

Low-pH Cola Beverages Do Not Affect Women's Iron Absorption from a Vegetarian Meal¹⁻³

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Abstract

Preliminary data in the literature indicate that iron absorption from a meal may be increased when consumed with low-pH beverages such as cola, and it is also possible that sugar iron complexes may alter iron availability. A randomized, crossover trial was conducted to compare the bioavailability of nonheme iron from a vegetarian pizza meal when consumed with 3 different beverages (cola, diet cola, and mineral water). Sixteen women with serum ferritin concentrations of 11–54 g/L were recruited and completed the study. The pizza meal contained native iron and added ferric chloride solution as a stable isotope extrinsic label; the total iron content of the meal was ~5.3 mg. Incorporation of iron from the meal into RBC was not affected by the type of drink (9.9% with cola, 9.4% with diet cola, and 9.6% with water). Serum ferritin and plasma hepcidin were correlated ($r = 0.66$; $P < 0.001$) and both were significant predictors of iron bioavailability, but their combined effect explained only 30% of the inter-individual variation ($P < 0.001$) and illustrates the current lack of understanding of mechanisms responsible for the fine-tuning of iron absorption. Although there was no effect of low-pH drinks on iron bioavailability in healthy women, their effect on absorption of fortification iron that requires solubilization in dilute acid, such as reduced iron, and in individuals with low gastric acid production, such as older people and individuals with *Helicobacter pylori* infection, warrants further investigation. J. Nutr. 141: 805–808, 2011.

Introduction

Iron deficiency anemia is the most common nutrient deficiency disorder affecting ~500 million people worldwide (1). Infants, children, and premenopausal women are most at risk. Because only a fraction of iron ingested in the diet is actually absorbed, the most common cause of nutritional deficiency is assumed to be related to low bioavailability rather than inadequate intake of iron. Simple dietary strategies for reducing the risk of iron deficiency by improving iron bioavailability are therefore important for public health on a global scale.

Previous studies have reported high bioavailability of iron when given with a cola drink. For example, when 1 mg hydrogen-reduced iron was added to a bread roll and consumed with 300 mL of cola, the mean iron absorption in 10 women who had fasted overnight and had a plasma ferritin concentration of 24 $\mu\text{g/L}$ was 64% (2). The explanation put forward for this very high absorption was the small particle size of the elemental iron (specially prepared as stable isotopically enriched reduced iron), unlike some commercial forms of elemental iron, and increased solubility in the gastrointestinal tract caused by the low pH of the cola drink, as illustrated by the observation that cola drinks are excellent extractants for micronutrients in soil (3). In 1982,

Hallberg and Rossander (4) reported results from a series of radio-iron tests examining the effect of different beverages on nonheme iron absorption from a mixed meal of hamburger, beans, and potatoes. Coca-Cola increased absorption of the total iron in the meal (3.5 mg) from 0.40 mg with water to 0.54 mg, but this did not reach significance (P -value not provided) in the small group of participants (7 men and 3 women). From the totality of data, the authors highlighted the importance of gastric pH on iron solubilization and hence bioavailability.

To investigate this further and test the hypothesis that low-pH drinks increase iron absorption in nonanemic women, a randomized, crossover trial was conducted to measure iron absorption from a standard test meal using stable isotope techniques. Erythrocyte incorporation of the absorbed isotopic label 14–18 d after oral administration of the test meal was used as a direct measure of iron bioavailability (5–7). Serum ferritin and plasma hepcidin were also measured on the day of the absorption test to examine the magnitude of effect of known modulators of iron absorption on both inter- and intra-individual variability in iron absorption.

Materials and Methods

Participants. Nonanemic women ($n = 16$) aged 18–65 y with serum ferritin concentrations of 11–54 g/L were recruited to investigate the effect of different beverages on nonheme iron absorption. Individuals with acute or chronic illness or who taking any medications known to interfere with iron absorption were excluded from participation. The study was approved by the University of East Anglia's Institute of Health

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Ethics Committee and conducted at the Human Nutrition Unit at the Institute of Food Research, Norwich. Informed written consented was obtained from all individuals prior to participation.

Experimental design. The study was a randomized crossover trial in which participants consumed cheese and tomato pizza for lunch, extrinsically labeled with ^{58}Fe stable isotope, on 2 consecutive days, together with 330 mL cola (pH 2.5), diet cola (pH 2.8), or mineral water (Tesco Still Mountain Scottish Spring Water, pH 7.1). Each pair of test meals plus drink was separated by a period of 14–18 d and the order in which the volunteers consumed the test drinks was randomized prior to the start of the study using a computer-generated randomization model. The pizza was prepared by spreading 125 g of tomato passata (Passata Di Pomodoro) evenly over a standard 150-g pizza base (Napolina Authentic Italian Pizza Base). The pizza was cut into 2 sections, 1 approximately two-thirds of the total size and a one-third portion. A 3-mg dose of ^{58}Fe -enriched ferric chloride was evenly applied over the passata of the larger portion using a plastic pipette and the dose container was rinsed twice with water, which was then also added to the base. The whole pizza was topped with 45 g of grated cheddar cheese, 15 g of mozzarella cheese, and 35 g of sliced fresh tomato, and cooked at gas mark 7 for 10–15 min. The sections of the cooked pizza were weighed before serving and participants were asked to eat at least the larger, two-thirds portion and consume the whole drink. The quantity of pizza consumed by each volunteer on the first day was repeated throughout the study; the iron content of a full serving of pizza was calculated to be 2.3 mg.

The iron absorption from the pizza meals was determined using the erythrocyte incorporation technique (6). To minimize intra-individual variation in iron absorption, participants were asked to complete a household measures food diary for the 2 d prior to their test meal appointments and to replicate these meals before the remaining 2 pairs of test meals. Each test meal and drink (1, 2, or 3) was consumed on 2 consecutive days to provide an average absorption value for the 2-d period.

A 20-mL baseline blood sample was taken from fasting participants on d 1 and they consumed a standard breakfast of white toast with butter and jam and a choice of fruit (banana or melon) and unlimited water. Three hours later, they were asked to consume the first test meal of pizza, labeled with ^{58}Fe , plus drink 1. On d 2, participants consumed an identical controlled breakfast at home before returning to the Human Nutrition Unit for their test meal (pizza + drink 1 as on d 1). After ~15 d, a fasting blood sample was taken prior to consumption of a second pair of test meals (pizza + drink 2). During the 2 d prior to their second test meals, participants were asked to repeat the food diary from phase 1, eating exactly the same foods. Controlled breakfasts, identical to phase 1, were again provided. Iron isotope enrichment of the second blood sample was used as a baseline for the second pair of test meals and to calculate RBC incorporation of iron from the first pair of test meals. A fasting blood sample was taken after ~30 d and the iron isotope enrichment was used as a baseline for the 3rd pair of test meals (pizza + drink 3) and to determine RBC incorporation of iron from the second pair of test meals. Again, the food diary was repeated before the 3rd phase and participants were given controlled breakfasts. At ~45 d, a final fasting blood sample was taken to determine RBC incorporation of iron from the 3rd pair of test meals.

Iron isotope preparation. Ferric chloride solution was prepared from isotopically enriched elemental iron (^{58}Fe 93.18 atom%; Chemgas) dissolved in 6 mol/L hydrochloric acid. The solution was diluted to an appropriate concentration and the pH was adjusted to 3.0 by the addition of sodium hydroxide. The ferric chloride solution was filtered through a 0.22- μm filter (Millipore) and aliquoted into individual vials, each containing 3 mg iron in total. The vials were flushed with nitrogen and stored frozen at -20°C until required.

Analytical methods. Hemoglobin, serum ferritin, total iron-binding capacity, serum iron, and C-reactive protein (CRP) were determined at each time point by an accredited laboratory using an automated analyzer (Immulite 2000, Vitros 250 and Horiba Pentra 80 analyzers, SPIRE Laboratory, Norwich, UK). The iron concentration of whole blood was

calculated from the hemoglobin concentration and the iron-isotope enrichment of RBC was measured (6). The concentration of hepcidin was determined in fasting plasma samples collected on the first day that participants consumed test meals by a combination of weak cation exchange chromatography and surface-enhanced laser-desorption/ionization time-of-flight MS by using synthetic analog hepcidin-24 as an internal standard (8).

Statistical analysis. A sample size of 16 allowed for the detection of a difference in absorption of 1 SD with 80% power at the $P < 0.05$ significance level using ANOVA, assuming that the largest difference is between one test and the other 2 tests. Based on previous, similar studies, the SD of iron absorption was estimated to be 3% and a difference in iron absorption of this magnitude was assumed to be nutritionally important. The statistical software R [R software version 2.6.1 [Hornik (2009), The R FAQ, <http://CRAN.R-project.org/doc/FAQ/R-FAQ.html>, ISBN 3-900051-08-9] was used to analyze the data (9). Linear models were employed to investigate the effect of cola drinks on iron absorption. Serum ferritin and plasma hepcidin were included as covariates in the models. For all models, regression diagnostics were performed to check for outliers and to determine whether data transformations were required to satisfy the assumptions of linear models. Results are presented as means \pm SD. All results were considered significant if $P < 0.05$.

Repeated-measures ANOVA models were used to examine how much of the inter- and intra-individual variation in iron bioavailability could be explained by hepcidin and ferritin both individually and collectively. Hepcidin concentrations that were below the limit of detection of L were included in the analysis. L were modeled using a random number generated from the uniform distribution in a range of 0–L were included in the analysis. L.

Results

All 16 participants completed the study and all concentrations of CRP, a marker of inflammation that is associated with changes in iron metabolism, were assessed and considered normal (10) during the study, with the exception of 1 study phase for 1 volunteer who had a concentration of 36 mg/L. This data point was excluded from the analysis, leaving 15 bioavailability data points from the test meal served with water. The mean age of the women was 46.3 ± 11.4 y and BMI was 24.7 ± 3.9 kg/m² (Table 1). The iron status of the women, as measured by serum ferritin, did not change throughout the course of the study (Table 2). Fourteen of the 42 samples (33%) analyzed for hepcidin were below the limit of detection (LOD) (8 of the 14 volunteers had at least 1 hepcidin measurement below the LOD and 3 had all 3 measurements below the LOD) and data for these were generated from a model. Serum ferritin and plasma hepcidin were correlated ($r = 0.66$; $P < 0.001$) (Fig. 1), but the correlation was slightly lower ($r = 0.41$; $P < 0.05$) when only volunteers who had a measured hepcidin concentration > 0.5 nmol/L were included in the analysis.

Incorporation of iron from the pizza meal into RBC was not affected by the beverage consumed with the meal (Table 2). There were 2 women who had much higher iron absorption from the test meals (Fig. 2). The hemoglobin concentration decreased in

TABLE 1 Characteristics of female volunteers at recruitment¹

Characteristic	
Age, y	46.3 \pm 11.4
Height, m	1.65 \pm 0.06
Weight, kg	66.1 \pm 10.3
BMI, kg/m ²	24.7 \pm 3.9

¹ Values are mean \pm SD, $n = 16$.

TABLE 2 Iron status of women before each test meal and RBC incorporation of iron from the vegetarian meal consumed with mineral water, cola, and diet cola on 3 separate occasions¹

	Mineral water	Cola	Diet cola
<i>n</i>	15	16	16
Hemoglobin, g/L	137 ± 8	135 ± 8	135 ± 9
Serum ferritin, ² μg/L	33.6 ± 16.2	27.1 ± 13.8	34.9 ± 17.0
Serum iron, μmol/L	17.0 ± 5.1	13.5 ± 6.1	14.6 ± 7.3
Total iron-binding capacity, μmol/L	67.6 ± 8.7	65.6 ± 8.8	63.9 ± 8.6
Transferrin saturation, %	25.1 ± 6.3	20.4 ± 8.6	22.6 ± 10.5
Serum CRP, mg/L	3.5 ± 2.4	3.3 ± 2.5	3.4 ± 1.9
Plasma hepcidin, ³ nmol/L	1.6 ± 1.1	1.0 ± 0.9	1.5 ± 1.2
RBC incorporation, % of iron ingested	9.6 ± 7.7	9.9 ± 8.4	9.4 ± 6.3

¹ Values are mean ± SD.

² *n* = 14 for mineral water and cola.

³ *n* = 14, hepcidin concentrations that were below the LOD of 0.5 nmol/L were modeled using a random number generated from the uniform distribution in a range of 0–0.5 nmol/L.

1 woman between the screening and study days, which could perhaps explain the high absorption, but no potential explanatory factor could be elucidated for the second woman (normal iron status markers and BMI) apart from the fact that she was a vegetarian. Meal type did not influence bioavailability and the percentage of intra-individual variation explained by meal type alone was just 2.3%. Serum ferritin and plasma hepcidin concentration together explained 30% of the inter-individual variation in iron bioavailability. Ferritin on its own accounted for 27% of the variation and hepcidin on its own accounted for 38% of the variation. When intra-individual variation in iron absorption was examined, ferritin on its own accounted for 44% of the variation and hepcidin for 36% of the variation.

Discussion

The results of this study provide no evidence that the iron bioavailability of a vegetarian pizza meal, extrinsically labeled with ferric chloride solution (enriched with stable isotopes of iron) and containing no fortification iron, was significantly increased when accompanied by low-pH cola drinks in healthy nonanemic women aged 30–63 y. Under normal conditions,

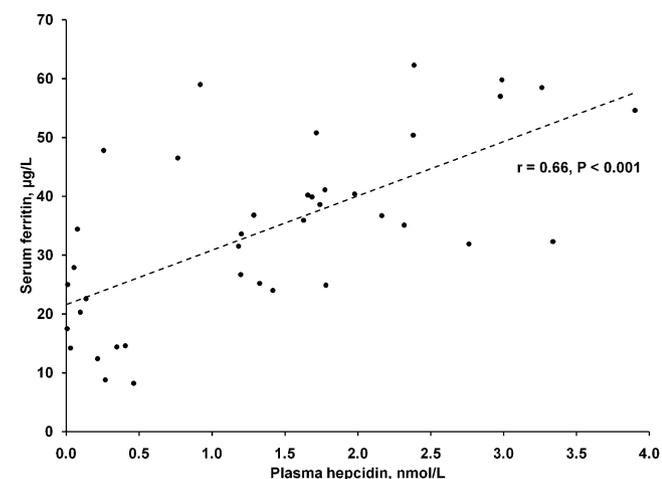


FIGURE 1 Relationship between serum ferritin and plasma hepcidin concentrations in fasting women prior to the study.

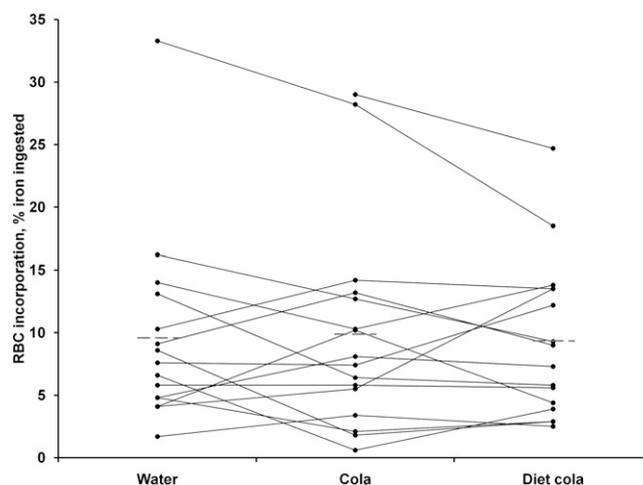


FIGURE 2 Women's RBC incorporation of iron from the vegetarian meal consumed with mineral water (*n* = 15), cola (*n* = 16), and diet cola (*n* = 16) on 3 separate occasions. Dashed lines indicate the means.

more gastric acid is secreted than is required for optimal iron absorption (11), so an acidic beverage, such as a cola drink, may improve iron absorption only in conditions such as achlorhydria, where iron absorption may be compromised (12), or perhaps assist the solubilization of elemental iron added as a fortificant to food, because it has been demonstrated that the solubility of elemental iron predicts efficiency of absorption in rats (13). It is also possible that women who were iron deficient, with or without anemia, may have responded differently to our study group.

The protective role of gastric acid in preventing iron deficiency anemia has been known for decades (14) and, more recently, the role of *Helicobacter pylori* in increasing gastric pH and its subsequent impact on iron status has been recognized (15). Reduced gastric acidity causes lower absorption of ferrous sulfate, carbonate, and fumarate (16) as well as elemental iron (17). Further, the gastric pH of a simulated digestion has been reported to be a key determinant of uptake of elemental iron by Caco-2 cells, presumably through its effect on iron solubilization (18). Therefore, there appeared to be adequate evidence from the literature to underpin the hypothesis that low-pH drinks increase iron absorption. In addition, if a sugar-iron complex were formed when iron is consumed with a cola beverage, it could prevent the production of insoluble iron hydroxides in the lumen of the small intestine in a similar fashion to iron-ascorbate complexes. This explanation has been proposed for the enhancing effect of fructose on iron absorption in rats (19) and was the reason for comparing normal, sugar-containing cola with diet cola.

One of the key determinants of the efficiency of iron absorption is the concentration of circulating hepcidin, which is expressed mainly in the liver. However, attempts to understand the relationship between circulating hepcidin and iron absorption have been hampered by various technical difficulties in successfully measuring plasma levels (8,20–22). With the development of more robust techniques, efforts have been made to clarify and characterize the effect of hepcidin on iron absorption. Our observation that only ~30% of inter-individual variation could be explained by a model that includes ferritin and hepcidin suggests that there are other physiological factors that modulate iron absorption (8,23). The key to regulation of iron within individuals is still unclear, but recent studies (8,23,24)

indicate that, in addition to hepcidin, other factors significantly influence iron absorption, including inflammation, hypoxia, and erythropoietic drivers. These factors promote changes in expression of proteins associated with the hepatocyte plasma membrane, including HFE, hemojuvelin, and transferrin receptor 2, and influence hepcidin gene expression (25). In addition, analysis of mother-child pairs has indicated that maternal iron absorption is the one significant predictor of iron absorption in children, once corrected for iron status (ferritin) and meal type (26). Whether this familial trend is inherited or caused by common shared environmental factors remains to be elucidated. Thus far, the influence of hepcidin on iron absorption within individuals requires further clarification, and the mechanisms underpinning the large day-to-day variation in efficiency of iron absorption, which is necessary for fine-tuning iron homeostasis, have yet to be fully identified and characterized.

The results of this study demonstrate that low-pH drinks do not enhance nonheme iron bioavailability in healthy women, but their effect on elemental iron added as a food fortificant and/or in individuals with low gastric acid secretion and/or iron deficiency remains to be explored.

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