Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages

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The effects of different polyphenol-containing beverages on Fe absorption from a bread meal were estimated in adult human subjects from the erythrocyte incorporation of radio-Fe. The test beverages contained different polyphenol structures and were rich in either phenolic acids (chlorogenic acid in coffee), monomeric flavonoids (herb teas, camomile (*Matricaria recutita* L.), vervain (*Verbena officinalis* L.), lime flower (*Tilia cordata* Mill.), pennyroyal (*Mentha pulegium* L.) and peppermint (*Mentha piperita* L.), or complex polyphenol polymerization products (black tea and cocoa). All beverages were potent inhibitors of Fe absorption and reduced absorption in a dose-dependent fashion depending on the content of total polyphenols. Compared with a water control meal, beverages containing 20–50 mg total polyphenols/serving reduced Fe absorption from the bread meal by 50–70 %, whereas beverages containing 100–400 mg total polyphenols/serving reduced Fe absorption by 60–90 %. Inhibition by black tea was 79–94 %, peppermint tea 84 %, pennyroyal 73 %, cocoa 71 %, vervain 59 %, lime flower 52 % and camomile 47 %. At an identical concentration of total polyphenols, black tea was more inhibitory than cocoa, and more inhibitory than herb teas camomile, vervain, lime flower and pennyroyal, but was of equal inhibition to peppermint tea. Adding milk to coffee and tea had little or no influence on their inhibitory nature. Our findings demonstrate that herb teas, as well as black tea, coffee and cocoa can be potent inhibitors of Fe absorption. This property should be considered when giving dietary advice in relation to Fe nutrition.

Iron absorption: Polyphenols: Coffee: Tea

Fe deficiency is the most common micronutrient deficiency in the world (World Health Organization, 1992). When severe, it leads to Fe-deficiency anaemia which is associated with poor health, increased risk of maternal perinatal death, and serious functional impairments that diminish human development and productivity (International Nutritional Anemia Consultative Group, 1993). A major cause of Fe deficiency is the impaired absorption of non-haem Fe (Charlton & Bothwell, 1983) due to the presence of potent inhibitors of absorption such as phytic acid or polyphenol compounds in many plant foods (Fairweather-Tait & Hurrell, 1996).

Polyphenol compounds are widely present in the human diet as components of fruits, vegetables, spices, pulses and cereals, and they are especially high in tea, coffee, red wine, cocoa and the different herb teas. In the USA, consumption of polyphenols is estimated at 1 g/d (Kuehnau, 1979) and in the UK as much as 0·5 g polyphenol/d is ingested from tea alone (Stagg & Millin, 1975). The phenolic compounds are released from the food or beverage during digestion, and can complex with Fe in the intestinal lumen making it unavailable for absorption. The consumption of black tea and coffee has been shown to strongly inhibit Fe absorption from composite meals (Disler *et al*. 1975; Hallberg & Rossander, 1982; Morck *et al*. 1983), with coffee having about half the inhibitory effect of tea.

Other beverages such as red wine (Bezwoda *et al*. 1985; Cook *et al*. 1995) and cocoa (Gillooly *et al*. 1984), as well as various vegetables with a high polyphenol content (Gillooly *et al*. 1983; Tuntawiroon *et al*. 1991), have also been reported to inhibit Fe absorption. Red wine polyphenols would appear to be less inhibitory than the phenolics of tea or coffee. They reduced Fe absorption from a simple bread-roll meal (Cook *et al*. 1995), but had little effect on Fe absorption from more complex composite meals (Hallberg & Rossander, 1982). On the other hand a serving of yod kratin (leaves of the lead tree, *Leucaena glauca*), a vegetable consumed widely in Thailand and that is high in phenolics, reduced Fe absorption

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from a composite meal of rice, fish and vegetables by almost 90 % (Tuntawiroon *et al*. 1991). Fe absorption from other vegetables rich in polyphenols, such as spinach and aubergine, has also been observed to be low and a significant negative correlation has been reported between the total polyphenol content of vegetable foods and absorption of their Fe content in man (Gillooly *et al*. 1983).

The present study was designed to investigate further the relative influence of the different polyphenolic-containing beverages on Fe absorption in man, by feeding beverages rich in either phenolic acids (coffee), monomeric flavonoids (herb teas) or complex polyphenol polymerization products (black tea and cocoa). Using the extrinsic tag radio-Fe technique, we have compared the effect of the herb teas, peppermint (*Mentha piperita* L.), vervain (*Verbena officinalis* L.), lime flower (*Tilia cordata* Mill.), pennyroyal (*Mentha pulegium* L.) and camomile (*Matricaria recutita* L.), with the influence of coffee, cocoa and different concentrations of black tea on Fe absorption from a bread meal. The effect of adding milk on the inhibitory nature of tea and coffee was also investigated.

Subjects and methods

Subjects

Eight separate Fe absorption studies were performed in a total of seventy-seven volunteer subjects. Each study contained between eight and ten subjects. The composite group included twenty-three males and fifty-four females ranging in age from 19 to 40 years. All subjects were in good health and denied a history of disorders known to influence the gastrointestinal absorption of Fe. None of the subjects was anaemic although serum ferritin concentrations ranged from 9 to 193 μg/l, indicating a wide variation in Fe status. Five female subjects were Fe-deficient as defined by a serum ferritin concentration $\leq$12 μg/l. Written informed consent was obtained from each volunteer before the investigation and all experimental procedures were approved by the Human Subjects Committee at the University of Kansas Medical Center.

Absorption measurements

Fe absorption was measured using the extrinsic tag radio-Fe technique with ^{55}Fe and ^{59}Fe (Cook *et al*. 1972). The added radio-Fe uniformly labels all the non-haem food Fe in the meal. Fe absorption was measured from different meals labelled with either ^{55}Fe or ^{59}Fe and consumed on consecutive days. At 2 weeks after consuming the labelled meals, Fe absorption was estimated from the radioactivity incorporated into circulating erythrocytes.

In our studies four separate Fe absorption measurements were performed in each subject by using radio-Fe tracers administered after an overnight fast and nothing but water was allowed for a further 3 h after the meal was given. The test meals were labelled as previously described (Cook *et al*. 1972) by adding either 37 kBq $^{59}FeCl_3$ or 111 kBq $^{55}FeCl_3$ to a 1 ml solution containing 0·1 mg Fe as $FeCl_3$ in 0·01 M-HCl.

On the day preceding administration of the first test meal, 30 ml blood was obtained from each subject for measurement of packed cell volume, serum ferritin (Flowers *et al*. 1986), and background radioactivity. The first and second test meals, labelled with ^{55}Fe and ^{59}Fe respectively, were given on days 1 and 2 of the study. At 14 d after administration of the second of these meals (day 16), 30 ml blood was drawn for measurement of incorporated blood cell radioactivity. A third and a fourth test meal tagged with separate radio-Fe labels were given on days 16 and 17 and a final blood sample was obtained on day 31 to determine the increase in erythrocyte radioactivity. Measurements of blood radioactivity were performed on duplicate 10 ml samples of whole blood by a modification of the method of Eakins & Brown (1966). Percentage absorption was calculated on the basis of the blood volume estimated from height and weight (Wennesland *et al*. 1959; Brown *et al*. 1962) and an assumed erythrocyte incorporation for absorbed radioactivity of 80 % (Hosein *et al*. 1967).

Test beverages

Ten different beverages were used in the absorption studies. The polyphenol and Fe contents of these beverages are shown in Table 1. Total polyphenols were measured in the tea, herb teas and cocoa beverages using the Folin-Ciocalteau method (Singleton & Rossi, 1965) with catechin as a standard. Coffee was analysed for chlorogenic acid by the AOAC method (Association of Official Analytical Chemists, 1990). Fe was determined by atomic absorption spectroscopy after dry ashing.

The Assam tea A (*Camellia sinensis* L.) and the herb teas, camomile, vervain and lime flower were purchased from local shops in Switzerland. Assam tea B was purchased from a local store in Kansas City as were the herb teas, peppermint A and B and pennyroyal. The instant coffee was Tasters Choice (Nestlé Beverage Company, San Francisco, CA, USA).

The black tea and the herb teas were prepared in an identical way. Boiling water (300 ml) was added to 3 g tea, and the mixture was left to infuse for 10 min before straining and serving. The coffee and cocoa were prepared by adding 2 g and 5 g powder respectively to 275 ml boiling water.

Table 1. Polyphenol and iron contents of test beverages

Beverage	Polyphenols* (mg/serving)	Iron (μg/serving)
Assam tea A	274	<4
Assam tea B	396	12
Peppermint A	177	173
Peppermint B	209	83
Pennyroyal	121	204
Vervain	116	NA
Lime flower	58	NA
Camomile	52	NA
Cocoa	116	NA
Instant coffee	120	66

NA, not analysed.
* For details of analytical methods see p. 290. Polyphenol content of instant coffee is given as mg chlorogenic acid per 275 ml serving, values for all other beverages are given as mg catechin equivalents per 275 ml serving.

Test meals

The four test meals for each of the eight studies are shown in Table 2. In studies 1–3 the inhibitory effects of various herb teas on Fe absorption were compared with the inhibitory effects of black tea and cocoa. Fe absorption from an Fe-fortified bread roll, consumed together with water, was compared with Fe absorption from a similar roll consumed together with either black tea, cocoa, peppermint tea, pennyroyal tea or infusions of vervain, lime flower or camomile. In studies 4 and 5 the influence of the concentration of black tea on Fe absorption was investigated by progressively diluting the full strength tea with water to give 50, 25, 10 and 5 % of the original concentration. Fe absorption from an Fe-fortified bread roll, consumed together with water, was compared with Fe absorption from a similar bread roll consumed together with different concentrations of black tea. In study 6, we studied whether the addition of milk to tea and coffee influenced their inhibitory effects on Fe absorption. Fe absorption from an Fe-fortified bread roll consumed together with black tea or coffee was compared with Fe absorption from a similar bread roll consumed together with tea or coffee plus 30 ml homogenized regular whole milk. In study 7, we investigated whether bread influenced Fe absorption from tea or water and in study 8, we compared the relative inhibitory nature of the different beverages on Fe absorption in the absence of the bread meal.

Except in study 8, all test meals contained 50 g bread, 10 g butter and 275 ml either water, herb tea, black tea, cocoa or instant coffee. In study 8, the beverages only were consumed. Sugar (10 g) was added to all beverages except water. The radio-Fe tracers were pipetted onto the bread rolls (studies 1–7) or added directly to the beverage (study 8). In studies 1, 3, 4, 5 and 6 a commercial bread roll (Butternut Enriched

Table 2. Iron absorption from test meals consisting of a polyphenol-containing beverage with or without bread

Study, no. of subjects, sex and mean age	Serum ferritin† (μg/l)	Meals	Iron absorption‡ (% dose)	Absorption ratio *v.* meal D (water)‡
1	34	A Bread, Assam tea A	0·74 (0·57, 0·95)	0·06*** (0·04, 0·07)
(*n* 2M, 7F, 25 years)	(9–90)	B Bread, peppermint tea A	2·01 (1·50, 2·70)	0·16*** (0·13, 0·19)
		C Bread, pennyroyal tea	3·53 (2·52, 4·96)	0·27*** (0·23, 0·33)
		D Bread, water	12·9 (10·7, 15·6)	–
2	39	A Bread, Assam tea A	0·89 (0·68, 1·16)	0·16*** (0·13, 0·19)
(*n* 5M, 5F, 23 years)	(18–144)	B Bread, vervain tea	2·32 (1·99, 2·70)	0·41*** (0·37, 0·46)
		C Bread, lime flower tea	2·71 (2·17, 3·39)	0·48** (0·41, 0·57)
		D Bread, water	5·63 (4·64, 6·84)	–
3	61	A Bread, Assam tea B	0·92 (0·72, 1·18)	0·21*** (0·17, 0·25)
(*n* 6M, 4F, 27 years)	(13–193)	B Bread, camomile tea	2·35 (1·75, 3·14)	0·53*** (0·45, 0·61)
		C Bread, cocoa	1·29 (1·02, 1·63)	0·29*** (0·26, 0·33)
		D Bread, water	4·46 (3·47, 5·72)	–
4	33	A Bread, Assam tea B (100 %)§	0·59 (0·45, 0·78)	0·09*** (0·08, 0·11)
(*n* 3M, 6F, 23 years)	(20–59)	B Bread, Assam tea B (50 %)	1·05 (0·72, 1·53)	0·16*** (0·12, 0·21)
		C Bread, Assam tea B (25 %)	1·18 (0·84, 1·66)	0·18*** (0·14, 0·23)
		D Bread, water	6·58 (4·76, 9·10)	–
5	50	A Bread, Assam tea B (25 %)	0·66 (0·45, 0·96)	0·15*** (0·11, 0·21)
(*n* 9F, 22 years)	(13–104)	B Bread, Assam tea B (10 %)	1·48 (0·99, 2·21)	0·34* (0·25, 0·47)
		C Bread, Assam tea B (5 %)	1·47 (1·03, 2·12)	0·34** (0·27, 0·43)
		D Bread, water	4·33 (3·31, 5·67)	–
6	47	A Bread, Assam tea A	0·71 (0·51, 0·98)	–
(*n* 1M, 9F, 28 years)	(12–76)	B Bread, Assam tea, milk	0·96 (0·76, 1·20)	–
		C Bread, coffee	2·81 (2·04, 3·86)	–
		D Bread, coffee, milk	2·88 (2·18, 3·18)	–
7	35	A Bread, Assam tea B	0·83 (0·56, 1·23)	–
(*n* 2M, 8F, 29 years)	(12–156)	B Assam tea B	1·00 (0·73, 1·38)	–
		C Bread, water	8·64 (6·00, 12·4)	–
		D Water	29·4 (24·4, 35·3)	–
8	36	A Assam tea B	0·75 (0·58, 0·96)	0·03*** (0·02, 0·03)
(*n* 4M, 6F, 26 years)	(9–48)	B Peppermint tea B	4·66 (3·41, 6·37)	0·16*** (0·13, 0·21)
		C Cocoa	1·61 (1·19, 2·18)	0·06*** (0·05, 0·07)
		D Water	28·5 (21·8, 37·1)	–

M, male; F, female.
Mean values were significantly different from one: *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$.
† Geometric mean values with ranges in parentheses.
‡ Geometric mean values with the variance (−1 SE, +1 SE) in parentheses.
§ Assam tea contained 396 mg polyphenols/275 ml serving, 5–50 % concentrations were made by diluting 100 % Assam tea with hot water and giving as a 275 ml serving.

Dinner Roll, Interstate brand, Kansas City, KS, USA), enriched with $FeSO_4$ and containing 2·1 mg Fe per 50 g serving, was used. In study 2, a similar commercial $FeSO_4$-enriched roll (Wonder Brown and Serve Enriched Roll, Continental Bakery Company, St Louis, MO, USA) was used. For study 7, the bread roll was prepared and baked in the laboratory from 60 % extraction non-enriched wheat flour (Nestlé Research and Development Centre, Orbe, Switzerland), salt, sugar, yeast and water. Each 50 g roll contained 2·1 mg Fe, of which 1·72 mg was added as $FeSO_4$ during preparation.

Statistical methods

Percentage absorption values were converted to logarithms before performing statistical analysis and the results were re-transformed to antilogarithms to recover the original units (Cook *et al*. 1969). Paired tests were used to compare absorption from selected test meals within each study by determining whether the mean log absorption ratios differed significantly from zero.

Results

There was a wide variation in the total polyphenol content which ranged from 52 mg per serving of camomile tea to 396 mg in one sample of black Assam tea (Table 1). The beverages all contained a small amount of Fe (< 4–204 μg/serving) which increased slightly the Fe intake of 2·1 mg coming from the Fe-fortified bread roll.

In studies 1–3 (Table 2) which compared peppermint teas, cocoa and black tea, the black tea had the greatest inhibitory effect on Fe absorption. The Fe absorption ratio of the black tea meals compared with water control meals was 0·06–0·21, with the absolute Fe absorption from the water meal varying from 4·46 % in study 3, to 12·9 % in study 1. All herb teas and cocoa also significantly reduced Fe absorption from the bread meal. Compared with the water control meal, the absorption ratios were: peppermint 0·16, pennyroyal 0·27, cocoa 0·29, vervain 0·41, lime flower 0·48 and camomile 0·53.

Black tea was very inhibitory even at low concentrations. In studies 4 and 5, Fe absorption and the Fe absorption ratio compared with the water meal increased as the tea was progressively diluted. However, even a 5 % concentration of the original black tea, containing only 20 mg polyphenol/serving, still reduced Fe absorption by almost 70 % (absorption ratio compared with the water meal 0·34), demonstrating the potent inhibitory effect of black tea polyphenols on Fe absorption.

The addition of milk had little or no effect on the inhibitory nature of tea or coffee (study 6). Unlike studies 1–5, study 6 did not include a water control meal. Fe absorption from the bread meal with black tea was 0·71 % compared with 2·81 % with coffee. Adding milk to tea increased absorption slightly to 0·96 % (absorption ratio 1·36; $P < 0·05$) but had no influence on Fe absorption from coffee (absorption ratio 1·04; $P > 0·05$).

Bread likewise had little influence on the inhibitory effect of black tea although adding it to water greatly reduced Fe absorption (study 7). Fe absorption from black tea and bread was similar to that from tea alone (0·83 *v.* 1·00 %; absorption ratio 1·21; NS). Fe absorption from bread and water however (8·64 %) was 3-fold lower than from water alone

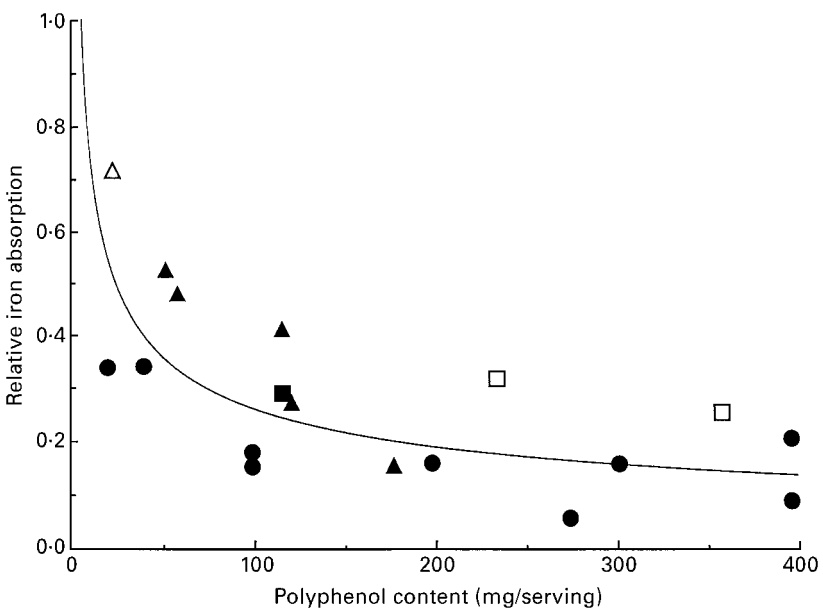

Fig. 1. Variation in relative iron absorption from a bread and beverage meal according to the polyphenol content of the beverage. Relative iron absorption is defined as iron absorption (% dose) from a bread meal consumed together with a beverage relative to iron absorption in the same subject from a bread meal consumed with water. (●), Black tea; (▲), herb teas; (△), white wine; (■), cocoa; (□), red wine. Values for red and white wine are taken from Cook *et al.* (1995).

(29·4 %; absorption ratio 3·40; $P < 0·001$). In the absence of the bread roll, Fe absorption from black tea, peppermint and cocoa was still far lower than from water alone, the absorption ratios being 0·03 for black tea, 0·16 for peppermint and 0·06 for cocoa (study 8).

In Fig. 1, the relative inhibitory effects of the different polyphenol-containing beverages have been compared by plotting the polyphenol content of the beverages (mg/serving) against relative Fe absorption. Fig. 1 also includes data on red wine from the study of Cook *et al*. (1995) which we made using exactly the same methodology. Polyphenols from the different beverages inhibited Fe absorption in a dose-dependent fashion and relative Fe absorption from different concentrations of polyphenols fitted best onto the curve following a potential law (relative Fe absorption $= 2 \times$ (mg polyphenol/serving)$^{-0·5}$). At low polyphenol concentrations (about 50 mg/serving) black tea was more inhibitory to Fe absorption than the herb teas, camomile and lime flower, however, at higher polyphenol concentrations (about 200 mg/serving), black tea and peppermint tea were equally inhibitory. At equivalent polyphenol concentrations, black tea was more inhibitory than white wine, red wine and cocoa.

Discussion

Plant polyphenols

The functional group of polyphenol compounds is an aromatic ring structure with one or more hydroxyl groups, and at least 5000 different compounds have been described in plant tissues (Harborne, 1993) including over 2000 naturally occurring flavonoids. The smaller molecules may polymerize either in the plant tissue or during food processing with the largest polymers having a molecular mass in the region of 5000 kDa (Gupta & Haslam, 1980). Due to their widely different structures, the different compounds could be expected to bind more or less strongly to Fe in the intestinal lumen and influence Fe absorption in different ways. Dietary polyphenols can be divided into three main groups; the phenolic acids, the flavonoids and the complex polymerization products formed from flavonoids alone or from a combination of flavonoids and phenolic acids. In relation to the polyphenol composition of the common beverages, the main phenolic compound in coffee is caffeoyl-quinic acid, or chlorogenic acid, which is a phenolic acid (Clifford & Ramirez-Martinez, 1991), whereas herb teas such as peppermint, contain mainly the monomeric flavonoids (Lallement & Bezanger, 1970). Flavonoids are also the major phenolics present in green tea leaves, the cocoa bean and red wine. Green tea flavonoids also contain gallic acid esters and during fermentation to black tea complex polymers are formed containing both flavonoids and gallic acid esters (Ballentine, 1991). The flavonoids present in the raw cocoa bean similarly undergo complex polymerization processes during fermentation and roasting (Shahidi & Naczk, 1995), whereas red wine contains all three classes of polyphenols including phenolic acids, monomeric flavonoids and a variety of polymerization products including dimers and trimers (Lunte *et al*. 1988) and larger molecules with molecular masses from 2000 to 4000 kDa (Somers, 1966).

Influence of polyphenols on iron absorption

Brune *et al*. (1989) looked at the relative inhibitory effect of the different polyphenol structures on Fe absorption. They measured Fe absorption in subjects given a bread meal to which they added equivalent amounts of gallic acid, chlorogenic acid and catechin, as an example of a flavonoid. They also added increasing amounts of tannic acid, a phenolic compound containing ten galloyl residues and glucose, which they used as a model for the larger polymerized polyphenol structures. Gallic acid, in an amount commonly found in foods, reduced Fe absorption from the bread meal by about 50 %, compared with 30 % for chlorogenic acid and no effect with catechin. Tannic acid inhibited Fe absorption in a dose-dependent fashion equivalent to its content of gallic acid.

Our results indicate that all major types of food polyphenols can strongly inhibit dietary non-haem Fe absorption. The inhibitory phenolic compounds would appear to include phenolic acids, such as chlorogenic acid from coffee, monomeric flavonoids such as found in herb teas, and the complex polymerization products found in black tea and cocoa. We have previously demonstrated a similar inhibitory effect of red wine on Fe absorption from an identical bread meal (Cook *et al*. 1995). Our results agree only in part with those of Brune *et al*. (1989) who, using model compounds, demonstrated that phenolic acids, such as gallic acid and chlorogenic acid, inhibited Fe absorption from a bread meal. However, these workers could show no effect of the monomeric flavonoid catechin on Fe absorption, and they suggested that flavonoids and their polymers would not interfere with Fe absorption in man. Our results with cocoa, red wine and herb teas would not support this conclusion.

Our results (Fig. 1) indicate that black tea polyphenols are more inhibitory than the polyphenols from herb teas, cocoa or wine. This could be due to their higher content of galloyl esters. Unlike the different concentrations of Assam tea, the different concentrations of herb tea represented in Fig. 1 would be expected to contain different types of polyphenols and this could explain the less clear relationship that was obtained between polyphenol concentrations and relative Fe absorption for herb teas than was obtained for Assam tea. Despite these differences, our results would indicate that any beverage providing 20–50 mg total polyphenols would reduce Fe absorption from a bread meal by 50–70 %, whereas beverages containing 100–400 mg total polyphenols would reduce Fe absorption by 60–90 %. Other workers have also demonstrated a dose-dependent inhibitory effect of polyphenols on Fe absorption either with model compounds such as tannic acid (Brune *et al*. 1989; Siegenberg *et al*. 1991) or with a green leafy vegetable (Tuntawiroon *et al*. 1991).

Based on these findings, it is tempting to suggest that the approximate inhibitory effect of a food or a meal on Fe absorption can be predicted by measuring its content of total polyphenols, and that it would not be necessary to specify the levels of different types of polyphenols. Our studies, however, were made using a simple bread meal and it may not be possible to extrapolate our results directly to more complex meals containing a wider range of Fe absorption inhibitors and enhancers. While both tea and coffee have

been shown to strongly inhibit Fe absorption from both simple bread meals and more complex composite meals (Hallberg & Rossander, 1982; Morck *et al*. 1983), red wine had little influence on Fe absorption from a composite meal containing meat and vegetables (Hallberg & Rossander, 1982). There is a possibility, therefore, that herb teas and cocoa may similarly have a less inhibitory effect on a composite meal even though they strongly reduced Fe absorption from the bread meal. The explanation could lie in the interactions that take place between the inhibitors and enhancers of Fe absorption in the intestinal tract, and it could be that the enhancers of Fe absorption, such as ascorbic acid and muscle tissue, are more or less effective depending on the type of polyphenol present.

Influence of milk on iron absorption from tea or coffee

In study 6 (Table 2) we also investigated whether adding milk to coffee or black tea, as is the custom in many countries, could decrease their inhibitory effect on Fe absorption. Some polyphenols are well known to bind strongly to proteins (Hagerman & Butler, 1981; Kumar & Singh, 1984), and it was hypothesized that such binding might prevent the polyphenols from complexing with Fe. Unfortunately this did not seem to be so, and there was little or no improvement in Fe absorption from the bread meal with tea or coffee when milk was added. As in other studies (Hallberg & Rossander, 1982; Morck *et al*. 1983), we found coffee to be less inhibitory to Fe absorption than tea.

Iron absorption from beverages alone

Assam tea, peppermint tea and cocoa inhibited Fe absorption by 84–97 % in the absence of the bread roll (study 8, Table 2). This is much higher than the 20–25 % inhibition reported for red wine in our previous study (Cook *et al*. 1995) but indicates that a polyphenol–Fe complex was formed in the intestinal lumen and that Fe within the complex was unavailable for absorption. We had hypothesized that perhaps the peptide degradation products formed on the digestion of wheat proteins might also be necessary to form a polyphenol–Fe–peptide complex. Percentage Fe absorption from black tea with bread (0·83 %) (study 7, Table 2) was slightly but not significantly ($P > 0{\cdot}05$) lower than from black tea alone (1·00 %). Although the white bread rolls would be expected to contain little or no phytic acid, Fe absorption from water was reduced from 29·4 to 8·6 % when consumed with bread. This could be due to the presence of the digestion products physically reducing access of Fe to the brush-border cells.

Influence of polyphenolic-containing beverages on iron status

In relation to public health, the important question is whether regular consumption of polyphenol-containing beverages could influence Fe status. Tea-drinking by infants in Israel (Merhav *et al*. 1985) and coffee-drinking by pregnant women in Costa Rica (Munoz *et al*. 1988) have been shown to have a negative effect on Fe status. However these population groups presumably consume a more simple and less varied diet than do people in the USA or Europe. In the USA, coffee and tea drinking could not be found to contribute significantly to the 5·3 % anaemia that was diagnosed in the >11 000 participants of the Second National Health and Nutrition Education Survey (NHANES II) (Mehta *et al*. 1992). Polyphenol-containing beverages, however, do strongly inhibit Fe absorption in the single meal studies and, even though this inhibition may be less pronounced when averaged over the many meals of a whole diet (Cook *et al*. 1991), it would still seem wise to consider this property when giving dietary advice to individuals who are most susceptible to developing Fe deficiency. On the other hand, the consumption of polyphenol-containing beverages, and in particular black tea with meals, could be a useful strategy in reducing Fe absorption in patients with Fe overload disorders (deAlareon *et al*. 1979).

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References

Association of Official Analytical Chemists (1990) *Official Methods of Analysis*, 15th ed., pp. 757–759 [K Helrich, editor]. Arlington, VA: Association of Official Analytical Chemists.

Ballentine DA (1991) Manufacturing and chemistry of tea. In *Phenolic Compounds in Food and Their Effect on Health. ACS Symposium Series* no. 506, pp. 102–117 [CT Ho, CY Lee and MT Huang, editors]. Washington, DC: American Clinical Society.

Bezwoda WR, Torrance JD, Bothwell TH, McPhail AP, Graham B & Mills W (1985) Iron absorption from red and white wines. *Scandinavian Journal of Haematology* **34**, 121–127.

Brown E, Hopper J Jr, Hodges JL Jr, Bradley B, Wennesland R & Yamauchi H (1962) Red cell, plasma, and blood volume in healthy women measured by radiochromium cell-labelling and hematocrit. *Journal of Clinical Investigation* **41**, 2188–2190.

Brune M, Rossander L & Hallberg L (1989) Iron absorption and phenolic compounds: importance of different phenolic structures. *European Journal of Clinical Nutrition* **43**, 547–558.

Charlton R & Bothwell TH (1983) Iron absorption. *Annual Reviews of Medicine* **34**, 55–68.

Clifford MN & Ramirez-Martinez JR (1991) Phenols and caffeine in wet processed coffee beans and coffee pulp. *Food Chemistry* **40**, 35–42.

Cook JD, Dassenko SA & Lynch SR (1991) Assessment of the role of non-heme iron availability in iron balance. *American Journal of Clinical Nutrition* **54**, 717–722.

Cook JD, Layrisse M & Finch CA (1969) The measurement of iron absorption. *Blood* **33**, 421–429.

Cook JD, Layrisse M, Martinez-Torres C, Monsen E & Finch CA (1972) Food iron absorption measured by an extrinsic tag. *Journal of Clinical Investigation* **51**, 805–815.

Cook JD, Reddy MB & Hurrell RF (1995) The effect of red and white wine on non-heme iron absorption in humans. *American Journal of Clinical Nutrition* **61**, 800–804.

deAlareon PA, Donovan ME, Forbes GB, Landau S & Stockman JA (1979) Iron absorption in thalassemic syndromes and its inhibition by tea. *New England Journal of Medicine* **300**, 5–8.

Disler PB, Lynch SR, Charlton RW, Torrance JD, Bothwell TH, Walker RB & Mayet F (1975) The effect of tea on iron absorption. *Gut* **16**, 193–200.

Eakins JD & Brown DA (1966) An improved method for the simultaneous determination of iron-55 and iron-59 in blood by liquid scintillation counting. *International Journal of Applied Radiation and Isotopes* **17**, 391–397.
Fairweather-Tait S & Hurrell RF (1996) Bioavailability of minerals and trace elements. *Nutrition Research Reviews* **9**, 295–324.
Flowers CA, Kuizon M, Beard JL, Skikne BS, Covell AM & Cook JD (1986) A serum ferritin assay for prevalence studies of iron deficiency. *American Journal of Hematology* **23**, 141–151.
Gillooly M, Bothwell TH, Charlton RW, Torrance JD, Bezwoda WR, McPhail AP, Derman DP, Novelli L, Morrall P & Mayer F (1984) Factors affecting the absorption of iron from cereals. *British Journal of Nutrition* **51**, 37–46.
Gillooly M, Bothwell TH, Torrance JD, McPhail AP, Derman DP, Bezwoda WR, Mills W, Charlton RW & Mayet F (1983) The effects of organic acids, phytates and polyphenols on iron absorption from vegetables. *British Journal of Nutrition* **49**, 331–342.
Gupta RK & Haslam E (1980) Vegetable tannins – structure and biosynthesis. In *Polyphenols in Cereals and Legumes*, pp. 15–24 [JH Hulse, editor]. Ottawa, Ont., Canada: IRDC.
Hagerman AE & Butler L (1981) The specificity of the proanthocyanin in protein interactions. *Journal of Biological Chemistry* **256**, 4494–4497.
Hallberg L & Rossander L (1982) Effect of different drinks on the absorption of non-heme iron from composite meals. *Human Nutrition: Applied Nutrition* **36**, 116–123.
Harborne JB (1993) *The Flavonoids: Advances in Research Since 1986*. London: Chapman & Hall.
Hosein F, Marsaglia G & Finch CA (1967) Blood ferrokinetics in normal man. *Journal of Clinical Investigation* **46**, 1–9.
International Nutritional Anemia Consultative Group (1993) *Iron EDTA for Food Fortification*. Washington, DC: Nutrition Foundation.
Kuehnau J (1979) The flavonoids. A class of semi-essential food components. Their role in human nutrition. *World Review of Nutrition and Dietetics* **24**, 117–191.
Kumar R & Singh M (1984) Tannins: their adverse role in ruminant nutrition. *Journal of Agricultural and Food Chemistry* **32**, 447–453.
Lallement GN & Bezanger BL (1970) Flavonoides de quelques labiate medicinales (rosmarin, menthe, sauge) (The flavonoid content of some medical plants from the labiatae family (rosemary, peppermint, sage)). *Plant Medicine and Phytotherapy* **4**, 92–107.
Lunte JM, Blankenship KD & Read SA (1988) Detection and identification of procyanidins and flavonols in wine by dual-electrode liquid chromatography–electrochemistry. *Analyst* **113**, 99–102.
Mehta SW, Pritchard ME & Stegman C (1992) Contribution of coffee and tea to anemia among NHANES II participants. *Nutrition Research* **12**, 209–222.
Merhav H, Amitai Y, Palti H & Godfrey F (1985) Tea drinking and microcytic anemia in infants. *American Journal of Clinical Nutrition* **41**, 1210–1213.
Morck TA, Lynch SR & Cook JD (1983) Inhibition of food iron absorption by coffee. *American Journal of Clinical Nutrition* **37**, 416–420.
Munoz LD, Lönnerdal B, Keen CL & Dewey KG (1988) Coffee consumption as a factor in iron deficiency anemia among pregnant women and their infants in Costa Rica. *American Journal of Clinical Nutrition* **48**, 645–651.
Shahidi F & Naczk M (1995) *Food Phenolics: Sources, Chemistry, Effects and Applications*, pp. 124–128. Lancaster, PA: Technomic Publ. Co. Inc.
Siegenberg D, Baynes RD, Bothwell TH, MacFarlane BJ, Lamparelli RD, Car NG, McPhail AP, Schmidt U, Tal A & Mayet F (1991) Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on non-heme iron absorption. *American Journal of Clinical Nutrition* **53**, 537–541.
Singleton VL & Rossi IA (1965) Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture* **16**, 144–158.
Somers TC (1966) Wine tannins – Isolation of condensed flavonoid pigments by gel filtration. *Nature* **209**, 368.
Stagg GV & Millin DJ (1975) The nutritional and therapeutic value of tea – A review. *Journal of the Science of Food and Agriculture* **26**, 1439–1459.
Tuntawiroon M, Sritongkul N, Brune M, Rossander-Hulthen L, Pleehachinda R, Suwanik R & Hallberg L (1991) Dose-dependent inhibitory effect of phenolic compounds in foods on non-heme iron absorption in men. *American Journal of Clinical Nutrition* **53**, 554–557.
Wennesland R, Brown E, Hopper J, Hodges JL, Guttentag OE, Scott KG, Tucker IN & Bradley B (1959) Red cell, plasma and blood volume in healthy men measured by radiochromium (Cr^{51}) cell tagging and hematocrit: influence of age, somatotype and habits of physical activity on variance after regression of volumes to height and weight combined. *Journal of Clinical Investigation* **38**, 1065–1077.
World Health Organization (1992) *National Strategies for Overcoming Micronutrient Malnutrition, 89th Session of the Executive Board*. Geneva: WHO.