The importance of dietary composition for efficacy of iron absorption measured in a whole diet that includes rye bread fortified with ferrous fumerate: a radioisotope study in young women

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Fe absorption is affected by many dietary factors. The objective of the present study was to measure the effects of high *v.* low content of vitamin C, meat and phytic acid in whole diets with Fe-fortified bread on the efficacy of Fe absorption. Thirty-two healthy women with low Fe stores were randomised to three groups, each of which was given two of six test diets containing either low/high amounts of vitamin C, meat or phytic acid, respectively, in a cross-over design. Each diet was served throughout a 5 d period. Fe-fortified rye bread, extrinsically labelled with ^{59}Fe, was given with all main meals. Fe absorption was determined from whole-body counter measurements of ^{59}Fe retention. The fractional non-haem Fe absorption (corrected to a 40 % standard absorption by measurements from the reference dose) was 1·9 % *v.* 3·4 % ($P=0.04$) for the low/high vitamin C diets, 3·0 % *v.* 3·5 % ($P=0.58$) on the low/high meat diets and 4·9 % *v.* 3·8 % ($P=0.24$) on the low/high phytic acid diet, respectively. The total Fe absorbed (geometric mean with standard error) varied from 0·43 (SE 0·11) mg from the diet with lowest bioavailability to 1·09 (SE 0·18) mg from the diet with highest bioavailability ($P<0.001$). The present whole-diet study indicates that diet composition is a strong predictor of Fe absorption. In the diet with a low content of enhancers and a high content of inhibitors, vitamin C improved non-haem Fe absorption. The total Fe absorption varied 2·5-fold after small alterations of the content of enhancers and inhibitors in the diet.

Iron deficiency: Iron fortification: Vitamin C: Meat: Phytic acid

Fe deficiency and its anaemia (iron-deficiency anaemia) are common micronutrient deficiencies in both low-income and industrialised countries (Administrative Committee on Coordination/Sub-Committee on Nutrition, 2000). Fe deficiency may arise from inadequate intakes of dietary Fe, poor absorption, excessive loss, or a combination of two or more of these. The amount of Fe absorbed from the diet is influenced by several factors, with the most influential being the Fe status of the individual (Reddy *et al.* 2000) but the Fe bioavailability of the diet plays a significant role (Wienk *et al.* 1999; Reddy *et al.* 2000; Heath & Fairweather-Tait, 2002). The inhibiting effect of phytic acid (Hallberg *et al.* 1987; Brune *et al.* 1992) and polyphenols (Hallberg & Rossander, 1982; Morck *et al.* 1983; Samman *et al.* 2001), and the enhancing effect of vitamin C (Hallberg *et al.* 1986, 1989), on the absorption of non-haem Fe are well established, whereas findings of the possible inhibiting effect of a concomitant intake of Ca are equivocal (Lynch, 2000).

Food fortification has long been considered an effective strategy for the prevention of iron-deficiency anaemia, but the long-term benefits of Fe fortification have not been established. The potential efficiency of dietary fortification depends on a number of factors, including the Fe status of the target group, total Fe intake, selection of food vehicle, the bioavailability of the Fe, and the balance of inhibitors and enhancers of Fe absorption. A potential strategy to reduce a high prevalence of Fe deficiency is through fortification of food components often consumed by the target population (Hurrell, 2002). Rye bread, which is consumed on a daily basis in some of the Scandinavian countries, is a suitable vehicle for Fe fortification due to its dark colour and low risk of organoleptic deterioration caused by pro-oxidative properties (Hansen *et al.* 2005).

Studies have found a difference between Fe absorption from single-meal and from whole diets, indicating that the results

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from single-meal studies overestimate the effects on enhancers and inhibitors in the diet on Fe absorption (Cook *et al.* 1991), and that short-term measurements of absorption overestimate differences in Fe bioavailability (Hunt & Roughead, 2000).

The aim of the present study was to measure the effects of high or low vitamin C, meat and phytic acid content in a whole diet over five consecutive days on the efficacy of Fe absorption from a diet with Fe-fortified rye bread. Cereal flours are currently the most common vehicles for Fe fortification, and rye bread is the major bread source in Denmark, where the study was conducted.

Materials and methods

Subjects

Thirty-two women, aged 21–29 years, with a BMI of $22.4 \pm 2.3 \, kg/m^2$, participated in the study. All subjects had low serum ferritin stores (between 12 and 30 µg/l) but were non-anaemic (Hb > 110 g/l). The subjects were all apparently healthy, non-pregnant/lactating, non-smokers, and none of them took any vitamin or mineral supplements 2 months before or during the study. None used medicine regularly and 53 % took oral contraceptives. None of the subjects donated blood within 2 months prior to or during the study. The subjects were given written and oral information about the study and written consent was obtained from all subjects. The study was approved by the Municipal Ethical Committee of Copenhagen and Frederiksberg (KF 01-100/97) and the National Institute of Radiation Hygiene, Denmark.

Experimental design

The subjects were randomised to participate in one of three completely controlled dietary cross-over arms of the study in which the effects of low/high vitamin C content (study A, diets A1 and A2), low/high meat content (study B, diets B1 and B2) and low/high phytic acid content (study C, diets C1 and C2) on Fe bioavailability were investigated (Table 1). In studies A and B the effect of a promoting factor was investigated and the diet was designed to have a high content of inhibitory factors and a low content of other enhancers. In study C the effects of an inhibitor was investigated and the diet had a high content of promoting factors and a low content of other inhibitors. Each study consisted of two 5 d intervention periods during which Fe-fortified rye bread extrinsically labelled with ^{59}Fe was given with all main meals. Fe absorption was calculated from whole-body counting measurements performed 14 d after the last day of each 5 d period. With this design the whole-body counting measurements served as baseline for the following intervention

period. After the second dietary 5 d period a ^{59}Fe-labelled iron chloride oral reference dose solution was given without any accompanying meal, and absorption of this dose was determined by whole-body counting another 2 weeks later.

Iron-fortified rye bread

The rye bread consisted of water, rye flour, rye grains, sourdough, breadcrumbs, dark syrup, salt, vinegar and yeast. Ferrous fumarate (Sigma, St Louis, MO, USA) was added to the dough before baking, corresponding to 6 mg Fe per 100 g bread. The bread was prepared and baked by a commercial bakery (Schulstad Bread A/S, 2650 Hvidovre, Denmark) with experience in baking with fortification.

Composition of test meals and serving procedure

Each subject was assigned to an energy intake based on individual energy requirements estimated from body weight and physical activity level. Apart from different contents of vitamin C, meat and phytic acid (Table 1), the diets had either a relatively high (diets A1 and A2) or relatively low (diets C1 and C2) Ca content and were served either with (diets A1, A2, B1, B2) or without (diets C1 and C2) tea. A 1 day menu was repeated for all 5 d in each diet period. The serving order of the two diet types was randomised. The menu consisted of typical Danish food items (Table 2) and the dietary factors mentioned earlier were equally distributed between the main meals. Diets A1, A2 and B1 had a low content of meat (given as pork *Longissimus dorsi*) with 0–10 g at breakfast and 15–25 g at lunch and dinner, per 10 MJ/d. Diets B2, C1 and C2 had a high content of pork meat (150 g/d) with 30 g eaten at breakfast, 45 g at lunch and 75 g at dinner, per 10 MJ/d. Each subject was given 120 g rye bread/d per 10 MJ, which was evenly distributed over the three main meals. Ca and Fe content were matched in order to compare the diets both pair-wise in each study group and in all six diets.

All main meals were consumed at the Department of Human Nutrition under supervision within fixed time limits (breakfast at 07.30–08.30 hours, lunch at 12.00–13.00 hours and dinner at 17.00–18.30 hours). A snack meal consisting of a white wheat roll with jam was consumed at home each evening (minimum 3 h after dinner). The meals were separated by more than 3 h 30 min and the subjects fasted for 12 h before receiving the first meal of the 5 d period. The meals were served on disposable material and the subjects were instructed to consume the meal within 30 min and to alternate between eating and drinking. Mineral water, low in Ca and without any EDTA or other ingredients known to affect Fe absorption, was given *ad libitum* and the

Table 1. Study design*

Study A		Study B		Study C	
Diet A1	Diet A2	Diet B1	Diet B2	Diet C1	Diet C2
Low vitamin C	High vitamin C	Low vitamin C	Low vitamin C	High vitamin C	High vitamin C
Low meat	Low meat	Low meat	High meat	High meat	High meat
High phytic acid	High phytic acid	High phytic acid	High phytic acid	Low phytic acid	High phytic acid

* In studies A, B and C the two test diets had a low or a high content of vitamin C, meat and phytic acid, respectively. In studies A and B the basic diets had a low content of Fe absorption-promoting dietary factors and a high content of inhibitive dietary factors, while the diets in study C had a high content of promoting dietary factors and a low content of inhibitive dietary factors.

Table 2. Menus for the six test diets with either high or low contents of vitamin C, meat and phytic acid

	Diet A1	Diet A2	Diet B1	Diet B2	Diet C1	Diet C2
Breakfast	Tea	Tea	Tea	Tea	Orange juice	Orange juice
	Oatmeal	Orange juice	Oatmeal	Oatmeal	Yoghurt	Oatmeal
	Milk	Milk	Milk	Milk	Rye bread	Sugar
	Rye bread	Rye bread	Rye bread	Rye bread	White wheat roll	Rye bread
	Sugar	Sugar	Sugar	Sugar	Butter	Butter
	Water	Cheese	Cheese	Cheese	Meat (pork)	Meat (pork)
		Water	Meat (pork)	Meat (pork)	Liver paste	Cod roe
			Water	Water	Water	Water
Lunch	Tea	Tea	Tea	Tea	Orange juice	Orange juice
	Milk	Milk	Milk	Milk	Rye bread	Rye bread
	Rye bread	Rye bread	Rye bread	Rye bread	White wheat roll	Whole wheat bread
	Whole wheat bread	Whole wheat bread	Whole wheat bread	Whole wheat bread	Meatball	Meatball
	Meatball	Meatball	Meatball	Meatball	Carrots	Mayonnaise
	Egg	Egg	Egg	Egg	Raisins	Peas
	Mayonnaise	Mayonnaise	Mayonnaise	Mayonnaise	Liver paste	Carrots
	Lettuce	Lettuce	Peas	Peas	Butter	Cod roe
	Peas	Peas	Carrots	Carrots	Orange	Orange
	Carrots	Carrots	Sugar	Sugar	Water	Water
	Sugar	Orange juice	Cod roe	Orange		
	Water	Water	Orange	Water		
			Water			
Dinner	Chilli con carne	Chilli con carne	Chilli con carne	Chilli con carne	Chilli con carne	Chilli con carne
	Rye bread	Rye bread	Rye bread	Rye bread	Rye bread	Rye bread
	Tea	Tea	Tea	Tea	Sponge cake	Chocolate
	Chocolate	Chocolate	Cheese	Chocolate	Orange juice	Orange juice
	Sugar	Orange juice	Chocolate	Sugar	Liver paste	Water
	Water	Water	Sugar	Water	Water	
			Water			
Snack	White wheat roll	White wheat roll	White wheat roll	White wheat roll	White wheat roll	White wheat roll
	Jam	Jam	Jam	Jam	Jam	Jam

amount consumed was recorded during the first study period. Each individual was instructed to drink the same amount of water on the corresponding days of the second dietary period. Macronutrient values and contents of vitamin C were calculated using the Dankost® dietary assessment software, accounting for the fortification of the bread (Danish Catering Center, Herlev, Denmark). Duplicate portions from each dietary intervention were collected in acid-washed polyethylene containers for chemical analyses of Fe, Ca, N and phytic acid.

Reference dose

Each subject consumed a standard Fe reference dose after the whole-body counting at the conclusion of the second dietary period. The solution contained 3·0 mg elemental Fe as ferrous sulphate (Merck, Darmstadt, Germany) (Hallberg & Hultén, 1996) and 30 mg L-(+)-ascorbic acid (Merck) in 0·01 mol/l HCl. The reference dose was labelled with ^{59}Fe (as high specific activity chloride solution) given on two consecutive mornings. The solution was prepared and served in a plastic vial, which was subsequently rinsed twice in 50 ml ultra-pure water, which was also consumed by the subjects. The subjects had fasted for 12 h prior to ingestion of the solution on both occasions and were asked not to consume any food or beverage for a further 3 h. Whole-body counting was carried out 12–13 d later.

Isotopes and labelling procedures

Breakfast, lunch and dinner meals were extrinsically labelled with ^{59}Fe by drop-wise addition of isotope solution (0·5–1·0 ml) to the rye bread. The radioisotope was purchased as ^{59}Fe-labelled ferric chloride in 0·1 M-HCl (Amersham Pharmacia Biotech, Amersham, UK) at a specific activity of 150 GBq/g Fe. The activity and specific activity was standardised by dilution with 0·1 M-HCl and addition of stable ferric chloride to 3·6 kBq/ml and 0·625 GBq/g Fe at time of administration. In each dietary period a dose of 8 kBq/d, i.e. 40 kBq per 5 d period, was distributed over the meals in relation to the Fe content in order to obtain equal specific radioactivity in the meals. Each of the two reference doses was labelled with 20 kBq ^{59}Fe each time, i.e. a total of 40 kBq. The extrinsic food labelling was undertaken approximately 16 h before serving. The estimated total activity of ^{59}Fe given to each subject was 120 kBq, which corresponds to calculated committed radiation dose of 0·288 mSv to each subject (International Commission on Radiological Protection, 1988).

Determination of iron absorption

Fe absorption was estimated by measurement of ^{59}Fe whole-body retention in a whole-body counter 12–13 d after termination of each dietary period, and after the reference dose. This time period was chosen to allow for the excretion of non-absorbed isotopes. The whole-body counter, located at Rigshospitalet, Copenhagen University Hospital, Denmark, consists of a low-activity lead-lined steel chamber with four plastic scintillation blocks (NE110; Nuclear Enterprises Ltd, Edinburgh, UK), two placed above and two below the subject. The counting efficiency and window settings were established through measurements of water-filled phantoms with known concentrations of ^{59}Fe and outlines and weights approximately equal to human subjects. The window setting was optimised prior to the experiment to cover the entire compton spectrum from ^{59}Fe down to a practical

lower limit of approximately 150 keV. The window setting was kept constant in between individuals and between phantom and subject measurements The counting efficiency for a 67 kg phantom was approximately 150,000 counts/600 s per kBq ^{59}Fe, corresponding to an absolute detection efficiency of 25 %. In order to minimise influence from external contamination due to natural or manmade background activity, the subjects showered and washed their hair and were dressed in hospital clothing before each measurement. Each whole-body counting session lasted 10 min. The results were corrected for chamber and individual subject background activity (mainly ^{40}K, as found from whole-body counter measurement before the start of the first test meal period) as well as for physical isotope decay.

Calculation of non-haem and haem iron absorption

Non-haem Fe absorption was corrected for the absorption from the reference dose to enable comparison between the three individual cross-over intervention studies. Based on Fe absorption from the reference dose, haem Fe absorption from each diet was estimated according to the following equation (Gleerup *et al.* 1995):

$$\text{Haem Fe absorption (\%)} = \text{absorption from reference dose (\%)}$$
$$\times 0.322 + 15.71.$$

Dietary analyses

Duplicate portions from each dietary period were homogenised (Tecator AD 1094; Tecator, Höganäs, Sweden), freeze dried (FD 8.0; Heto, Allerød, Denmark), ground to a powder, and analysed in duplicates for Fe, Ca, N and phytic acid content. Fe and Ca contents were analysed by atomic absorption spectrometry (Spectr-AA 200; Varian, Mulgrave, Victoria, Australia) after microwave destruction. Heat-treated meat (vacuum packed in plastic bags and heated in water at 70°C for 20 min) was analysed separately for haem Fe content according to Carpenter & Clark (1995). The absorbance of hemine was measured at 640 nm on a Lambda 15 spectrometer (Perkin-Elmer, Norwalk, CT, USA). N was analysed by GC after combustion (NA 1500 Carlo Erba; Fisions Instruments, Milan, Italy) using the Dumas method. Protein was calculated by multiplying measured N content by 6.25. A reference material (Standard Reference Material 1548, total diet, National Institute of Standards and Technology, Gaithersburg, MD, USA) was analysed for Fe, Ca and N in the same run as the diet samples (Fe: 35.3 (SE 3.8) μg/g, certified value: 33.0 (SE 3.9) μg/g; Ca: 1967 (SE 113) mg/kg, certified value: 2050 (SE 30) mg/kg; N: 29.7 (SE 1.6) mg N/g, certified value: 30.3 (SE 3.1) mg N/g). Phytic acid content was assessed as myoinositol hexaphosphate and its degradation products, myoinositol tri-, tetra- and pentaphosphates, by HPLC as described by Skoglund *et al.* (1998).

Blood sampling and analysis

Blood samples of 10 ml were taken at screening and analysed for serum ferritin and Hb concentration. Samples were drawn after a 12 h fast (consumption of 0.5 litre of water was allowed) and 10 min of rest in the supine position. Subjects were asked to refrain from physical exercise for 36 h and alcohol/medication for 24 h before blood sampling. In order to prevent artificially high values of serum ferritin due to infections, subjects were carefully instructed to report any sign of infection at the time of blood sampling. In the case of infection blood samples were postponed until 14 d after the end of infection as judged by the subject.

For analysis of Hb, 4.5 ml blood was collected in a vacutainer tube containing disodium EDTA (0.01 %). A separate blood sample (5 ml) was collected into plain tubes for the determination of serum ferritin concentration. The samples were centrifuged at 3000 *g* for 15 min at room temperature within 1 h of collection and the serum withdrawn was stored at -20 °C until ferritin analysis. Hb was analysed within 4 h of collection.

Blood samples were analysed for Hb on a Sysmex KX-21 (Automated Hematology Analyser, Kobe, Japan). An internal control was included in all runs with intra-assay CV of 0.59 % (*n* 5), 0.95 % (*n* 10) and 2.2 % (*n* 5). Serum ferritin was analysed by delayed fluorometry (Delfia Fluorometer 1235/514; Wallac Oy, Turku, Finland) with a kit adapted for the Delfia system (Delfia Ferritin Kit B069/101; Wallac Oy). The ferritin assay was calibrated against WHO ferritin 94/572 Third International Standard. The detection limit was 0.5 μg/l, the intra-assay CV was 2.1 % and the inter-assay CV was 5 % for an internal control. α-1 Anti-chymotrypsin in serum was analysed by turbidimetric detection of antigen–antisolid reaction on Cobas Miraplus (Roche Diagnostic Systems; F. Hoffmann-La Roche Ltd, Basel, Switzerland). The inter-assay CV was 2.8 % for an internal control. A reference material (DAKO Human Serum Protein Low (0939) and High (0940); DAKO, Glostrup, Denmark) was analysed in the same run as the blood samples (low: 0.27 g/l, certified value: 0.21–0.29 g/l; high: 0.72 g/l, certified value: 0.55–0.75 g/l).

Statistical analysis

The sample size was calculated from previous data collected at the Department on Fe absorption from single meals containing amounts of meat and phytic acid similar to those in the present study diets (Bæch *et al.* 2003). To obtain a power of 80 % it was calculated that nine subjects were needed to detect a 1.5 % change in Fe absorption at a significance level of 0.05 (Administrative Committee on Coordination/Sub-Committee on Nutrition, 2000). All analyses were performed using the Statistical Analysis System software package, version 8.2 (SAS Institute, Cary, NC, USA). Univariate mixed model ANOVA was performed in the procedure MIXED. In the statistical model, non-haem Fe corrected (%), non-haem Fe corrected (mg) and total Fe (mg) were evaluated as the dependent variable, and diet (low/high amounts of vitamin C, meat or phytic acid) was included as an independent fixed effect. The three studies (A, B and C) were regarded as blocks and were included both as independent main fixed effect and in interaction with diet. Subjects nested within a study were included as a random effect. Tukey's test was used for the *post hoc* detection of significant pairwise comparisons. Homogeneity of variance and normal distribution among random effects were investigated by plots of residuals. Shapiro–Wilk's test for normal distribution was performed. The investigations showed that it was necessary to log-transform values of non-haem Fe corrected (%), non-haem Fe corrected (mg) and total Fe (mg). Least square means and standard errors were estimated and transformed back where necessary before presentation.

Results

All main meals were extrinsically labelled and consumed at the Department under supervision by a staff member. Compliance to the dietary intervention was evaluated as high. Subjects' initial mean body weight (64·6 (SD 7·7) kg was maintained to within 0·1 % during the study. The screening mean Hb concentration was 123 (SE 0·89) g/l and the mean serum ferritin concentration was 20·2 (SE 1·4) μg/l (95 %CI 16·0, 18·0). Mean α-1 anti-chymotrypsin concentration was 0·28 (SE 0·01) g/l (range 0·17–0·35), indicating that concentration of serum ferritin was not increased due to infections.

The nutrient compositions of the diets, and content of potential inhibitors and enhancers of Fe absorption, are shown in Table 3. Mean energy intake for the subjects was 10·7 (SE 0·7) MJ/d. The total Fe content of the diets was similar, with an average non-haem Fe content of 17·4 mg/d per 10 MJ. Fe fortification amounted on average to 7·3 mg in a 10 MJ diet, corresponding to 42 % of the non-haem Fe content. The diet with a high phytic acid content contained 1248 (SE 19) μmol/10 MJ on average, while diet C1 with low phytic acid content contained almost four times less phytic acid (543 μmol/10 MJ). In the low vitamin C diets (A1, B1 and B2), the contents ranged from 26 to 34 mg/10 MJ. In the high vitamin C diets (A2, C1 and C2) the vitamin C content ranged from 115 to 123 mg/10 MJ. The Ca content was similar in the two test periods of each study. The macronutrient composition was similar in all six diets.

The fractional and absolute absorption of non-haem Fe, haem Fe and total Fe from the six diets is presented in Table 4. Fractional non-haem Fe absorption was 80 % lower from diet A1 with low vitamin C content (26 mg/d) than from diet A2 with high vitamin C content (115 mg/d) ($P = 0.04$), and total Fe absorption was significantly lower (74 %, $P = 0.02$). No significant difference was found between fractional non-haem Fe absorption from diet B1 (low meat content) and B2 (high meat content) and no significant difference was found between fractional non-haem Fe absorption from diet C1 (low phytic acid content) and C2 (high phytic acid content).

For comparison of the Fe absorption values between the three groups, absorption was corrected for absorption from the reference dose. The lowest percentage (geometric mean) non-haem Fe absorption was 1·9 (SE 0·5) from diet A1 and the highest percentage was from diet C1 with 4·9 (SE 0·9) ($P = 0.007$). Absorption of non-haem Fe was highest (0·91 (SE 0·17) mg) from diet C1 containing 121 mg vitamin C, 150 mg meat and 543 μmol phytic acid, and it was lowest (0·37 (SE 0·09) mg) from diet A1 containing 26 mg vitamin C, 50 g meat and 1951 μmol phytic acid ($P = 0.011$). Total Fe absorption was highest from the diets C1 and C2 (high vitamin C and meat content and a low or high phytic acid content, respectively) and lowest from the low bioavailability diet (A1), resulting in a 2·5-fold increase in the absorption ratio between the most promoting diet (C1) and the most inhibiting diet (A1) on non-haem Fe and total Fe absorption ($P < 0.001$).

Discussion

The present study shows that the overall efficacy of Fe absorption from a diet of which 42 % of total Fe was from Fe-fortified bread depends on the dietary composition of the total diet. Ascorbic acid improved Fe bioavailability in a meal with otherwise low bioavailability, in a diet with a low content of enhancers and a high content of inhibitors. Meat content was of less importance than vitamin C and phytic acid content for non-haem Fe absorption from a diet including Fe-fortified rye bread. Low phytic acid content and high vitamin C and meat content improved Fe absorption significantly compared to a meal with low bioavailability.

The design of the present study is ideal for studying the effects of enhancers and inhibitors on Fe absorption from the diet. Effects of intake of vitamin C, meat and phytic acid on the absorption of Fe are investigated separately in individual cross-over intervention studies, and the enhancing and inhibiting effects are compared in the three cross-over studies. The subjects consumed the high or low vitamin C diet, the high or low meat diet, or the high or low phytic acid diet, in a randomised design minimising the effects of naturally occurring biological variations. Apart from the Fe fortification of the rye bread and the total amount of rye bread that was double the amount of rye bread usually consumed by Danish women, the diets of the present study reflect diets that are normally consumed in Denmark.

An increase in the calculated daily intake of ascorbic acid from 26 to 115 mg in the whole diet used in the present study increased

Table 3. Nutrient composition and content of potential inhibitors and promoters of iron absorption (per 10 MJ)

	Diet A1	Diet A2	Diet B1	Diet B2	Diet C1	Diet C2
Inhibitors/promoters						
Meat (g)	50	50	50	150	150	150
Vitamin C (mg)*	26	115	34	30	121	123
Dietary fibre (g)*	31	31	30	31	23	31
Phytic acid (μmol)	1951	1951	1975	2002	543	2054
Ca (mg)	979	1016	766	750	323	327
Tea/egg with meals	+	+	+	+	−	−
Nutrient composition						
Protein (g; E%)	82 (14)	83 (14)	84 (14)	90 (15)	81 (14)	88 (15)
Fat (g; E%)*	82 (31)	82 (31)	85 (32)	81 (30)	81 (31)	82 (31)
Carbohydrate (g; E%)*	323 (55)	321 (55)	319 (54)	321 (54)	325 (55)	317 (54)
Total Fe (mg)	18·9	20·3	20·0	21·2	21·3	22·3
Haem Fe (mg)	0·17	0·17	0·17	0·5	0·5	0·5
Non-haem Fe (mg)†	18·7	20·1	19·8	20·7	20·8	21·8
Phytic acid:Fe molar ratio	5·8:1	5·4:1	5·5:1	5·1:1	1·4:1	5·2:1

* Calculated from food composition tables (Dankost, Dankost Catering Centre, Herlev, DK (2002)).
† Calculated as the difference between total Fe and haem Fe content.

Table 4. Fractional and absolute iron absorption*

	Diet A1		Diet A2		Diet B1		Diet B2		Diet C1		Diet C2	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
n	10				11				11			
Non-haem Fe (%)	1·5	0·57[b]	2·7	0·60[a]	2·9	0·59	3·4	0·50	4·9	0·93	3·7	0·90
Reference dose (%)	36·3	6·96			43·5	5·11			40·6	2·83		
Non-haem Fe corrected (%)†‡	1·9	0·5[b]	3·4	0·4[ab]	3·0	0·6[ab]	3·5	0·6[ab]	4·9	0·9[a]	3·8	0·7[ab]
Non-haem Fe corrected (mg)†‡	0·37	0·09[b]	0·69	0·08[c]	0·54	0·10[abc]	0·63	0·11[abc]	0·91	0·17[ac]	0·76	0·15[abc]
Haem Fe (%)	27·4	2·2			29·7	1·6			28·8	0·91		
Haem Fe (mg)	0·05	0·01			0·05	0·01	0·16	0·01	0·15	0·01		
Total Fe (mg)‡§	0·43	0·11[d]	0·75	0·13[c]	0·60	0·12[abcd]	0·80	0·09[abcd]	1·09	0·18[abc]	0·92	0·19[abc]

[a,b,c,d]Mean values within a column with unlike superscript letters were significantly different ($P<0.05$).

* For details of diets and procedures, see Tables 1–3 and p. 721.

† Geometric mean with standard error.

‡ Individual absorption values corrected to 40 % absorption from the reference dose.

§ Non-haem Fe absorption corrected to the reference dose + haem Fe absorption estimated from the reference dose.

the absolute absorption of non-haem Fe from a meal with otherwise low bioavailability. The vitamin C content was evenly distributed between the three main meals and the increase in non-haem Fe absorption is in accordance with findings from several single-meal studies showing improved Fe absorption when vitamin C was consumed in meals with high contents of phytic acid and polyphenols, the most potent inhibitors of Fe absorption (Hallberg *et al.* 1986, 1989; Siegenberg *et al.* 1991). Studies including a complete diet have shown a less pronounced effect of vitamin C on Fe absorption (Reddy *et al.* 2000; Cook & Reddy, 2001) and one study has shown that long-term supplementation with vitamin C did not result in improved indexes of Fe status (Hunt *et al.* 1994). Whether results of Fe absorption obtained from single-meal studies can be compared to results obtained over longer periods has been debated intensively during the last decade (Cook *et al.* 1991; Hunt & Roughead, 2000), but the reasons for the differences in results from single-meal and long-term studies are still not clear.

Meat is a rich source of minerals and has a dual role in relation to daily Fe supply due to the high bioavailability of haem Fe in muscle tissue and the enhancing effect on the bioavailability of non-haem Fe (Layrisse *et al.* 1969; Martinez-Torres & Layrisse 1971; Björn-Rasmussen & Hallberg, 1979). The enhancing effect of meat on Fe availability is not understood and is usually referred to as the "meat factor". The "meat factor" may be related to the potential ability of sulphydryl-containing amino acids or peptides to chelate non-haem Fe and thereby facilitate intestinal absorption (Taylor *et al.* 1986; Bæch *et al.* 2003). Recently, it was suggested that carbohydrates originating from glycosaminoglycans in the extracellular matrix of muscle tissue is responsible for the enhancing effect on Fe availability (Huh *et al.* 2004).

In the present study, the meat content of the whole diet ranged from 50 to 150 g/10 MJ diet but no significant enhancement of non-haem Fe absorption was measured. From single-meal studies, Reddy *et al.* (2000) found highly positive correlations between animal tissue and non-haem Fe absorption and negative correlations between phytic acid and non-haem Fe absorption. In the present study, the daily portion of 150 g meat was consumed as 30 g at breakfast, 45 g at lunch and 75 g at dinner. A recent study has demonstrated that ingestion of $\geqq 50$ g of pork meat significantly enhances non-haem Fe absorption in a dose-dependent

manner from a diet with a high content of inhibitors and a low content of enhancers of Fe absorption (Bæch *et al.* 2003), but the study also showed that adding only 25 g meat to the meal caused no significant increase in fractional Fe absorption. It is therefore not surprising that no significant enhancing effect on non-haem Fe absorption was found in the present study.

Phytic acid is thought to be one of the strongest inhibitors of non-haem Fe absorption but the 4-fold higher phytic acid content of the whole diet in diet C2 compared to diet C1 caused no significant difference in non-haem Fe absorption when the dietary intake of phytic acid was evenly distributed between meals. Previous studies have shown that the quantitative inhibitory effect of phytic acid is similar over a wide range of phytic acid concentrations and is only reduced when there is very little phytic acid present (Hallberg *et al.* 1989). However, in the present study the content of phytic acid of all diets was relatively high (Table 3) mainly due to the rye bread. When calculating the phytic acid:Fe molar ratio of the diets (Table 3) they were all higher than the optimal ratio for Fe absorption which is <1:1. This explains part of the relatively low Fe bioavailability of the present study. However, the high and low phytic acid diets had a relatively higher Fe bioavailability as they contained a high meat and vitamin C concentration and no intake of polyphenols from tea or coffee. The reason for a lack of difference between the high and low phytic acid diets on the Fe absorption could be the high intake of enhancers counteracting the effect of the high phytic acid content in diet C2.

Fractional Fe absorption from the low bioavailability diet of the present study (A1) was well in accordance with fractional Fe absorption found in a whole-diet study on young women by Hunt (2003). Fractional absorption from the high bioavailability diet was higher in the study by Hunt, as compared to the present study, most probably due to a lower phytic acid content and a higher vitamin C content in the diets in the study by Hunt (2003).

The results of the present study indicate that increased intake of vitamin C results in a relatively higher fractional Fe absorption from a diet with low bioavailability of Fe than do an increase in meat intake or a reduction in phytic acid intake. The relatively higher Fe bioavailability diet resulted in higher absorption of both fractional and total Fe as compared to the low bioavailability diet. The high bioavailability diet resulted in a 2·5-fold increased total Fe absorption.

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