International Food Research Journal 22(3): 997-1001 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Is Indian tea (chai) detrimental to dietary iron absorption?

[1*]Gaur, S. and [2]Miller, D.D.

[1]*Department of Food Science and Human Nutrition, University of Illinois, Urbana-Champaign, IL, USA-61820*
[2]*Department of Food Science, Cornell University, Stocking Hall, Ithaca, NY, USA-14853*

Article history

Received: 7 July 2013
Received in revised form: 1 December 2014
Accepted: 9 December 2014

Keywords

Iron
Tea
Milk
In vitro
Dialyzability

Abstract

Indian tea preparation involves addition of milk to brewed black tea. It is a well-known fact that individually both tea polyphenols and milk proteins inhibit iron absorption by forming complexes with dietary iron, rendering it insoluble in the gut. However, when present in combination these dietary components could have an ambiguous effect of either increasing the available iron by binding each other or decreasing iron availability by showing additive inhibitory effect. Our objective was to investigate effect of milk addition to tea on iron bioavailability using *in vitro* digestion method. Treatments namely, tea only (A), tea+milk (B) and milk only (C), were mixed with $FeCl_3$ to yield solutions that are 5 mM in iron. These solutions were subjected to simulated gastrointestinal digestion and were further estimated for percent dialyzability and solubility of iron using ferrozine as indicator. Results obtained showed that dialyzable iron in the treatment of tea with milk was lowest (19.83 ± 1.71% of total iron in the treatment) compared to treatment of tea only (30.8 ± 2.03%) and milk only (24.72 ± 3.73%). Our results suggest that tea and milk when taken together could have higher deleterious effect on iron availability than taken individually, and this might be a possible factor contributing to the prevailing iron deficiency throughout the country.

Introduction

Iron deficiency is one of the major causes for morbidity and mortality in India (Nair and Iyenger, 2009). Every year around 22,000 people, mainly pregnant women, die from severe anemia (National Nutrition Monitoring Bureau (NNMB), 2003). According to the National Family Health Survey (Arnold *et al*., 2009), 70-80% in children (6 to 59 months), 90% of adolescent girls, 55% of women, 70% of pregnant women, and 24% of adult men are suffering from anemia in the country (IIPS, 2007 and Arnold *et al.*, 2009). Iron deficiency causes a wide range of abnormalities such as iron deficiency anemia, cognitive dysfunction in young children, growth and development retardation, gastrointestinal tract abnormalities, and miscellaneous disease like pica and thrombocytosis. The primary reason for this deficiency is the presence of inhibitors such as phytic acids and polyphenols, and absence of absorption enhancers such as ascorbic acid in Indian vegetarian diets.

Chai (black tea with milk) is a widely consumed beverage throughout the country with annual per capita consumption of 0.68 Kg (23.5 oz) (Indiastat, 2007). It is often consumed with daily meals and thus both tea and milk play an important role in determining the bioavailability of dietary iron. Iron in the food systems exists in two oxidation states, ferric (Fe^{3+}) and Ferrous (Fe^{2+}). Both the species are unstable at physiological pH and tend to polymerize in the absence of appropriate ligands, thus reducing the bioavailability (Crichton, 1987). The stability and solubility of dietary iron are determined by the ligands present in food, which could chelate all six coordination positions of iron species (May and Williams, 1980). Tea polyphenols and milk proteins act as ligands, but mostly they result in forming high molecular weight compounds, which could not get absorbed in the gut, even though being in soluble form (Disler *et al.*, 1975; Hallberg, 1981; Hurrell *et al.*, 1999). However, chelators such as ascorbic acid form monomeric and soluble compounds with dietary iron and are found to significantly counteract the inhibitory effect of polyphenols (Hallberg *et al.*, 1989; Siegenberg *et al*., 1991).

The fate of dietary iron depends on the type of complex formed in the system. The possible complexes that could be formed in the system are polyphenol-iron, protein-iron, polyphenol-protein, and polyphenol-protein-iron complex. Polyphenol-iron complex is formed by catecholic complexation and polymerization reactions, whereas protein (casein)-iron complex is formed through binding of

*Corresponding author.
Email: *gaur2@illinois.edu*
Tel: +1 (217) 419 - 8286

iron to clusters of phosphoserine residues (Bernos *et al.*, 1997; Gaucheron *et al*., 1997).

Polyphenol-protein complexes could also significantly influence iron bioavailability. Tea polyphenols are reported to have greater affinity to complex with proteins than iron, with direct relation to molecular weight of polyphenols (Brown and Wright, 1963; Farkas and Riche, 1987; Kim and Miller, 2005; Yuksel *et al.*, 2010). Tea polyphenols being higher molecular weight compounds are highly reactive and could readily combine with sulfhydryl and amino groups of proteins (Gaucheron *et al.*, 1997). In a recent study, polyphenols were found to alter the structure of proteins by decreasing α-helix and β-sheet and resulting in protein unfolding (Liang and Xu, 2003; Hasni *et al.*, 2011). Kartsova and Alekseeva (2008) concluded that binding of casein and tea polyphenols reduced concentration of free polyphenols, using micellar electrokinetic chromatography. Previously, Farkas and Riche (1987) hypothesized that addition of milk to tea could improve iron bioavailability, as the casein-polyphenol complex thus formed could remain intact at the point of digestion, preventing any interaction with dietary iron, rendering it available and soluble for absorption.

In addition, minerals in milk such as calcium and phosphates could also affect dietary iron absorption (Monsen and Cook, 1976; Hallberg *et al.*, 1991), possibly by changing intraluminal ligand balance, modifying gastrointestinal transit time, competing for receptors on the brush border membrane, and disturbing of iron transport through the mucosal cells (Hallberg *et al.*, 1991). *In vitro* studies have been widely used for estimation of relative iron bioavailability (Miller and Berner, 1981; Kane and Miller, 1984; Hurrell *et al*., 1989; Hurrell *et al.*, 1999). In this study, a modification of in vitro digestion method (Miller *et al.*, 1981) was used to investigate the effect of milk addition to tea on iron bioavailability under simulated gastrointestinal conditions.

Materials and Methods

Materials

Indian black tea (Brooke bond Taj Mahal tea) and whole milk were procured from local market, Ithaca, NY. Nonheme ferric iron, $FeCl_3$, 1000 ppm in Fe (Certified Atomic Absorption Standard (SO-I-124) and Spectrapore® I dialysis tubing with a molecular weight cut-off of 6000-8000 (08-670C), were procured from Fisher Scientific. Porcine pepsin (P7000), pancreatin (1750), bile (B8631), PIPES buffer solution (piperazine-N, N'-bis[2-ethane-sulfonic acid]) (P3768), HEPES buffer (N-2-hydroxyethyl-piperazine- N'-2-ethanesulfonic acid) sodium salt (H7006), Ferrozine chromogen solution (3-(2-pyridyl)-5,6- bis (4-phenyl-sulfonic acid)-1,2,4-triazine), disodium salt (P9762), trichloroacetic acid and hydroxylamine monohydrochloride, were procured from Sigma-Aldrich.

Preparation of reagents

All the glassware was washed with detergent, rinsed with distilled water, soaked overnight in 1 N HCl, rinsed again with distilled water and dried. Pepsin solution was prepared by suspending 4.0 g pepsin in 0.1 N HCl and diluting to 100 ml with 0.1 N HCl. Pancreatin-bile mixture was prepared by suspending 0.5 g pancreatin and 3.0 g bile extract in 0.01 N $NaHCO_3$ and diluting to 250 ml with 0.1 N $NaHCO_3$. PIPES buffer solution, 0.15 M, was adjusted to pH 6.3 using concentrated HCl and HEPES buffer was prepared at 0.3 M with no pH adjustment (pH~10). Protein precipitant solution (reducing) was prepared by dissolving 100 g trichloroacetic acid and 50 g hydroxylamine monohydrochloride in distilled water with further addition of 100 ml concentrated HCl and making up volume to 1L with distilled water. Ferrozine chromogen solution was prepared at 5 mg/ml in distilled water. Dialysis bags were cut into 20 cm lengths and soaked in water for at least one hour prior to use.

Preparation of treatments

Black tea infusion was prepared by boiling 3 g of tea leaves in 100 mL boiling water for 10 minutes, followed by filtration using Whattman's filter paper. The mixture was allowed to stand for 15 minutes at room temperature. Three treatments namely tea only (A), tea+milk (B) and milk only (C), were prepared. In the case of treatment (A) 50 mL of infusion, for treatment (B) 25 mL of infusion and 25 mL of boiled milk, and for treatment (C) 50 ml of milk was transferred to 100 mL beaker and boiled for five minutes. 28 mL of 1000 ppm FeCl3 was added all the treatments, and the volume was diluted to 100 mL with 0.01 N HCl, yielding solutions that were 5mM in iron.

In vitro design

The *in vitro* design developed by Miller *et al.* (1981) and modified by Kane and Miller (1984) was used to assess relative iron bioavailability. Water bath was set at 37° C and dialysis bags were cut in 20 cm and soaked in water for one hour. 10 mL aliquots of each of the treatments, blank and control were transferred to vials, in duplicate and mixed with 10

mL of 0.01 N HCl. 1 mL of Pepsin suspension was added to each vial. The mixtures were incubated at 37°C in a shaking water bath for two hours. At the end of pepsin incubation, a dialysis bag containing 20 mL of PIPES buffer was placed in each vial, and the samples were incubated for 30 minutes. 5 mL of the pancreatin-bile mixture was added to each vial and incubation was continued for two more hours. At the end of pancreatin-bile incubation, the dialysis bags were removed and rinsed by dipping in distilled water. Bag contents were transferred to tared beakers and weighed. The pH of each dialysate and retentate was measured. The experiment was conducted on a timed schedule so that all samples were incubated for the same time.

Iron assay

Dialyzable iron and total soluble iron were measured using a modification of the method proposed by Reddy *et al.* (1986). For the measurement of total iron, reducing precipitant solution was added to 2 mL aliquots of dialysate and retentate. Samples were held overnight at room temperature. Subsequently they were centrifuged in a benchtop centrifuge at 2575 x g for 10 minutes. Aliquots of the supernatant were transferred to separate tubes. Ferrozine solution (0.25 ml) and HEPES buffer (2.0) ml were added to each tube. Absorbance at (562 nm) was measured after one hour for the total iron determination. Sample iron concentrations were calculated from absorbance readings using a regression equation derived from data generated from standards.

Ferrozine reagent reacts with divalent iron to form a stable magenta complex species.

Preparation of control and blank

$FeCl_3$ (5 mM) was used as control and blanks were prepared by the same procedure except iron was not added to it.

Calculations

Dialyzable total iron (D-Fe(II)$^+$Fe(III)), total soluble iron and soluble iron in retentate were expressed as percentages of the total added Fe(III) in each vial. It was assumed that dialyzable iron had equilibrated across the dialysis membrane by the time dialysis bag was removed at the end of digestion.

Dialyzable iron

$$\frac{\{[Fe(II) + Fe(III)]_D (\mu g/ml) \times totalvolume(46\ ml)\}}{Fe(III)\ in\ original\ sample(\mu g)} \times 100$$

Total soluble iron

$$\frac{\{[Fe(II) + Fe(III)]_D (\mu g/ml) \times dialysate\ volume(ml)\} + \{[Fe(II) + Fe(III)]_R (\mu g/ml) \times retentate\ volume(ml)\}}{Fe(III) in\ original\ sample(\mu g)} \times 100$$

Soluble iron in retentate

$$\frac{\{[Fe(II) + Fe(III)]_R (\mu g/ml) \times retentate\ volume(ml)\}}{Fe(III)\ in\ original\ sample(\mu g)} \times 100$$

Where D stands for dialysate and R stands for retentate. Blanks were used to blank the spectrophotometer.

Statistics

Data were analyzed using ANOVA and means of the samples were considered to be different at 5% significance level. Further, Dennett's test was conducted to compare the group means and to identify the sample/samples which is/are different from each other.

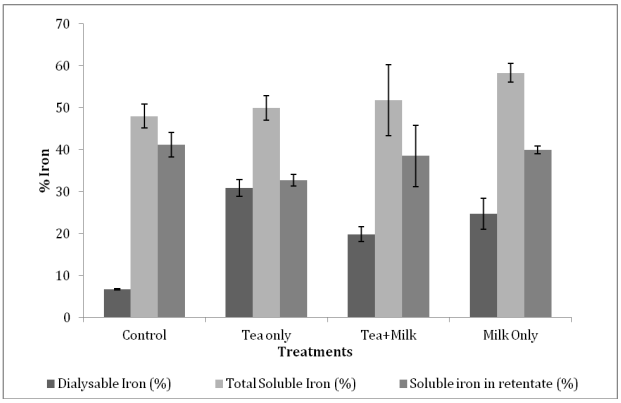
Results and discussion

The percentage values for total dialyzable iron, total soluble iron and soluble iron in retentate are provided in Figure 1. Results showed that, addition of milk to tea significantly reduced the concentration of dialyzable iron ($\alpha<0.05$) compared to tea or milk alone. The percentage of dialyzability from treatment of tea+milk was 35.65% less than treatment of tea and 19.78% less than treatment of milk. Dialyzable iron and total soluble iron concentration were significantly lower in control compared to all other treatments. Soluble iron in the retentate was lowest in tea and highest in control.

The lower dialyzability ($\alpha<0.05$) of iron from treatment of tea + milk was unexpected. We expected either an increase or no significant effect in iron dialyzability, based on previous studies (Disler *et al*., 1975; Hurrell *et al*., 1989). Numerous researchers have reported increase in iron bioavailability from solutions of tea polyphenols and proteins due to complex formation between tea polyphenol and milk protein, rendering dietary iron free and available for absorption (Farkas and Riche, 1987; Trevisanato and Kim, 2000; Kartsova and Alekseeva, 2008).

The dialyzability of iron through dialysis membrane was based on the molecular weight of compounds formed due to interactions between polyphenols, casein, and iron. Dialysis step allowed only the soluble iron species with molecular weight less than 6000-8000 Da to dialyze through the membrane. Lower dialyzability in the case of control can be attributed to polymerization of ferric iron into high molecular weight polymer, which was not able to cross the dialysis membrane (Crichton, 1987).

Iron in tea solution was seen quite soluble and dialyzable, possibly due to availability of tea polyphenols as soluble ligands for binding with iron. These ligands occupied all six coordination positions of iron and prevented its polymerization. The lower



Treatments	Dialysable Iron (%)	Total Soluble Iron (%)	Soluble iron in retentate (%)
Control	6.67 ± 0.09^{c}	47.99 ± 2.88^{c}	41.13 ± 2.91^{a}
Tea only	30.82 ± 2.03^{a}	49.94 ± 2.92^{b}	32.73 ± 1.41^{c}
Tea+Milk	19.83 ± 1.71^{b}	51.73 ± 8.47^{b}	38.51 ± 7.34^{b}
Milk Only	24.72 ± 3.73^{a}	58.32 ± 2.26^{a}	39.97 ± 0.91^{b}

Figure 1. Percentage of dialyzable iron, soluble and total soluble iron from in vitro digestion of control, tea, tea+milk and milk

Values are mean ± SD (n=3) *Mean in each column within each iron species not sharing a common superscript letter is significantly ($\alpha<0.05$) different

molecular weight complexes thus were able to dialyze through the membrane leaving lower percentage of soluble iron in retentate (May and Williams, 1980).

In the case of milk, addition of 0.01N HCl to milk caused the coagulation of milk proteins and formed high molecular weight compounds (≈20,000-25,000 Da). However, pepsin-pancreatin digestion of casein, released low molecular casein phosphopeptides (CPP), which were more soluble and available to react with iron in the solution (Saxena and Seshadri, 1988; Hurrell *et al*., 1989; Kitts and Yuan, 2004). This finding is explained by relatively higher total soluble iron and soluble iron in retentate, in case of milk treatment. The lower dialyzability from milk treatment could be attributed to high molecular casein phosphopeptides and presence minerals such as calcium in milk providing competition to iron for soluble ligands (Monsen and Cook, 1976; Kane and Miller, 1984; Hallberg *et al*., 1991).

Treatment tea + milk showed lowest dialyzability for iron. The two possible reasons for this behavior are: (a.) Low molecular peptides formed due to pepsin pancreatin digestion were smaller in size and rendered large surface area for binding with polyphenols. As a result, free polyphenols occupied all the available spaces on the peptides and made polyphenols unavailable as soluble iron ligands. (b.) Formation of polyphenol-peptide-iron complex precipitated iron and being high molecular weight compounds, these complex were not able to cross the membrane.

The results from *in vitro* studies accurately predicted the direction of response but further in vivo studies are essential to validate the results and to have detailed and quantitative understanding of iron bioavailability, mechanism involved, etc. Interpretation of *in vitro* studies data requires extra caution while dealing with complex systems involving proteins and polyphenols. For example, in-vitro results may only be valid for a person who never drinks tea. The chronic exposure to tea stimulates the secretion of proline-rich proteins (PRP's), which have the capability of binding the polyphenols. This prevents the interaction of iron with polyphenols and thus reduces the deleterious effects of polyphenols. Therefore, the role of PRP should be considered while determining the bioavailability from polyphenol containing diet (Kim and Miller, 2005). Similarly, other factors such as enzyme concentrations, sites of absorption, diffusion barriers, composition and enhancers or inhibitors in the diet, also play a significant role in determining the iron bioavailability and differ from person to person (Miller and Berner, 1989).

In conclusion, consumption of chai (tea with milk) with diets might significantly influence the iron bioavailability by inhibiting iron absorption in the gut. Further investigation in this area of research would help nutritionists in developing effective strategies to combat problem of iron deficiency.

References

Arnold, F., Parasuraman, S., Arokiasamy, P., and Kothari, M. 2009. Nutrition in India. National Family Health Survey (NFHS-3), India, 2005-06. Mumbai: International Institute for Population Sciences; Calverton, Maryland, USA: ICF Macro.

Bernos, E., Girardet, J.M., Humbert, G. and Linden, G. 1997. Role of the O-phosphoserine clusters in the interaction of the bovine milk αs1-, β-, κ-caseins and the PP3 component with immobilized iron (III) ions. Biochima et Biophysica Acta 1337: 149–159

Brown, P.J. and Wright, W.B. 1963. An Investigation of the interactions between milk proteins and tea polyphenols. Journal of Chromatography 11: 504-514.

Crichton, R.R. 1987. Iron metabolism and oxygen toxicity. Bioelectrochemistry and Bioenergetics 18 (1-3): 105-116.

Disler, P.B., Lynch, S.R., Charlton, R.W., Torrance, J.D., Bothwell, T.H., Walker and R.B., Mayet, F. 1975. The effect of tea on iron absorption. Gut 16 (3): 193-200.

Farkas, C.S. and Riche, W.H. 1987. Effect of tea and coffee consumption on nonheme iron-absorption - some questions about milk. Human Nutrition Clinical Nutrition 41c (2): 161-163.

Gaucheron, F., Le Graet, Y., Boyaval, E. and Piot, M. 1997. Binding of cations to casein molecules: importance of physicochemical conditions. Milchwissenschaft Milk Science International 52 (6): 322- 327.

Hallberg, L., Brune, M., Erlandsson, M., Sandberg, A. and Rossander, L. 1991. Calcium: effect of different amounts on nonheme and heme-iron absorption in humans. The American Journal of Clinical Nutrition 53 (1): 112-119.

Hallberg, L. 1981. Bioavailability of dietary iron in man. Annual Review of Nutrition 1: 123-147.

Hallberg, L., Brune, M. and Rossander, L. 1989. Iron-absorption in man – ascorbic acid and dose-dependent inhibition by phytate. American Journal of Clinical Nutrition 49 (1): 140-144.

Hasni, I., Bourassa, P., Hamdani, S., Samson, G., Carpentier, R. and Tajmir-Riahi, H. 2011. Interaction of milk α- and β-caseins with tea polyphenols. Food Chemistry 126 (2): 630-639.

Hurrell, R., Lynch, S., Trinidad, T., Dassenko, S. and Cook, J. 1989. Iron absorption in humans as influenced by bovine milk proteins. The American Journal of Clinical Nutrition 49 (3): 546-552.

Hurrell, R.F., Reddy, M. and Cook, J.D. 1999. Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. British Journal of Nutrition 81 (4): 289-295.

International Institute for Population Sciences (IIPS) & Macro International. 2007. National Family Health Survey (NFHS-3). 2005–06. IIPS, Mumbai, India.

Indiastat. 2007. Ministry of Finance & Company Affairs, Govt. of India and Ministry of Rural Development, Govt. of India [Online]. Downloaded from *http://www.indiastat.com/percapitaavailability/24/fooditems/103/stats.aspx* on 29/11/2014.

Kane, A.P. and Miller, D.D. 1984. In vitro estimation of the effects of selected proteins on iron bioavailability. American Journal of Clinical Nutrition 39 (3): 393-401

Kartsova, L. and Alekseeva, A. 2008 Effect of milk caseins on the concentration of polyphenolic compounds in tea. Journal of Analytical Chemistry 63 (11): 1107-1111.

Kim, H. and Miller, D.D. 2005. Proline-Rich Proteins Moderate the Inhibitory Effect of Tea on Iron Absorption in Rats. The Journal of Nutrition 135 (3): 532-537.

Kitts, D.D. and Yuan, Y.V. 2004. Caseinophosphopeptides and calcium bioavailability. Trends in Food Science and Technology 3: 31-35.

Liang, Y. and Xu, Y. 2003. Effect of extraction temperature on cream and extractability of black tea [*Camellia sinensis* (L.) O. Kuntze]. International Journal of Food Science and Technology 38: 37–45.

May, P.M. and Williams, D.R. 1980. The inorganic chemistry of iron metabolism. In: Jacobs, A. and Worwood, M., Editors, 1980. Iron in Biochemistry and Medicine II, Academic Press, New York/London, pp. 1–28.

Miller, D.D. and Berner, L. 1989. Is solubility in vitro a reliable predictor of iron bioavailability? Biological Trace Elemental Research 19 (1):11-24.

Miller, D.D., Schricker, B.R., Rasmussen, R.R. and Campen, D.V. 1981. An in vitro method for estimation of iron availability from meals. Am Journal of Clinical Nutrition 34 (10): 2248-2256.

Monsen, E.R. and Cook, J.D. 1976. Food iron-absorption in human subjects. IV. The effects of calcium and phosphate salts on the absorption of nonheme iron. The American Journal of Clinical Nutrition 29 (10): 1142-1148.

Nair, K.M. and Iyengar, V. 2009. Iron content, bioavailability & factors affecting iron status of Indians. Indian Journal Medical Research 130 (5): 634-645.

NNMB Technical Report No. 22. 2003. National Nutrition Monitoring Bureau (NNMB).Prevalence of micronutrient deficiencies. National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India.

Reddy, M.B., Chidambaram, M.V., Fonseca, J., Bates and G.W. 1986. Potential role of *in vitro* iron bioavailability studies in combating iron-deficiency - a study of the effects of phosvitin on iron mobilization from pinto beans. Clinical Physiology and Biochemistry 4 (1): 78-86.

Saxena, A. and Seshadri, S. 1988. The effect of whole milk, milk protein and some constituent amino acids on the in vitro availability of iron from cereal meals. Nutrition Research 8 (7): 717-724.

Siegenberg, D., Baynes, R.D., Bothwell, T.H., Macfarlane, B.J., Lamparelli RD, Car NG, MacPhall P, Schmidt U, Tal A and Mayet F. 1991. Ascorbic-acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. American Journal Clinical Nutrition 53(2): 537-541.

Trevisanato, S.I., and Kim, Young-In. 2000. Tea and health. Nutrition Reviews 58, no. 1, (January 1): 1-10

Yuksel, Z., Avci, E. and Erdem, Y.K. 2010. Characterization of binding interactions between green tea flavanoids and milk proteins. Food Chemistry 121 (2): 450-456.