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A Higher Proportion of Iron-Rich Leafy Vegetables in a Typical Burkinabe Maize Meal Does Not Increase the Amount of Iron Absorbed in Young Women[1–3]

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Abstract

Food-to-food fortification can be a promising approach to improve the low dietary iron intake and bioavailability from monotonous diets based on a small number of staple plant foods. In Burkina Faso, the common diet consists of a thick, cereal-based paste consumed with sauces composed of mainly green leaves, such as amaranth and jute leaves. Increasing the quantity of leaves in the sauces substantially increases their iron concentration. To evaluate whether increasing the quantity of leaves in sauces would provide additional bioavailable iron, an iron absorption study in 18 young women was conducted in Zurich, Switzerland. Burkinabe composite test meals consisting of the maize paste *tô* accompanied by an iron-improved amaranth sauce, an iron-improved jute sauce, or a traditional amaranth sauce were provided as multiple meals twice a day for 2 consecutive days. Iron absorption was measured as erythrocyte incorporation of stable iron isotopes. Mean fractional iron absorption from maize paste consumed with an iron-improved amaranth sauce (4.9%) did not differ from the same meal consumed with an iron-improved jute sauce (4.9%; $P = 0.9$), resulting in a similar quantity of total iron absorbed (679 vs. 578 μg; $P = 0.3$). Mean fractional iron absorption from maize paste accompanied by a traditional amaranth sauce (7.4%) was significantly higher than that from the other 2 meal types ($P < 0.05$), but the quantity of total iron absorbed was similar (591 μg; $P = 0.4$ and 0.7, respectively). A food-to-food fortification approach based on an increase in leafy vegetables does not provide additional bioavailable iron, presumably due to the high phenolic compound concentration of the leaves tested. Alternative measures, such as adding iron absorption enhancers to the sauces, need to be investigated to improve iron nutrition from Burkinabe maize meals. J. Nutr. 144: 1394–1400, 2014.

Introduction

Low dietary iron intake and bioavailability from monotonous diets based on a few staple crops are major contributors to low iron status (1), especially in developing countries, such as Burkina Faso where only 26% of the women living in Ouagadougou, the capital city, have adequate iron intakes (2). Iron fortification, iron supplementation, and dietary modification/diversification are the 3 conventional approaches to improve iron intake and bioavailability (3). With all having their advantages and limitations, dietary modification/diversification is a promising approach in settings in which iron supplementation or iron fortification of staple foods is difficult to implement due to logistics issues (1) or due to the lack of centralized food production (4,5). Food-to-food fortification using iron-rich foods has been proposed as a sustainable and relatively inexpensive dietary modification approach to increase iron intake in low socioeconomic classes (6). It requires the identification of locally available iron-rich foods whose consumption could be enhanced through recommendations and promotion campaigns.

In most developing countries, the traditional diet is composed of a starchy food prepared from cereals, roots, or tubers and eaten together with a plant-based sauce (7). In Burkina Faso, maize, sorghum, and millet are the main staple crops usually consumed in the form of a thick paste called *tô*, which is made by mixing and cooking the cereal flour with water. In Burkina Faso's capital city, Ouagadougou, *tô* is increasingly prepared

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[3] Supplemental Table 1 and Supplemental Figure 1 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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from maize and is frequently used for complementary feeding (8). Many of the different sauces eaten with *tô* are prepared with leafy vegetables (9), which play an important role in the food diversity of the Burkinabe people. The iron bioavailability from the composite meal, *tô* accompanied by a leafy vegetable sauce, is expected to be rather low (10) because of usually high concentrations of phytic acid (PA)[9] and phenolic compounds in the ingredients used to prepare the meal (11–13). The strong inhibitory effect of PA on iron bioavailability from cereal meals was shown in several previous studies (14,15), and phenolic compounds from leafy vegetables or sorghum were reported to decrease iron absorption (16,17).

With the exception of small amounts of dry fish powder, the sauces do not contain any ingredients that would be expected to be rich in bioavailable iron (18). However, leafy vegetables, such as amaranth (*Amaranthus cruentus)* or jute leaves (*Corchorus olitorius)*, which are often used to prepare the sauces, were reported to contain iron concentrations of between 22 and 77 mg/100 g dry matter (19–21). Sauces prepared with these types of leafy vegetables may play an important role in the coverage of iron needs, and increasing the proportion of the iron-rich leafy vegetables in the sauces could be a promising dietary approach to increase long-term iron intake.

This approach can only be effective in improving iron nutrition if the additional iron from the leafy vegetables is bioavailable and increases the amount of absorbed iron. The aims of the present study were therefore to investigate iron bioavailability from *tô* eaten with a traditional sauce formulation and to determine if consumption of *tô* with iron-improved sauces containing extra leaves results in an increase in total iron absorbed. The fractional iron absorption and total iron absorbed from composite meals consisting of the traditional Burkinabe maize paste *tô* accompanied by traditional amaranth sauce, iron-improved amaranth sauce, or iron-improved jute sauce were measured by using a 2-d multiple meal design and stable isotope technique.

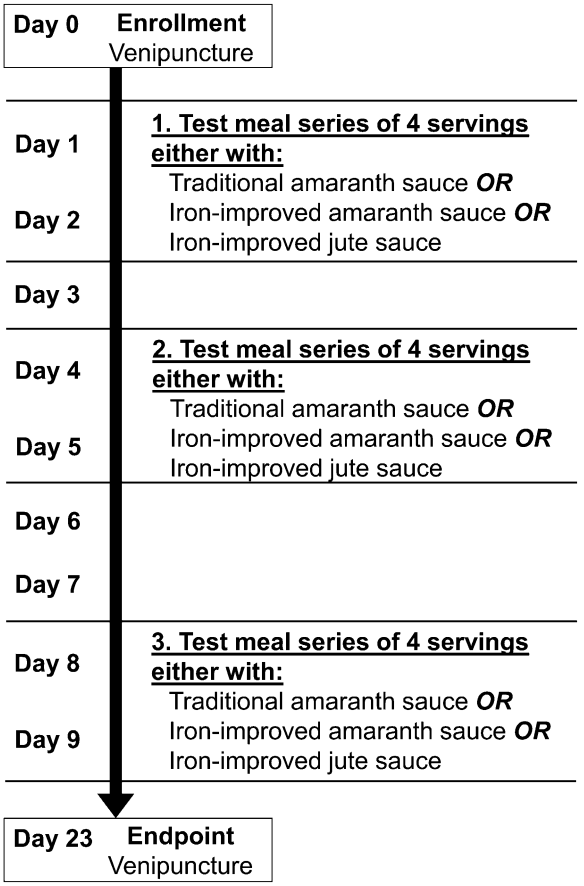


FIGURE 1 Schematic diagram of the study design including the administration of 3 different test meals in young women at ETH Zurich, Switzerland.

Participants and Methods

Participants. Eighteen apparently healthy, nonpregnant, nonlactating women aged between 18 and 40 y, with a normal BMI (18.5–25 kg/m^2) and a body weight <65 kg, were recruited from among the student and staff population of ETH Zurich and University Zurich, Switzerland. Women with a high iron status, defined as plasma ferritin (PF) >30 μg/L, were not included in the study. Women with known metabolic, chronic, and gastrointestinal disease, as well as woman taking long-term medication (except for oral contraceptives), were excluded from participating. Intake of vitamin and/or mineral supplements was not allowed 2 wk before and during the study. No women were recruited who had donated blood or who had experienced substantial blood loss within 4 mo of the beginning of the study. All study participants provided informed written consent. Ethical approval for the study was given by the ethical review committee at ETH Zurich.

Study design. A randomized crossover design with multiple meals was used with each woman serving as her own control. Each woman consumed 3 different test meals, each provided in series of 4 servings over 2 consecutive days (2 meals/d). This resulted in a total number of 12 servings, which were administered over 9 d (days 1–2, days 4–5, and days 8–9) including a 1- and 2-d break without test meals after the first and second series of test meals, respectively (day 3 and days 6–7; **Fig. 1**). The order of the 3 different test meal series was randomly assigned for each woman.

The 3 different test meals consisted of maize-based *tô*, accompanied with traditional amaranth sauce, iron-improved amaranth sauce, or iron-improved jute sauce. Servings of 1 test meal type were always labeled with the same isotope: ^{54}Fe was used for the meals with iron-improved amaranth sauce, ^{57}Fe for the meals with iron-improved jute sauce, and ^{58}Fe for the meals with traditional amaranth sauce. On study days, the first labeled serving was administered in the morning between 0630 and 0900 h after an overnight fast and the second serving was administered at least 3 h after completion of the first serving. The participants consumed the test meals completely in the presence of the investigators and were not allowed to eat or drink between the test meals and for 3 h after the second meal.

During screening (baseline measurements), 3 wk before the first test meal series, body weight and height of the participants were measured and a first blood sample was drawn for iron status determination [hemoglobin, PF, C-reactive protein (CRP)]. Fourteen days after the last test meal (day 23; endpoint measurements), body weight and height measurements were repeated, and a second blood sample was drawn for iron isotopic analysis and confirmation of iron status. Iron absorption was determined by using a stable isotope technique in which the incorporation of isotopic iron labels into erythrocytes was measured 14 d after the administration of the last test meal (22).

Test meals. Test meals were composite meals of the traditional Burkinabe maize paste *tô* served with 1 of the leafy vegetable sauces to be tested in the study. Distilled water (300 g) was administered in 2 servings of 80 g and 220 g with each test meal serving. Each test meal serving consisted of 250 ± 2 g *tô* (17–18% of dry matter) accompanied by 125 ± 2 g leafy vegetable sauce. The 3 different sauces were prepared

[9] Abbreviations used: CRP, C-reactive protein; PA, phytic acid; PF, plasma ferritin.

with the same ingredients according to the recipes shown in **Supplemental Table 1**. Changes made to the traditional recipe relied on the increase in the proportion of leafy vegetables from 15% to 25% on a dry matter basis, the decrease in the proportions of PA-rich ingredients, such as groundnut paste, and the increase in the overall dry matter content of the sauce from 16% to 20%. The 3 different sauces were prepared in bulk in Burkina Faso. All fresh ingredients, and particularly the leaves, were washed several times to remove any trace of soil or dust that could lead to iron contamination (23). The sauces were kept frozen during storage and transport to Switzerland.

Whole maize grains (*Zea mays*) were bought from a local market in Ouagadougou, Burkina Faso. Special attention was paid during the transformation of maize into flour to avoid iron contamination by dust or equipment, because it has been shown that this contamination could increase iron concentration by several times (24). The maize grains were decorticated as described in Hama et al. (13). The decorticated maize grains were cleaned by winnowing and afterward soaked in water for 24 h according to the traditional way of processing. After thorough washing, soaked maize grains were sundried and then milled by using a laboratory sample mill (Cyclotec 1093; Foss). The obtained maize flour was shipped to Switzerland for the preparation of maize paste.

The maize paste *tô* was freshly prepared for each study day according to a standardized procedure (23). The preparation started by adding a cold suspension of maize flour (385 g) and distilled water (1.1 kg) to boiling distilled water (3.85 kg). After the mixture boiled again, a part of it (1.65 kg) was withdrawn from the cooking pot and put into a separate bowl. Then, 715 g additional maize flour was added to the remaining suspension in the cooking pot and cooked for 3.5–4.5 min under continuous stirring. After that, the portion that had been withdrawn was returned and the whole preparation was cooked for 7–9 min under continuous stirring. After cooling, servings of 250 g were stored in a refrigerator until the administration on the following day.

All servings of *tô* accompanied by traditional amaranth sauce, iron-improved amaranth sauce, or iron-improved jute sauce were extrinsically labeled with 0.8 mg ^{58}Fe, 0.8 mg ^{54}Fe, or 0.8 mg ^{57}Fe, respectively. The stable iron isotopes were in the form of a solution and were diluted in the first serving of water (80 g) and administered after one-half of the maize paste and sauce was consumed. To ensure complete intake of isotopic labels, the second serving of water (220 g) was administered in the same plastic tumbler after completion of the meal.

Test meal analysis. Iron concentrations in the maize flour and sauces were analyzed by air-acetylene flame atomic absorption spectrophotometry (AAnalyst 800; Perkin Elmer) after mineralization by microwave digestion (ETHOS-1; Milestone) by using a mixture of HNO_3 and H_2O_2. The PA concentration was determined by anion-exchange HPLC measuring the myoinositol hexaphosphate (IP6) concentration according to the method of Talamond et al. (25) with the modifications of Lestienne et al. (26) including the use of an AS-11 precolumn and column kit (Dionex). The concentration of iron-binding galloyl and catechol groups was measured after extraction with 50% dimethylformamide in acetate buffer according to the method of Brune et al. (27) by using a ferric ammonium sulfate reagent that enabled the measurement of galloyl (expressed as tannic acid equivalents) and catechol group concentrations (expressed as catechin equivalents). The L-ascorbic acid concentration in the sauces was measured by using an L-ascorbic acid assay kit (K-ASCO; Megazyme).

Preparation of isotopically labeled iron. Isotopically labeled $^{54}FeSO_4$, $^{57}FeSO_4$, and $^{58}FeSO_4$ were prepared from isotopically enriched elemental iron (^{54}Fe-metal, 99.9% enriched; ^{57}Fe-metal, 96.8% enriched; ^{58}Fe-metal, 93.5% enriched; all from Chemgas) by dissolution in 0.1 mol/L sulfuric acid. The solutions were flushed with argon to keep the iron in the +2 oxidation state. Prepared iron tracer solutions were analyzed for iron isotopic composition and tracer iron concentration by reversed isotope dilution MS.

Blood analysis. Hemoglobin was measured in whole blood on the day of collection by using a CoulterCounter (Beckman Coulter); anemia was defined as hemoglobin <120 g/L (28). PF and CRP were measured by using an Immulite automatic system (Siemens). Iron deficiency was defined as PF <15 μg/L and iron deficiency anemia as hemoglobin <120 g/L plus PF <15 μg/L (28). Expected high-sensitivity CRP concentrations for healthy individuals were <5 mg/L (29).

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 (30). Iron isotope ratios were determined by a multicollector inductively coupled plasma MS instrument (Neptune; Thermo Finnigan).

Calculation of iron absorption. The amounts of ^{54}Fe, ^{57}Fe, and ^{58}Fe labels in the blood were calculated on the basis of the shift in iron isotope ratios and the estimated amount of iron circulating in the body. Circulating iron was calculated on the basis of the blood volume estimated from height and weight and measured hemoglobin concentration (31). The calculations were based on the principles of isotope dilution, taking into account that iron isotopic labels were not monoisotopic, by using the methods described by Turnlund et al. (32). The calculation of iron absorption is shown in detail in **Supplemental Figure 1**. For the calculation of fractional absorption, 80% incorporation of the absorbed iron into RBCs was assumed (33).

Statistical analysis. Analyses were conducted by using R software (version 2.15.1; The R Foundation for Statistical Computing), Statgraphics Plus (version 5.1; Statpoint Technologies), and Excel (Windows 7; Microsoft). Results of fractional and total iron absorption, food analysis, age, anthropometric measurements, hemoglobin, PF, and CRP are presented as means ± SDs if normally distributed. If not normally distributed, the results are presented as geometric mean values with 95% CIs in parentheses. Comparison of test meal composition (iron, PA, phenolic compounds) was made by a 1-factor ANOVA followed by Fisher's least significant difference tests for multiple comparisons. The fractional and total iron absorptions from different test meals were compared by using linear mixed-effects models taking into account that the same woman was repeatedly measured on the dependent variables (fractional or total iron absorption). In the models, the type of test meal was defined as a fixed effect and the subject as a random effect. All data were converted to their logarithms for statistical analysis and reconverted for reporting. Differences were considered significant at $P < 0.05$. The study was powered to detect an intrasubject difference of 30% in fractional iron absorption with an α level of 0.05.

Results

Participant characteristics. At baseline, all women had PF concentrations <30 μg/L, and none of the women had elevated CRP concentrations (>5 mg/L) (**Table 1**). Five of the women were iron deficient without anemia and 2 were iron-deficient anemic. At the study endpoint, the PF concentrations of 3 women were >30 μg/L (32.1, 38.2, and 42.9 μg/L). The woman with the PF concentration of 38.2 μg/L had an elevated CRP concentration (6.4 mg/L) at the study endpoint.

TABLE 1 Age, anthropometric measurements, and concentrations of hemoglobin, PF, and CRP in young women at baseline[1]

Variable	Summary value
Age, *y*	25.2 ± 4.1
Weight, *kg*	58.2 ± 6.7
Height, *cm*	166 ± 6
BMI, *kg/m^2*	21.1 ± 2.3
Hemoglobin, *g/L*	130 ± 12
PF, *μg/L*	15.1 (10.9, 20.9)
Plasma CRP, *mg/L*	0.48 (0.29, 0.78)

[1] Values are means ± SDs or geometric means (95% CIs); n = 18. CRP, C-reactive protein; PF, plasma ferritin.

Test meal composition. The iron concentrations of the washed amaranth and jute leaves were 39.5 ± 0.4 and 30.1 ± 0.8 mg/100 g dry matter, respectively. The leaves provided 49% of the total iron in the traditional amaranth sauce, 71% in the iron-improved amaranth sauce, and 56% in the iron-improved jute sauce. The iron concentrations of the iron-improved amaranth sauce and iron-improved jute sauce were ~2.0 and ~1.7 times that of the traditional amaranth sauce ($P < 0.0001$; **Table 2**). The iron concentration of the maize paste alone was 0.4 ± 0.0 mg/serving and, depending on the sauce, contributed only 5–10% to the total iron concentration of the composite test meal including the iron isotopic tag. The iron isotopic tag was 13%, 15%, and 25% of the native iron concentrations in the meals with iron-improved amaranth sauce, iron-improved jute sauce, and traditional amaranth sauce, respectively. The PA concentrations of the 3 different sauces did not differ ($P = 0.7$) and were generally low (Table 2). The PA from the sauces contributed to approximately one-fourth to one-third of the total PA in 1 test meal serving. The remaining PA in 1 test meal serving came from the maize paste, whose PA concentration was 60 ± 12 mg/serving. The PA:iron molar ratios in the 3 different test meals were similar, ranging from 1.0:1 to 1.8:1 (Table 2). The PA:iron molar ratio was highest in the meals with the traditional amaranth sauce followed by meals with the iron-improved jute sauce and was lowest in the meals with the iron-improved amaranth sauce. The galloyl and catechol concentrations of the iron-improved amaranth sauce were approximately twice those of the traditional amaranth sauce ($P < 0.001$; Table 2). The iron-improved jute sauce had higher concentrations of galloyl and catechols than the other 2 sauces ($P < 0.0001$). The galloyl and catechol concentrations in the maize paste were 10 ± 2 and 3 ± 2 mg/serving, respectively. L-Ascorbic acid concentrations were 0.6, 2.3, and 0.4 mg/100 g fresh matter in the traditional amaranth sauce, iron-improved amaranth sauce, and iron-improved jute sauce, respectively.

Iron absorption measurements. The mean fractional iron absorption from the maize paste consumed with the iron-improved amaranth sauce did not differ ($P = 0.9$) from that obtained with the iron-improved jute sauce (**Table 3**), resulting in a similar quantity of total iron absorbed from the 2 meals ($P = 0.3$). According to the bioavailability thresholds established by WHO (34), the mean iron bioavailability from both meals with iron-improved sauces was low (<5%). The mean fractional iron absorption from the meal with traditional amaranth sauce was ~34% higher than that from the meals with iron-improved amaranth and jute sauces ($P < 0.05$). However, the amount of total iron absorbed from the meal with traditional amaranth sauce was not significantly different from that from the meals with iron-improved amaranth and iron-improved jute sauces ($P = 0.3$ and 0.9, respectively). The mean iron bioavailability from the meal with the traditional amaranth sauce was between 5% and 10%, which is defined as moderate iron bioavailability (34).

Discussion

The major finding of the current study is that there was no increase in the quantity of iron absorbed by young women from a maize paste meal accompanied by iron-improved leaf-based sauces, which increased daily iron intake by 4.0 and 5.8 mg (~1.5-fold), compared with the traditional sauce. The mean quantity of total iron absorbed per meal including the iron from the paste and the sauce, but excluding the isotopic tag, covered between 16% and 20% of the median daily iron requirements of menstruating women older than 18 y (34). Our data were generated in apparently healthy women, and when extrapolating the data to Burkinabe women, impaired iron absorption by chronic infections, such as malaria (35), needs to be considered. Nevertheless, on the basis of our data, infection-free Burkinabe women with a mean consumption of 419 g *tô* and 176 g sauce per meal (C. Icard-Vernière, unpublished results, 2013) are expected to cover between 23% and 29% of their 1.5 mg of absorbed iron required per day (34). Burkinabe women and young children usually eat *tô* twice per day, and sometimes even 3 times per day (8,18). In the case of 2 meals per day, our results indicate that the leafy vegetable sauces consumed with *tô* considerably contribute to iron needs (46–58%); however, coverage of iron needs would remain clearly inadequate. Consumption data for young Burkinabe children 12–24 mo of age showed an average *tô* and sauce consumption of 84 ± 17 g and 66 ± 11 g/meal, respectively (24). Assuming equal fractional iron absorption in young children, they would absorb between 0.12 and 0.15 mg iron/meal, and in the case of 2 meals per day this would cover 52–66% of the 0.46 mg absorbed iron required per day (34).

The fractional iron absorption from the meals with iron-improved sauces most likely decreased because increasing the amount of leafy vegetables in the sauces also increased the concentrations of phenolic compounds able to inhibit iron absorption (36). It has been suggested that galloyl groups, and to a lesser extent catechol groups, are the structures responsible for the inhibition of iron absorption by phenolic compounds (37). In

TABLE 2 Total iron-binding catechols and galloyls, PA, and iron in the 3 different sauces and the composite test meals consumed by young women[1]

Meal components	Catechols	Galloyls	PA	Iron	PA:iron[2]
	mg catechin equivalents/serving	*mg tannic acid equivalents/serving*	*mg/serving*	*mg/serving*	
Traditional amaranth sauce	100 ± 10	49 ± 3	27 ± 5	2.8 ± 0.1	0.8:1
Composite test meal (including maize paste *tô*)[3]	103 ± 10	59 ± 4	87 ± 12	4.0 ± 0.1[4]	1.9:1
Iron-improved amaranth sauce	189 ± 2	81 ± 2	23 ± 3	5.7 ± 0.1	0.3:1
Composite test meal (including maize paste *tô*)[3]	192 ± 3	91 ± 4	83 ± 12	6.9 ± 0.1[4]	1.0:1
Iron-improved jute sauce	382 ± 2	146 ± 7	25 ± 2	4.8 ± 0.5	0.4:1
Composite test meal (including maize paste *tô*)[3]	385 ± 3	156 ± 8	85 ± 12	6.0 ± 0.5[4]	1.2:1

[1] Values are means ± SDs unless otherwise indicated; $n = 3$. PA, phytic acid; PA:iron, phytic acid:iron molar ratio.
[2] Values are molar ratios of the sauces or the composite meals.
[3] Values are based on the means from the analysis of single components (maize paste + sauce). SDs were adapted by calculating the square root of the sum from the square of the SDs from the single analysis of the paste and sauces. Concentrations of iron, PA, catechols, and galloyls of the maize paste alone are described in text.
[4] Iron concentrations of the composite test meals included the native iron and the isotopic tag (0.8 mg iron as ^{54}Fe, ^{57}Fe, or ^{58}Fe).

TABLE 3 Fractional and total iron absorption from traditional Burkinabe composite meals consumed by young women[1]

Test meal	Fractional iron absorption	Total iron absorption[2]	Ratio of fractional absorption (meal A:meals B and C)[3]
	% of dose	*mg/d*	
Maize paste with traditional amaranth sauce	7.4 (5.1, 10.8)[a]	0.59 (0.41, 0.86)	—
Maize paste with iron-improved amaranth sauce	4.9 (2.8, 8.7)[b]	0.68 (0.39, 1.20)	0.7
Maize paste with iron-improved jute sauce	4.9 (2.8, 8.6)[b]	0.58 (0.33, 1.02)	0.7

[1] Values are geometric means (95% CIs); $n = 18$. Labeled means in a column without common letter differ, $P < 0.05$.
[2] Based on the fractional absorption and iron concentrations of 2 servings of maize paste (500 g) with the respective leafy vegetable sauce (250 g) including the isotopic tag. Iron concentrations of the test meals are shown in Table 2.
[3] The fractional iron absorption from the maize paste with traditional amaranth sauce (A) was compared with that from the maize paste with iron-improved amaranth sauce (B) and the maize paste with iron-improved jute sauce (C).

our study, substantial concentrations of galloyls and catechols were measured, particularly in the iron-improved jute sauce, which had much higher concentrations than the other 2 sauces. Iron absorption from the iron-improved jute sauce was lower compared with the traditional amaranth sauce but similar to the iron-improved amaranth sauce. This suggests that, above a certain concentration, galloyls and catechols do not further inhibit iron absorption in a dose-dependent way and confirms the findings from a previous study with a Thai leafy vegetable, in which iron absorption was inhibited dose-dependently between 0 and 146 mg tannic acid equivalents per meal, but no further inhibition was observed when tannic acid equivalents were steadily increased up to 584 mg/meal (17). Similarly, no further inhibition of iron absorbed was observed in a more recent study when the concentration of brown sorghum polyphenols was increased from 72 to 166 mg gallic acid equivalents/test meal (16).

In our test meals, the concentrations of ascorbic acid, which is able to counteract the inhibitory effects of phenolic compounds and PA (16,38), were low because of the long boiling time, which decreased initial ascorbic acid concentrations of the leaves during sauce preparation (39,40). Contrary to our expectations, the iron bioavailability from the meal with the traditional amaranth sauce was in the range for diets rich in cereals but including sources of ascorbic acid (34) and not in the range for diets low in ascorbic acid and animal protein. This moderate iron bioavailability from the meal with traditional amaranth sauce can most likely be explained by the low PA concentrations in the meals. The PA concentration of the maize flour was reduced because of the pre-preparation of the maize grains including decortication and soaking, which are known to significantly decrease PA concentration (41,42). The negligible amounts of PA in the sauces came from groundnut paste and *soumbala*, a Burkinabe condiment made from fermented legume (43).

Our study is 1 of the rare studies investigating iron absorption from a composite meal frequently consumed in sub-Saharan Africa by using a multiple meal design. In our study, the fractional and total iron absorption from the meal with traditional amaranth sauce was similar to that from a traditional Beninese composite millet meal based on regular-iron millet (7.5% and 0.53 mg/d, respectively) measured in a recent study using multiple meals for 5 d (44). It should be stressed that the PA concentrations in the millet study (394–519 mg/meal) were noticeably higher, but the concentrations of phenolic compounds in the millet meals were lower. In another study that used multiple meals, fractional iron absorption from typical Rwandese meals based on regular-iron beans was reported to be between 6.3% and 7.4%. The fractional iron absorption decreased to 3.8% when the iron concentration of the test meals was increased by using high-iron beans (45). As in our study, the reason was most likely the increase of an iron absorption inhibitor when using the high-iron beans, which had a substantially higher PA concentration than the regular-iron beans.

A previous single meal study using radioisotopes measured iron absorption from 3 typical Beninese maize meals, which were very similar to the meals in our study. The fractional (1.2–3.2%) and total (45–106 μg) iron absorptions by young men were much lower compared with our results. However, when applying the experimentally derived algorithms (46) to adjust PF in this study to the same mean value as in our present study, the fractional iron absorption increases to 4.5–12.1%. The higher absorption in the previous radioisotope study occurred in meals with sauces containing 16% and 43% of fresh fish, whose proteins are able to increase nonheme-iron absorption (47). In our study, the sauces had only 2.8% of fish powder and we assumed no effect of fish proteins on nonheme-iron absorption.

In conclusion, our study shows that the total amount of iron absorbed from a typical Burkinabe dish cannot be increased by a food-to-food fortification approach, taking advantage of the relatively high iron concentration of the traditional Burkinabe leafy vegetables, without considering further measures. These further measures could include adding iron absorption enhancers to the sauces (e.g., ascorbic acid or muscle tissue), fortifying the sauces directly with micronutrient powders including iron, decreasing the concentrations of inhibiting phenolic compounds in the sauces, or direct iron fortification of cereal flours. However, all of the alternative strategies have their difficulties, such as heat sensitivity of ascorbic acid, availability and affordability of animal-source foods and micronutrient powders in rural communities, lack of decentralized milling, and the difficulties in fortifying flour at the community level. In our study, increasing the iron concentrations of the sauces by using increased amounts of leafy vegetables also increased the concentration of the inhibiting phenolic compounds. We conclude therefore that food-to-food fortification can only be promising if the food used to fortify does not contain substantial concentrations of iron absorption inhibitors. In the case of the typical Burkinabe diet, the use of liver or fresh fish in the sauces could be valuable options to increase absorbed iron.

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