the intention-to-treat analyses in this article.

Results

In total, 161 women were screened for eligibility of which 137 were excluded because they did not meet the study criteria, did not show up for deworming, or did not show up during the first day of the study (see **Figure 1** for study flow chart). Twenty-four women were included in the study of which 1 was excluded from the statistical analyses as she used medication which could interfere with iron absorption and did not consume

all study meals. Of the 23 women that were included in the statistical analyses, 21 were 100% compliant in completing meal consumption.

The women were on average 22.8 y old with a range of 18–35 y and had a BMI of 20.5 kg/m^2 (see **Table 1** for baseline characteristics). None of the women were overweight or obese, but 46% of the subjects were anemic, 26% had ID (serum ferritin <15 μg/L or serum soluble transferrin receptor >8.3 mg/L), and 17% had BIS <0 mg/kg bodyweight. Only 1 of the women had increased markers of infection (CRP >5 mg/L and AGP >1 g/L).

Analysis of the bouillon cubes revealed that the total Fe and ^{57}Fe content closely met with the targeted value of 2.52 mg Fe with little variation between the cubes intended for the different treatments and study periods (see **Table 2**). Details of the nutritional composition of the meals can be found in **Table 3**. The calculated energy content of the breakfasts ranged from 1.8 to 2.6 MJ and that of lunches from 3.6 to 4.0 MJ with an average 0.66 MJ/100 g meal. Meals included on average 54 g muscle tissue from beef or fish per meal, which would provide 0.25 mg heme iron per 100 g meal (data calculated from food composition tables). Food composition analyses showed ranges of 1.4–2.0 mg/100 g meal for total iron, 21–30 mg/100 g for calcium, and 0.8–1.4 mg/100 g for zinc in the 9 meal composites of the different treatments and study periods. Analyzed phytate content was on average 43 mg/100 g meal and ranged from 27 to 56 mg/100 g meal for different treatments and study periods. The molar ratio of phytate: total iron was 2.0:1 on average in the meals. Vitamin C concentrations were below the detection limit of <1 mg/100 g meal.

There was no dose-response effect of NaPP on the bioavailability of FePP ($\beta \pm$ SE: 0.003 ± 0.028, $P = 0.45$). Iron bioavailability from FePP-fortified bouillon cubes was not significantly different among the 0, 3, and 6 NaPP: ^{57}Fe ratios (see **Figure 2**), with an average for all 3 treatments of 10.5% ranging from 3.4 to 39.1%.

TABLE 1 Baseline characteristics of study participants

	N	Mean ± SD
Age, y	24	22.8 ± 3.9
Height, m	24	1.59 ± 0.05
Weight, kg	24	52.0 ± 7.0
Body mass index, kg/m^2	24	20.5 ± 2.3
Blood hemoglobin, g/L	24	120.9 ± 8.4
Serum ferritin, μg/L	23	30.08 ± 20.65
Serum transferrin receptor, mg/L	23	6.27 ± 1.38
Body iron stores, mg/kg BW	23	3.54 ± 20.65
Serum CRP, mg/L	23	0.30 (0.22, 0.45)[1]
Serum AGP, mg/L	23	0.55 ± 0.19

[1]Median (25th, 75th percentiles).

TABLE 2 Total iron, isotopic label, and NaPP concentrations of the 3 different types of intrinsically ^{57}Fe-labeled bouillon cubes[1]

Bouillon cube treatment type (molar ratio NaPP: ^{57}Fe)	Total iron (mg Fe/cube)	Isotopic label ^{57}Fe concentration (% of total iron)
0:1	2.54 ± 0.06	94.3 ± 0.04
3:1	2.56 ± 0.04	94.4 ± 0.04
6:1	2.59 ± 0.03	94.3 ± 0.05

[1]Values are means ± SD; $n = 3$ independent samples. FePP, ferric pyrophosphate; NaPP, sodium pyrophosphate.

TABLE 3 Composition of dietary factors influencing iron bioavailability of the 10 study meals by treatment and study period[1]

Bouillon cube treatment (molar ratio NaPP: [57]Fe)	Iron (mg/100 g)	Calcium (mg/100 g)	Zinc (mg/100 g)	Phytate (mg/100 g)	Phytate:iron (molar ratio)
Period 1					
0:1	1.9	27	1.1	45	2.0:1
3:1	2.0	29	1.1	54	2.2:1
6:1	1.8	26	1.1	37	1.7:1
Period 2					
0:1	2.2	30	1.4	47	1.8:1
3:1	1.4	21	0.8	53	3.1:1
6:1	1.6	22	0.9	31	1.7:1
Period 3					
0:1	2.0	29	1.2	56	2.4:1
3:1	1.6	25	0.9	37	2.0:1
6:1	1.8	24	1.0	27	1.3:1

[1] Based on calculations from food composition tables, meals contained per 100 g on average 660 KJ and 11.8 g muscle tissue, which would provide 0.25 mg heme iron. For all meal samples of different treatments and periods, vitamin C values were below the detection limit of <1 mg/100 g. NaPP, sodium pyrophosphate.

Baseline BIS strongly and inversely correlated with iron bioavailability in both the primary and secondary analyses ($\beta \pm$ SE: -0.0964 ± 0.0266, $P = 0.0008$ for both models), suggesting that an increase of 1 mg/kg bodyweight in BIS would decrease iron bioavailability by 9.2% (see **Figure 3**).

Discussion

The current study showed that the bioavailability of iron from meals prepared with FePP-fortified bouillon cubes was on average 10.5% and that increasing amounts of NaPP added to these meals did not result in a significantly higher iron bioavailability. The bioavailability of iron in this study was higher than found in our previous study with a FePP-fortified bouillon drink; in that study, iron bioavailability was 4.4% and increased to 6.4% when NaPP was added in a 1:1 molar ratio to iron (7). There are several differences that may explain these

divergent findings. First, in the current study the iron-fortified bouillon cubes were provided with typical Nigerian meals that contain dietary factors which influence iron absorption compared with a bouillon drink in the previous study. Although the vitamin C content of the meals could not be detected, most meals contained a piece of meat or fish which could have had a positive effect on iron absorption (21). Furthermore, the molar ratio of phytate to iron in the meals was on average 2:1, which is well below the recommended <6:1 for composite meals to improve iron absorption (8). Therefore, the Nigerian meals provided in our study most likely had an overall positive impact on the bioavailability of iron. Secondly, solid foods have a longer gastric transit time than bouillon drinks and therefore there may have been more time for the FePP to dissolve in the gastric acid, resulting in higher iron absorption. Thirdly, the designs of the two studies differed; the current study was a multiple meals isotope study with 10 meals with [57]Fe provided over a period of 5 d, whereas Cercamondi et al. (7) conducted a single meal isotope study. Another multiple meal study also found relatively high bioavailability of iron from composite pearl
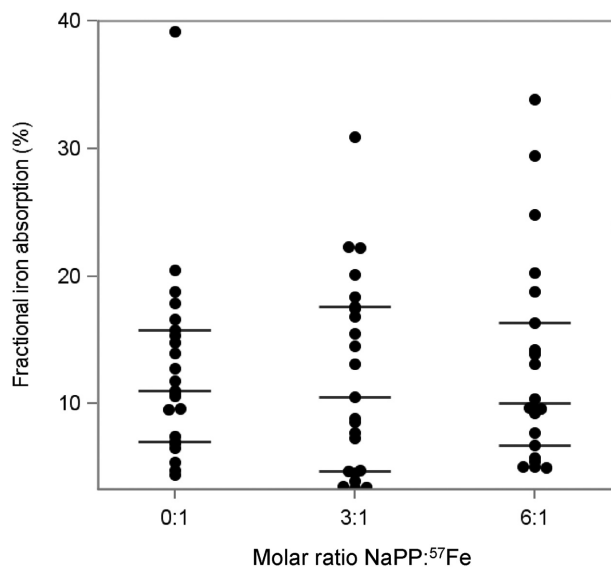

FIGURE 2 Bioavailability of FePP (geometric means (95% CI, $n = 23$) from meals prepared with bouillon cubes with 3 different ratios of NaPP:[57]Fe including 0:1, 3:1, and 6:1. NaPP, sodium pyrophosphate.
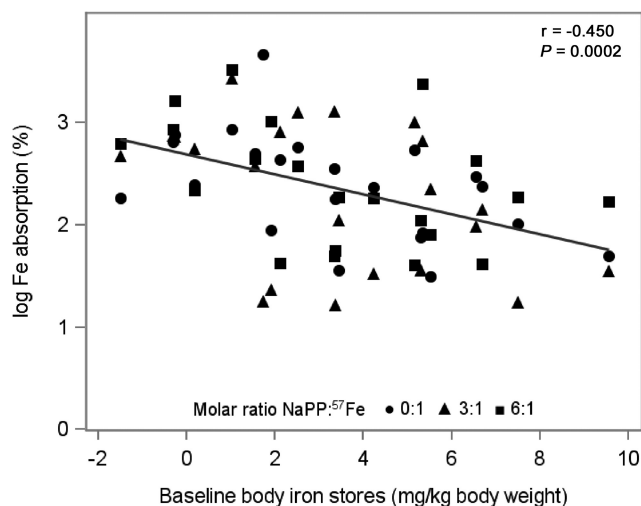

FIGURE 3 Relation between baseline body iron stores of study participants ($n = 23$) and log iron bioavailability (%) from meals prepared with bouillon cubes with 3 different ratios of NaPP:[57]Fe. NaPP, sodium pyrophosphate.

millet meals (22) compared with a single meal radio isotope study with similar meals (23). However, it is currently unclear how factors such as study design, food matrix, iron status, and study population would lead to differential effects on iron absorption. The study population was largely Caucasian in the Cercamondi study (7). Even though the West-African women in the current study were apparently healthy, almost 50% of them were anemic and some may have been heterozygote for genetic hemoglobin disorders such as thalassemia given the high prevalence of thalassemia in the African region (24). Both anemia and some heterozygote thalassemia types have been shown to upregulate iron absorption and could therefore explain the higher iron absorption observed in this study (25).

The bioavailability of iron found in this study is more than double that reported in other studies in women consuming test meals containing labeled FePP, where bioavailability was 0.3–3.3%. These studies were all single meal isotope studies and were conducted with a range of different food matrices (26–33). In two of these studies, the addition of vitamin C in a molar ratio of 4 vitamin C:1 iron has been shown to double the iron bioavailability from FePP to 2.3–5.8% (27, 28), which is still lower than the iron bioavailability of 10.5% found in the current study. (34, 35)

Our study did not confirm the enhancing effect of NaPP on the bioavailability of FePP which was found by Cercamondi et al. (7). It may be that dietary factors known to influence iron absorption overcome the enhancing effect of NaPP. In fact, these dietary factors were present in abundance in the meals compared with NaPP. This finding implies that the addition of NaPP may only improve the bioavailability of FePP for products consumed under conditions with minimal influence of diet. However, NaPP remains important to ensure optimal sensory, technical, and stability properties of FePP-fortified bouillon cubes.

Our study has several strengths. We chose a multiple dose design with 10 typical Nigerian meals which would best assess the effect of iron fortification in a real-life situation. Cumulative bioavailability over 10 meals takes into account the influence of day-to-day subject physiological variations and meal differences that could affect iron absorption. Another strength of the study was the accurate intrinsic labeling which has the advantage that the isotope iron is incorporated into bouillon cubes in the same way as the FePP in the native fortified bouillon cubes that are on the market (36). Finally, the study had a good compliance with 91% of the subjects consuming 100% of the study meals.

A limitation was that upon study completion it was found that screening visit serum ferritin values did not match with those of the blood samples taken at baseline and the end of the intervention, which were analysed at the ETH laboratory in Zurich. Based on ETH data only, 26% of the subjects were iron deficient and 46% had anemia, which is similar to figures reported by the latest food and nutrition survey for Nigeria (37). Because of the strong inverse association between BIS and iron bioavailability, also found in our study, it is likely that iron bioavailability would have been even higher than 10.5% if all study participants were iron deficient as targeted for during screening. Furthermore, it could be argued that Nigerian meals, as provided in our study, may not be representative of Nigerian populations from lower socio-economic backgrounds, consuming monotonous diets, high in phytate, with almost no meat or fish. In fact, the consumption of poultry, meat, and seafood varies significantly across Nigeria, with limited consumption in the north (34, 35).

In conclusion, the results of the current study are encouraging for the potential effect of FePP-fortified bouillon cubes on improving iron status in low- and middle-income countries. As bouillon cubes are consumed on a regular basis in both urban and rural areas in Africa, these are suitable vehicles to also reach populations most vulnerable to micronutrient deficiencies (4, 5, 38). The current study showed a relatively high iron bioavailability, at 10.5%, of FePP-fortified bouillon cubes in typical Nigerian meals, with pieces of meat and fish and containing vegetables which could have provided vitamin C. Iron bioavailability may be lower in meals that are largely based on staple foods. Future research could therefore focus on assessing the efficacy and effectiveness of FePP-fortified bouillon cubes on improving iron status in populations from lower socio-economic backgrounds with low iron intake.

References

1. Allen L, de Benoist B, Dary O, Hurrell R, editors. Guidelines on Food Fortification with Micronutrients. Geneva: World Health Organization, Food and Agriculture Organization of the United Nations; 2006.

2. de Benoist B, Mclean E, Egli I, Cogswell M. WHO Global Database on Anaemia: Worldwide Prevalence of Anaemia 1993–2005. Geneva: World Health Organization; 2008.

3. Stevens GA, Finucane MM, De-Regil LM, Paciorek CJ, Flaxman SR, Branca F, Peña-Rosas JP, Bhutta ZA, Ezzati M. Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995–2011: a systematic analysis of population-representative data. Lancet Glob Health 2013;1:e16–25.

4. Garcia-Casal MN, Pena-Rosas JP, Malave HG. Sauces, spices, and condiments: definitions, potential benefits, consumption patterns, and global markets. Ann N Y Acad Sci 2016;1379:3–16.

5. Engle-Stone R, Ndjebayi AO, Nankap M, Brown KH. Consumption of potentially fortifiable foods by women and young children varies by ecological zone and socio-economic status in Cameroon. J Nutr 2012;142:555–65.

6. Osendarp SJM, Martinez H, Garrett GS, Neufeld LM, De-Regil LM, Vossenaar M, Darnton-Hill I. Large-scale food fortification and biofortification in low- and middle-income countries: a review of programs, trends, challenges, and evidence gaps. Food Nutr Bull 2018;39:315–31.

7. 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.

8. Hurrell R, Egli I. Iron bioavailability and dietary reference values. Am J Clin Nutr 2010;91:1461S–7S.

9. Casgrain A, Collings R, Harvey LJ, Hooper L, Fairweather-Tait SJ. Effect of iron intake on iron status: a systematic review and meta-analysis of randomized controlled trials. Am J Clin Nutr 2012;96:768–80.

10. Tian T, Blanco E, Smoukov SK, Velev OD, Velikov KP. Dissolution behaviour of ferric pyrophosphate and its mixtures with soluble pyrophosphates: potential strategy for increasing iron bioavailability. Food Chem 2016;208:97–102.

11. World Health Organization. Iron Deficiency Anemia: Assessment, Prevention and Control. Geneva: World Health Organization; 2001.

12. Dati F, Schumann G, Thomas L, Aguzzi F, Baudner S, Bienvenu J, Blaabjerg O, Blirup-Jensen S, Carlstrom A, Petersen PH, et al. 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). International Federation of Clinical Chemistry. Community Bureau of Reference of the Commission of the European Communities. College of American Pathologists. Eur J Clin Chem Clin Biochem 1996;34:517–20.

13. Erhardt JG, Estes JE, Pfeiffer CM, Biesalski HK, Craft NE. Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and C-reactive protein by an inexpensive, sensitive, and simple sandwich enzyme-linked immunosorbent assay technique. J Nutr 2004;134:3127–32.

14. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. Blood 2003;101:3359–64.

15. 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.

16. Brown E, Hopper J, Jr, Hodges JL, Jr, Bradley B, Wennesland R, Yamauchi H. Red cell, plasma, and blood volume in the healthy women measured by radiochromium cell-labeling and hematocrit. J Clin Invest 1962;41:2182–90.

17. Kastenmayer P, Davidsson L, Galan P, Cherouvrier F, Hercberg S, Hurrell RF. A double stable isotope technique for measuring iron absorption in infants. Br J Nutr 1994;71:411–24.

18. Hosain F, Marsaglia G, Noyes W, Finch CA. The nature of internal iron exchange in man. Trans Assoc Am Physicians 1962;75:59–63.

19. Stadlmayr B, Charrondiere UR, Enujiugha VN, Bayili RG, Fagbohoun EG, Samb B, Addy P, Barikmo I, Ouattara F, Oshaug A, et al. West African Food Composition Table. Rome: Food and Agriculture Organization of the United Nations; 2012.

20. Oguntona EB, Akinyele IO, editors. Nutrient Composition of Commonly Eaten Foods in Nigeria - Raw, Processed and Prepared. Ibadan: O B T Printing Ventures Ibadan; 1995.

21. Lynch SR, Hurell RF, Dassenko SA, Cook JD. The effect of dietary proteins on iron bioavailability in man. In: Dintzis FR, Laszlo JA, editors. Mineral Absorption in the Monogastric GI Tract. New York: Plenum Press; 1989, p. 117–32.

22. 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.

23. Guiro AT, Galan P, Cherouvrier F, Sall MG, Hercberg S. Iron absorption from African pearl millet and rice meals. Nutr Res 1991;11:885–93.

24. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ 2008;6:480–7.

25. Zimmermann MB, Fucharoen S, Winichagoon P, Sirankapracha P, Zeder C, Gowachirapant S, Judprasong K, Tanno T, Miller JL, Hurrell RF. Iron metabolism in heterozygotes for hemoglobin E (HbE), alpha-thalassemia 1, or beta-thalassemia and in compound heterozygotes for HbE/beta-thalassemia. Am J Clin Nutr 2008;88:1026–31.

26. Walczyk T, Kastenmayer P, Storcksdieck Genannt Bonsmann S, Zeder C, Grathwohl D, Hurrell RF. Ferrous ammonium phosphate (FeNH$_4$PO$_4$) as a new food fortificant: iron bioavailability compared to ferrous sulfate and ferric pyrophosphate from an instant milk drink. Eur J Nutr 2013;52:1361–8.

27. Fidler MC, Davidsson L, Zeder C, Walczyk T, Marti I, Hurrell RF. Effect of ascorbic acid and particle size on iron absorption from ferric pyrophosphate in adult women. Int J Vitam Nutr Res 2004;74:294–300.

28. Moretti D, Zimmermann MB, Wegmuller R, Walczyk T, Zeder C, Hurrell RF. Iron status and food matrix strongly affect the relative bioavailability of ferric pyrophosphate in humans. Am J Clin Nutr 2006;83:632–8.

29. Hackl L, Speich C, Zeder C, Sanchez-Ferrer A, Adelmann H, de Pee S, Tay F, Zimmermann MB, Moretti D. 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. J Nutr 2017;147:2319–25.

30. Hurrell RF, Reddy MB, Burri J, Cook JD. An evaluation of EDTA compounds for iron fortification of cereal-based foods. Br J Nutr 2000;84:903–10.

31. Hurrell RF, Furniss DE, Burri J, Whittaker P, Lynch SR, Cook JD. Iron fortification of infant cereals: a proposal for the use of ferrous fumarate or ferrous succinate. Am J Clin Nutr 1989;49:1274–82.

32. Fidler MC, Walczyk T, Davidsson L, Zeder C, Sakaguchi N, Juneja LR, Hurrell RF. A micronised, dispersible ferric pyrophosphate with high relative bioavailability in man. Br J Nutr 2004;91:107–12.

33. Hurrell RF, Reddy MB, Dassenko SA, Cook JD. Ferrous fumarate fortification of a chocolate drink powder. Br J Nutr 1991;65:271–83.

34. Consumption Pattern in Nigeria 2009/10. Abuja: National Bureau of Statistics; 2012.

35. Ene-Obong HN, Sanusi RA, Udenta EA, Williams IO, Anigo KM, Chibuzo EC, Aliyu HM, Ekpo OO, Davidson GI. Data collection and assessment of commonly consumed foods and recipes in six geo-political zones in Nigeria: important for the development of a National Food Composition Database and Dietary Assessment. Food Chem 2013;140:539–46.

36. INTERNATIONAL ATOMIC ENERGY AGENCY, Assessment of Iron Bioavailability in Humans Using Stable Iron Isotope Techniques, Human Health Series 21, 2012.

37. Federal Government of Nigeria, United Nations Children's Fund. Participatory Information Collection (PICS) Study: Nutritional Status of Women and Children in Nigeria. Abuja: Federal Government of Nigeria; 1993.

38. Engle-Stone R, Nankap M, Ndjebayi AO, Brown KH. Simulations based on representative 24-h recall data predict region-specific differences in adequacy of vitamin A intake among Cameroonian women and young children following large-scale fortification of vegetable oil and other potential food vehicles. J Nutr 2014;144:1826–34.