The effect of different levels of wheat bran on iron absorption in rats from bread containing similar amounts of phytate

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1. Iron absorption was studied in weanling rats using balance techniques from semi-synthetic diets containing dried white bread (60·5 g dietary fibre/kg, White & Southgate, unpublished results), brown bread (130·2 g dietary fibre/kg) or wholemeal bread (221·2 g dietary fibre/kg) at a level of 300 g/kg and compared with a control group given a diet containing added $FeSO_4$ at a similar Fe level to that for the bread groups. The dried bread contained 6·2–6·4 g phytate/kg.

2. Absorption of Fe was significantly higher ($P < 0·001$) in the control group (0·45) than in the white (0·28), brown (0·31) or wholemeal (0·24) groups.

3. A second experiment was carried out on 6-week-old rats in which the dried bread was extrinsically labelled with ^{59}Fe and absorption from a single meal measured by both faecal excretion and incorporation of ^{59}Fe into the blood. Control animals were given $^{59}FeSO_4$ for comparison.

4. The excretion of ^{59}Fe (% of administered dose) was significantly lower ($P < 0·001$) in the control group (31) than in the white (48), brown (45) or wholemeal (47) groups. After 10 d the control group had significantly more ^{59}Fe in the blood than the bread groups, but there were no differences between the bread groups.

5. It appears that wheat bran fibre itself has no effect on the retention of Fe from the diet in the rat, when supplied in amounts similar to those found in commercially-available bread.

The relative importance of phytate and fibre in inhibting iron absorption is controversial. These studies were an attempt to investigate the effect of fibre alone on Fe absorption by varying the dietary level of wheat fibre but at the same time keeping the phytate level constant. This was done by adding appropriate amounts of sodium phytate to the flour (with due allowance for losses on baking) to equalize the final phytate concentration in the white, brown and wholemeal bread.

MATERIALS AND METHODS

Two experiments were carried out on rats to assess the effect of increasing levels of bran (baked into bread) on Fe absorption. The first was a balance study carried out over 2 weeks in which the intake and excretion of Fe from diets largely composed of dried bread were measured. The second was the measurement of Fe absorption from a single meal of dried bread, extrinsically labelled with ^{59}Fe, compared with absorption from labelled $FeSO_4$.

Balance experiment

This was designed as far as possible to follow the lines of a metabolic balance study carried out on human subjects at the Dunn Clinical Nutrition Centre, Cambridge, in which the same bread was used. Three types of bread were prepared with differing amounts of bran but similar phytate levels. They will be referred to as white, brown and wholemeal, and their composition is shown in Table 1. $FeSO_4$ was added to the white and brown bread to bring the total Fe content up to that of the wholemeal bread.

A control diet was formulated which contained the same proportion of protein, fat and carbohydrate as the diet of the human subjects i.e. providing (% total energy): protein 16, fat 34, carbohydrate 50. These were supplied as albumen, maize oil and starch–sucrose (50:50, w/w) respectively. The contribution of bread to the daily energy intake in the human

Table 1. *Composition of breads (g/kg dry wt)*

	White	Brown	Wholemeal
Total dietary fibre	60·5	130·2	221·2
(Non-cellulosic polysaccharides	47·6	98·4	168·2
Cellulose	12·9	22·0	36·3
Lignin)	< 1·0	9·8	16·7
Free sugars	52·3	70·9	62·5
Starch	637·0	557·0	462·0
Phytate	14·4	14·55	14·25
Iron (μg/g)	67·2	62·3	64·0

Table 2. *Composition of experimental diets (g/kg)*

Ingredients	Control diet	Bread diets
Albumen	174·8	130·0
Maize oil	166·4	167·0
Starch	299·4	166·5
Sucrose	299·4	166·5
Dried bread		310·0
Mineral mix*	40·0	40·0
Vitamin mix†	20·0	20·0

Chemical composition	Control	White	Brown	Wholemeal
Protein (nitrogen × 6·25)	134·0	149·0	147·0	145·0
Fat	164·0	168·0	167·0	169·0
Available carbohydrate	641·0	588·0	538·0	504·0
Total dietary fibre	4·0	16·1	38·1	72·4
Phytate	1·55	6·3	6·3	6·4
Fe (μg/g)	24·60	22·20	21·09	21·28

* Mineral mix (g/kg diet): calcium hydrogen phosphate 13·00, calcium carbonate 8·20, potassium chloride 7·03, disodium hydrogen phosphate 7·40, magnesium sulphate 4·00, manganese sulphate 0·18, zinc carbonate 0·10, ferrous sulphate 0·144, copper sulphate 0·015, potassium iodate 0·001.

† Vitamin mix (mg/kg diet): nicotinic acid 60, vitamin B12 in mannitol 50, calcium pantothenate 40, thiamin hydrochloride 10, riboflavin 10, pyridoxin 10, folic acid 5, D-biotin 1, vitamin K 1, Rovimix E-25 300, Rovimix A-500 25, Rovimix A-500/D3 15, choline bitartrate 1800.

subjects was calculated to be 26% and the appropriate weight of bread was therefore included in the test diets of the rats at the expense of starch–sucrose (carbohydrate) and albumen (protein). Vitamin and mineral mixes were also included to provide the requirements for rats, and Fe added as $FeSO_4$ to the control diet to make the diets similar in Fe content. The composition of the diets is shown in Table 2.

Forty weanling male Wistar rats were individually caged in stainless-steel and plastic cages with metal-grid floors for several days, and fed the control diet *ad lib*. The animals were then randomly divided into four groups of ten and given the test diets for 14 d. During this period food intakes were accurately measured and complete faecal collections carried out for each animal. Spilt food and faeces were separated by means of a nylon sieve. Urine was not collected as it contains negligible Fe. The faeces were dried, ground and analysed for Fe. The animals were weighed regularly, and at the end of the 14 d balance period.

Radioisotope experiment

This was carried out to confirm the results of the balance study and also to compare two different methods of measuring Fe availability.

Forty weanling male Wistar rats were randomly divided into four groups of ten and housed individually in cages similar to the previous experiment. They were given control diet for 1 week *ad lib.* They were then trained to meal feed for 7 d by allowing them access to food pots at limited times. When this was successfully accomplished, the animals were fasted overnight and the following day were given test meals labelled with ^{59}Fe as follows.

The animals received either 3 g dried bread (white, brown or wholemeal), containing approximately 175 μg Fe, made up into a paste with 5 ml distilled water, or they were given 8 g cooked starch–sucrose paste (50:50, w/w) with 175 μg Fe added as $FeSO_4$. The Fe was labelled extrinsically by the addition of approximately 1 μCi ^{59}Fe (Amersham International Bucks)/rat (as ferric chloride solution, contributing negligible Fe). The animals were given the meal, fasted for 5 h and then given control diet. They were fasted overnight and the next morning given 8 g starch–sucrose paste containing 175 μg Fe, labelled extrinsically with approximately 2 μCi ^{55}Fe (Amersham International, Bucks)/rat (as $FeCl_3$ solution, carrier-free). After a 5 h fast they were then given access to control diet *ad lib.*

Faeces were collected daily and the ^{59}Fe content measured using a Gamma Counter. After 10 d the animals were anaesthetized under diethyl ether and blood was removed by cardiac puncture and ^{59}Fe and ^{55}Fe content measured using a liquid-scintillation counter. The total ^{59}Fe and ^{55}Fe- content of the blood was calculated assuming that blood volume is 5·7% body-weight (Huang & Bondurant, 1956).

Analytical methods

Fe analysis. Portions of dried, finely-ground samples were dry-ashed at 480° in silica crucibles, the residue taken up in 5 ml warm concentrated hydrochloric acid, and the solution diluted to an appropriate concentration for analysis by atomic absorption spectrometry.

Phytate analysis. This was carried out by a modification of the Holt method, as decribed by Davies & Reid (1979).

Dietary fibre analysis. This was carried out using a modification (White & Southgate, in preparation) of the Southgate method (Southgate, 1969).

Radioactivity measurements. Radioactivity in untreated samples was measured using a Philips Automatic Gamma Counter, PW 4580, with a 75 mm × 75 mm sodium iodide crystal, Centre 535, width 30, gauge 20, with a counting efficiency of approximately 3·2%.

Liquid-scintillation counting. Portions of 0·1 ml heparinized blood were counted by a dual-label technique. The samples were digested with 1·5 ml Soluene 350 (Packard Instrument Ltd, Caversham, Berks)–propan-2-ol (1:1, v/v) for 12 h, and then 0·5 ml hydrogen peroxide (300 g/l) added and left to stand for 3 h. Finally 15 ml Instagel (Packard)–0·5 M-HCl (9:1, v/v) was added and the vials counted in a Philips liquid-scintillation counter. The settings used were channel 1: attenuation 3·9, 40–1000; channel 2: attenuation 3·9, 40–200; channel 3: attenuation 0·0, 40–400. Quench curves were prepared using channels ratio and values for the efficiency of counting were approximately 74% for ^{59}Fe and 12% for ^{55}Fe.

Statistical methods

Differences between groups were tested by using unpaired *t* tests (Snedecor & Cochran, 1967).

Table 3. *Iron balance results for rats given diets containing bread for 14 d*
(Mean values with their standard errors)

	Control		White		Brown		Wholemeal	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Faecal dry wt (g)	8·18*	0·29	11·45[a]	0·37	14·62[b]	0·42	19·84[c]	0·64
Total Fe intake (mg)	4·84*	0·10	4·26[a]	0·11	4·00[b]	0·08	3·93[b]	0·09
Total Fe excreted (mg)	2·63	0·06	3·07[a]	0·11	2·77[a]	0·09	2·99[a]	0·12
Fe absorbed (mg)	2·21*	0·24	1·19[a]	0·08	1·22[a]	0·06	0·93[b]	0·05
Absorption	0·455*	0·033	0·279[a]	0·019	0·307[a]	0·017	0·240[b]	0·017

Mean values for control group were statistically significantly different from those for the bread diets: * $P < 0·001$.
[a, b, c] Values with unlike superscript letters were statistically significantly different: $P < 0·02$.

RESULTS

Balance experiment

The Fe intake and excretion over the 14 d experimental period are shown in Table 3. The faecal dry weight increased significantly ($P < 0·001$) with each increase in fibre content. The Fe content of the control diet (24·66 μg/g) was marginally higher than the bread diets (21·09–22·20 μg/g) and consequently there was a significant difference ($P < 0·001$) in Fe intake between the control group and the three bread groups. As Fe excretion is related to intake, the group means for excreted Fe cannot be directly compared.

The absorption of Fe from the control diet was 0·45, which was significantly higher ($P < 0·001$) than that from the bread diets, 0·24–0·31. Differences between the bread diets were very small, but the wholemeal-bread groups absorbed slightly less Fe ($P < 0·02$) than the brown-bread group.

Isotope experiment

The mean daily excretion of ^{59}Fe, expressed cumulatively, for each group is shown in Fig. 1. As would be expected, dietary fibre affected transit time and initially excretion was most rapid in the wholemeal-bread group, and slowest in the white-bread group. There were no differences in excretion of ^{59}Fe between the three bread groups, but the control group excreted significantly less ^{59}Fe ($P < 0·001$) over the 5 d period. Total excretion of ^{59}Fe over the 5 d was 31% of administered dose in the control group, and 45–48% of administered dose in the three bread groups as shown in Table 4.

Uptake of absorbed Fe into the blood is shown in Table 5. As in the faecal excretion results there were no significant differences in ^{59}Fe uptake between the three bread groups whose values ranged from 35–38% of administered dose, but uptake was significantly greater ($P < 0·001$) in the control group (49% of administered dose) given $FeSO_4$.

There was greater inter-individual variation in the absorption of Fe from $^{55}FeSO_4$. In the control group 44% of the administered dose was found in the blood which was significantly less ($P < 0·05$) than the value of 53% of administered dose for the wholemeal group, but not significantly different from the brown (40% of administered dose) or white (41% of administered dose) bread groups.

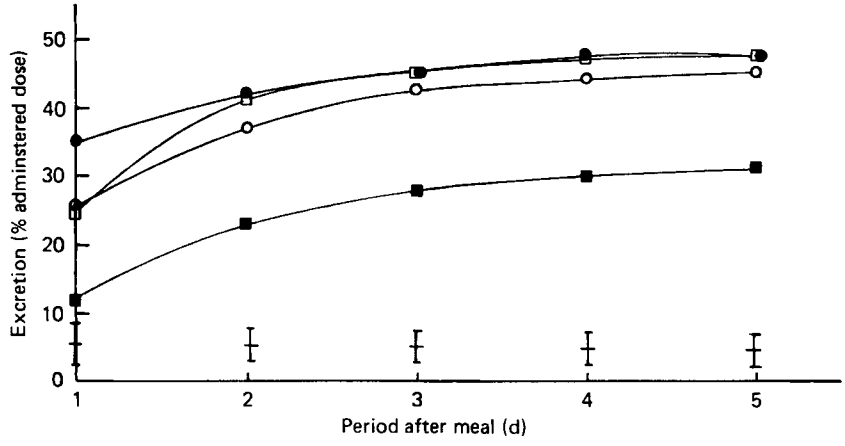

Fig. 1. Cumulative excretion of ^{59}Fe (% administered dose) by rats given a single meal of dried bread extrinsically labelled with ^{59}Fe (■), control; (□), white bread; (○), brown bread; (●), wholemeal bread. For details of diets, see p. 000 and Tables 1 and 2. Points are mean values with their standard errors represented by vertical bars.

Table 4. *Excretion of ^{59}Fe (% administered dose) by rats given a single meal of dried bread extrinsically labelled with ^{59}Fe*

(Mean values with their standard errors)

	Fe excretion (% of administered dose)	
	Mean	SE
Control	30·8*	1·36
White	47·7	2·09
Brown	45·0	2·85
Wholemeal	47·4	2·21

* Significantly different from other groups: $P < 0{\cdot}001$.

Table 5. *Iron in blood (% administered dose) of rats given dried bread extrinsically labelled with ^{59}Fe or $FeSO_4$ in a starch–sucrose paste extrinsically labelled with ^{55}Fe*

(Mean values with their standard errors)

	^{59}Fe		^{55}Fe	
	Mean	SE	Mean	SE
Control	49·3**	0·98	44·0	2·72
White	35·2	1·87	41·0	3·64
Brown	35·5	1·71	39·6	3·13
Wholemeal	37·7	2·31	52·7*	2·53

* Significantly different from other groups: * $P < 0{\cdot}05$, ** $P < 0{\cdot}001$.

Table 6. *Summary of results of experiments investigating the effects of (a) primarily phytate, (b) primarily fibre, and (c) both fibre and phytate on iron absorption*

Experimental model	Dietary manipulation	Results	Reference
(a) Human	Addition of sodium phytate to bread containing ferric and ferrous salts	Sodium phytate decreased iron absorption	McCance *et al.* (1943)
Human	Addition of 26 g wheat bran (5 g phytate-phosporus/kg) or 26 g maize bran (0 g phytate/kg) to control diet	No effect on Fe absorption	Sandstead *et al.* (1978)
Human	Addition of sodium phytate (2·5 g/d per subject) or phytate present in tanok bread for several weeks	Serum Fe fell initially but then returned to normal	Reinhold *et al.* (1973)
Rat	Sodium phytate added to semi-synthetic diet (10 g/kg) for 21 d	Whole-body retention of Fe fell	Davies & Nightingale (1975)
Rat	Dephytinized bran *v.* raw bran	Fe absorption higher with dephytinized bran	Morris & Ellis (1980)
Rat	Sodium phytate added to basal diet (7–12 g/kg)	No effect on Fe absorption	Cowan *et al.* (1966)
Rat	Sodium phytate added to wheat-based diets (6·2–22·7 g/kg)	No effect on Fe absorption	Ranhotra *et al.* (1974)
In vitro	Sodium phytate plus metals	Complexes formed at pH 7·4	Vohra *et al.* (1965)
(b) Rat	Addition of cellulose to diet (160 g/kg) for 9 weeks	No effect on Fe absorption	Tsai & Lei (1979)
In vitro	Bran and hemicellulosic fraction of bran plus ferrous-Fe	Very marked binding at pH 6·8	Ismail-Beigi *et al.* (1977)
(c) Human	Metabolic balance of white bread v. brown bread	Absorption from white bread higher	Widdowson & McCance (1942)
Human	Enriched white bread *v.* unenriched brown bread	Absorption from brown bread higher	Callender & Warner (1970)
Human	70% extraction flour *v.* 95–100% extraction flour for 7–19 weeks	No difference in Fe absorption	Walker *et al.* (1948)
Human	Addition of bran to wheat bread (7%)	Fe absorption reduced by 50%	Björn-Rasmussen (1974)
Human	Addition of 31 g wheat fibre/d for 3 weeks	Significant fall in serum Fe	Jenkins *et al.* (1975)
Rat	Addition of wheat bran to diet	Reduced Fe availability	Ranhotra *et al.* (1979)
In vitro	Dephytinized wholemeal bread, non-dephytinized bread, bran and cellulose plus Fe	Dephytinized bread higher binding capacity than non-dephytinized bread. Also cellulose binds Fe	Reinhold *et al.* (1975)

DISCUSSION

There are conflicting results in the literature concerning the effect of added sodium phytate on Fe absorption in man and the rat, as summarized in Table 6. McCance *et al.* (1943) showed that sodium phytate reduced Fe absorption in man from bread, but more recently Sandstead *et al.* (1978) found that the daily addition of 26 g wheat bran (containing 5 g phytate/kg) or maize bran (containing no phytate) to the diet had no effect on Fe absorption in man. In the rat, Davies & Nightingale (1975) found the addition of sodium phytate to the diet reduced whole-body Fe retention, whereas Cowan *et al.* (1966) and Ranhotra *et al.* (1974) found it had no effect.

Much of the work in this field is concerned with the effect of bran on Fe absorption, and it is difficult to assess the individual roles played by fibre and phytate in inhibiting Fe absorption. Tsai & Lei (1979) showed that the addition of cellulose (up to 160 g/kg diet) to the diet of rats had no effect on Fe absorption. Yet in vitro work of Ismail-Beigi *et al.* (1977) and Reinhold *et al.* (1975) has shown that bran, cellulose and hemicellulosic fractions of bran bind Fe strongly at neutral pH, such as would be found in the jejunum.

Results from both experiments described in this paper show a higher Fe absorption from $FeSO_4$ than from Fe present in bread whether results are expressed as mg Fe absorbed or percentage Fe absorption. The balance study showed a small difference in Fe absorption between the three bread diets. The brown-bread group appeared to have absorbed significantly more ($P < 0{\cdot}02$) iron, 31% of administered dose, than the wholemeal-bread group, 24% of administered dose. Since the value for absorption for the white-bread group, 28% of administered dose, fell between the brown- and wholemeal-bread groups, but the fibre content was lowest, it is unlikely that the difference between Fe absorption from brown- and wholemeal-bread diets is related to the fibre content of the diet.

The radioisotope experiment clearly demonstrated that there was no difference in Fe absorption between the three breads. It can therefore be concluded that the addition of wheat-bran fibre (in amounts similar to those found in commercially-available bread) to flour of similar phytate concentration had no effect on Fe absorption in the rat in short- or long-term experiments. This supports the findings of Tsai & Lei (1979) that the addition of cellulose (160 g/kg diet), one of the components of wheat bran, to the diet had no effect on Fe availability to the rat. Fe absorption from control diets containing $FeSO_4$ was higher which may be because the Fe from $FeSO_4$ is more available than the Fe in the bread, or it may be the absence of phytate in the control diet which rendered the dietary Fe more available, or both.

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