Iron and zinc absorption in human subjects from a mixed meal of extruded and nonextruded wheat bran and flour[1-3]

Susan J Fairweather-Tait, David E Portwood, Lisa L Symss, John Eagles, and Margaret J Minski

ABSTRACT The effect of extrusion cooking of a bran-flour mixture on iron and zinc retention was measured in normal adults. The stable isotopes ^{58}Fe (1.253 mg) and ^{67}Zn (5.13 mg) were administered with 40 g nonextruded or extruded cereal with milk and isotopic retention was measured from fecal excretion over the next 4–7 d by neutron-activation analysis (Fe) and fast-atom-bombardment mass spectrometry (Zn). ^{58}Fe retention was 15.1 ± 2.4% ($\bar{x}$ ± SEM) with the nonextruded meal and 16.5 ± 2.7% with the extruded meal. ^{67}Zn retention was 18.9 ± 1.7% with the nonextruded meal and 18.3 ± 1.5% with the extruded meal. Extrusion cooking had no effect on ^{58}Fe or ^{67}Zn retention. *Am J Clin Nutr* 1989;49:151–5.

KEY WORDS Extrusion cooking, iron retention, zinc retention, stable isotopes, bran

Introduction

Concern has been expressed recently over the possible deleterious effects of thermal processing of foods on nutrient availability. One report (1) showed that extrusion cooking of a high-fiber cereal product reduces the bioavailability of zinc, magnesium, and phosphorus when compared with the nonextruded product. Because the extrusion cooking process is becoming more widely applied in the food industry, these adverse effects on mineral availability require further investigation. Wheat bran was selected for this study because it is used for the production of high-fiber extruded products and has been shown to reduce the absorption of Zn (2) and iron (3) when consumed at sufficiently high levels.

Methods

Subjects and diets

Eleven adult volunteers were recruited from the staff at the Institute of Food Research, Norwich Laboratory; the subject details are described in **Table 1.** At the beginning of the study, a 10 mL blood sample was taken from the antecubital vein for hemoglobin determination (4) and plasma ferritin analysis using a radioimmunoassay (DuPont UK Ltd, New England Nuclear Products Division, Stevenage, Herts, UK). By these criteria none of the subjects was considered to be Fe deficient.

The study took place over a 4-wk period. During this time, all subjects were given a 7-d self-selected rotating menu, starting one week before the administration of the first test meal. At the beginning of weeks 2 and 4 after an overnight fast, each subject was given one of two test meals in random sequence. The test meals comprised 100 mL pasteurized full-fat milk plus 40 g of a 1:1 mixture of finely milled wheat bran and white (72% extraction, unfortified) wheat flour. A portion of the cereal mixture had been subjected to extrusion cooking using a Daker-Perkins MPF 50D twin-screw extruder (Baker Perkins Ltd, Westfield Road, Peterborough, UK) as outlined in **Table 2** and was consumed with cold milk. The nonextruded flour was heated with the milk to form a porridge and the bran was mixed in just before consumption. Each subject was allowed to add sugar to taste; the same quantity was added to both meals. With each meal the subjects were given 170 ml of a cola drink containing 1.3 mL of a solution of ^{58}Fe (1.230 mg Fe/mL in 0.5 mol HCl/L containing 78.13 atom % [78.35 wt %] ^{58}Fe, AERE, Harwell, Oxfordshire, UK) and 1.0 mL of a solution of ^{67}Zn (5.51 mg Zn/mL in 0.5 mol HCl/L containing 93.11 atom % [93.16 wt %] ^{67}Zn, ORNL, Oak Ridge, TN). Simultaneously they swallowed two capsules, each containing 10 barium-impregnated radioopaque markers, to check for completeness of fecal collections. The protocol was similar to that described in a previous study (5). After the test meal no food or drink was allowed for 4 h, after which time subjects returned to their 7-d rotating menu.

Fecal collections

Once the test meal had been consumed, all feces were collected in plastic bags that can be treated in an autoclave. Sub-

[1] From the AFRC Institute of Food Research, Norwich Laboratory, Norwich and the Imperial College Reactor Centre, Silwood Park, Ascot, Berks, UK.

[2] Supported by the Ministry of Agriculture, Fisheries and Food, UK.

[3] Address reprint requests to SJ Fairweather-Tait, AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA, UK.

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TABLE 1
Details of subjects*

	Men	Women
Subjects (*n*)	6	5
Age (y)	26 ± 2.5	27 ± 2.5
Weight (kg)	68.2 ± 4.0	56.7 ± 3.3
Height (m)	1.782 ± 0.033	1.628 ± 0.019
Hemoglobin (g/L)	147.9 ± 4.7	138.6 ± 2.1
Ferritin (mg/L plasma)	114.3 ± 27.6	39.0 ± 9.0

* $\bar{x}$ ± SEM.

jects took 500 mg carmine (in two capsules) with their evening meal on the day of the test meal and collected all stools until the carmine had been excreted plus one further collection. All the unabsorbed isotope was assumed to be present in the collected feces. A separate stool sample was collected 1–2 d before the second test meal and the isotopic enrichment of both ^{58}Fe and ^{67}Zn was measured to confirm that the fecal material contained isotopes of natural abundance with no enrichment as was assumed to be the case for the first test meal.

The nonextruded and extruded mixtures of wheat bran and flour were analyzed for dietary fiber (6) and phytate (7). The Fe and Zn contents were determined by atomic absorption spectroscopy (AAS) with a PU9000 atomic absorption spectrophotometer (Pye Unicam, Cambridge, UK) after being burned to ash (dry) for 48 h at 480 °C in silica crucibles and dissolved in hot concentrated hydrochloric acid.

Fecal samples were stored at −18 °C until the completion of the study when all collections from each subject for each period were combined, thawed, heated in an autoclave at 0.776 m Hg and 121 °C for 20 min, freeze-dried, ground to a fine powder in a Moulinex coffee grinder (Moulinex Ltd, Coulsdon, Surrey, UK), and passed through a 30-mm sieve to recover all the radiopaque markers. Fecal collections were deemed to be complete in all cases, as measured by full recovery of radiopaque markers. After mixing in a powder homogenizer for 30 min, subsamples were taken for analysis. The Fe and Zn contents were determined by AAS with the method outlined above, total ^{58}Fe was measured by neutron-activation analysis (NAA), and total ^{67}Zn was measured by fast-atom-bombardment mass spectrometry (FABMS).

Neutron activation analysis

Dried fecal material was burned to ash at 480 °C for 48 h in silica crucibles and the ash was analyzed for ^{58}Fe by NAA as outlined in an earlier study (5). The quantity of ^{58}Fe that came from the enriched dose was estimated by deducting naturally occurring isotope (0.33 atom %, 0.34 wt %) from the total with allowance for natural abundance of ^{58}Fe in the label. Retention of ^{58}Fe from ferric chloride was then calculated and compared.

TABLE 2
Extrusion conditions

	Bran-flour mixture	Bran
Die size (mm)	3	3
Barrel temperature (°C)	27/52/93/140/140	25/35/56/80/80
Feed rate (kg/h)	30	20
Water flow rate (kg/h)	2.25	4.8
Screw speed (rpm)	190	250
Mean die pressure (MPa)	4.48	2.07
Mean die temperature (°C)	141	85

TABLE 3
Iron, zinc, phytate, and dietary fiber content of nonextruded and extruded bran-flour (1:1) mixture*

	Fe	Zn	Phytate	Fiber
	mg/kg	*mg/kg*	*g/kg*	*g/kg*
Nonextruded bran-flour mixture	62.9 ± 0.7	43.4 ± 0.1	6.77 ± 0.07	161.1 ± 0.24
Extruded bran-flour mixture	75.3 ± 1.1	47.1 ± 1.2	5.34 ± 0.14	146.2 ± 3.1

* $\bar{x}$ ± SEM of duplicates.

Fast-atom-bombardment mass spectrometry

Dried fecal material was burned to ash at 480 °C for 48 h in a silica crucible. To increase the Zn concentration of the solutions, the Zn was selectively extracted before analysis by the method of Götz et al (8). Portions of ash (0.1 g) were dissolved in 10 ml of 2 mol HCl/L and added to an AG1-X8 anion exchange, 200–400 mesh, chloride-form gel (Bio-Rad Laboratories, Richmond, CA). The 70 mm packing was plugged (bottom and top) with glass wool and washed with 60 ml 2 mol HNO_3/L by use of a LKB 2120 Varioperpex II pump (LKB Instruments Ltd, S Croydon, Surrey, UK) operating at a flow rate of 1 mL/min, regenerated to the chloride form with 60 ml 0.5 mol HCl/L. After addition of the sample, the column was washed with another 30 ml 0.5 mol HCl/L and the Zn was eluted with 20 mL 0.04 mol HCl/L collected as 1-mL fractions. These were dried and redissolved in 20 μL of 2 mol HNO_3/L and 2 μL samples were used for analysis. The FABMS was carried out as previously described (9) on an MS80 RFA mass spectrometer (Kratos Analytical Instruments, Manchester, UK). The samples were analyzed in triplicate interspersed with two bracketing solutions used to correct for fractionation and other instrument variables. The ratio obtained was then used together with the total Zn concentration obtained by AAS to calculate the amount of ^{67}Zn in the feces and hence the percentage absorption of ^{67}Zn from the label.

Statistical analysis

Mean percent Fe and Zn retention from the isotopes given with the two test meals were compared in each subject by means of paired *t* tests.

Ethical consideration

The study was approved by the Institute of Food Research, Norwich Laboratory, Ethical Committee.

Results

Extrusion cooking increased the Fe level of the bran-flour mixture as shown in **Table 3.** This contaminant Fe, which must originate from the extruder, was shown to be

TABLE 4
Fecal iron and ^{58}Fe in subjects fed a nonextruded mixture and an extruded mixture of wheat bran and flour together with 1.253 mg ^{58}Fe as ferric chloride*

	Nonextruded cereal		Extruded cereal	
	Men	Women	Men	Women
Fecal dry weight (g)	185.2 ± 17.6	187.9 ± 22.6	182.3 ± 24.2	180.0 ± 24.9
Total Fe (mg)	81.6 ± 14.2	72.5 ± 12.9	71.3 ± 13.9	71.3 ± 13.8
Total ^{58}Fe (mg)	1.352 ± 0.055	1.236 ± 0.094	1.295 ± 0.089	1.228 ± 0.089
Naturally occurring ^{58}Fe (μg)†	277.5 ± 48.2	246.4 ± 43.7	242.3 ± 47.2	242.6 ± 46.8
^{58}Fe from oral dose (mg)‡	1.074 ± 0.028	0.989 ± 0.063	1.053 ± 0.056	0.986 ± 0.074
^{58}Fe retention (% of dose)§	14.3 ± 2.5	16.2 ± 4.3	15.7 ± 4.4	17.5 ± 3.1

* $\bar{x}$ ± SEM.
† Total Fe × natural abundance of ^{58}Fe (0.0034 wt%).
‡ Total ^{58}Fe − Naturally-occurring ^{58}Fe.
§ (1.253 − [1.253 × 0.0034] − ^{58}Fe from oral dose)/0.01249.

as well absorbed as endogenous food Fe in rats (10). On extrusion, the fiber concentration decreased slightly mainly because of a loss of pentose sugars although there was an apparent small increase in the cellulose fraction. Phytate also decreased as a result of the heat treatment. The nonextruded cereal contributed 1.6 mg Fe and 1.1 mg Zn and the extruded product contributed 1.9 mg Fe and 1.2 mg Zn per meal.

The mean fecal dry weights, Fe and ^{58}Fe content together with calculated ^{58}Fe enrichment values are given in **Table 4.** The total ^{58}Fe and naturally occurring ^{58}Fe, calculated from total Fe levels, are also shown. As anticipated, there was no detectable enrichment of the feces with ^{58}Fe 1–2 d before the second test meal, ie, 12–13 d after the first dose of isotope. The mean percent ^{58}Fe retention for all subjects from the ^{58}Fe dose was 15.1 ± 2.4% from the nonextruded meal and 16.5 ± 2.7% from the extruded meal. The difference was not significant. There was a large interindividual difference in Fe retention that ranged, for example, from 5.3 to 30.3% with the nonextruded meal and 6.2 to 35.7% with the extruded meal. No sex differences were observed nor was there any relationship between the level of body Fe stores, as indicated by plasma ferritin levels, and percent Fe retention.

The Zn and ^{67}Zn content of the feces and percent Zn retention are given in **Table 5.** Again, as found for Fe, there was a wide range of values among individuals. Mean ^{67}Zn retention for all subjects from the ^{67}Zn dose was 18.9 ± 1.7% with the nonextruded meal and 18.3 ± 1.5% with the extruded meal. No differences were observed between male and female subjects. As with Fe there was no significant difference between the two meals. Isotopic enrichment of the fecal samples collected just before the second test meal are shown in **Table 6.** When the ^{64}Zn:^{67}Zn fecal sample ratio was expressed as a fraction of the ^{64}Zn:^{67}Zn ratio for naturally occurring Zn, the value was close to unity for all samples, thus demonstrating that there was no enrichment of the feces with ^{67}Zn at the time of the second test meal. If anything, there was a slight reduction in ^{67}Zn, which must either be due to isotope effects or more likely to a small systematic error in the FABMS measurement. However, because the meals were given in random order there should have been no bias in the results and hence no effect on the interpretation of the data.

Discussion

Extrusion cooking was reported to adversely affect mineral absorption (1) but the mechanism of action is not yet clear. Undoubtedly there are chemical changes associated with the extrusion process and these may alter mineral bioavailability. For example, the high temperatures and intermediate water content associated with extrusion cooking favors the formation of Maillard products (11). There may also be a change in the complex polysaccharides on extrusion cooking, such as an increase in the lignin fraction caused by the heat treatment (12) and the formation of amylose-lipid complexes (13). These substances may affect mineral absorption by altering the degree of binding in the intestinal lumen.

Phytate was shown to reduce Zn availability by forming an insoluble complex (14); the extent to which this occurs is dependent upon the presence of other minerals, particularly calcium (15), and it is therefore important to consider the composition of a complete meal when investigating the effect of phytate on Zn availability. Although Sandberg et al (16) showed no change in phytate on extrusion cooking of a high-fiber product, the extruded bran-flour mixture used in the present study had a slightly lower phytate level than did the nonextruded starting material. Susceptibility to phytate hydrolysis is probably dependent on physicochemical composition, and the lack of agreement between the two studies can best be explained in terms of small variations in extrusion conditions together with the use of different raw materials to formulate the high-fiber cereal products. Kivisto et al (1) argue that the lower absorption of Zn in ileos-

TABLE 5
Fecal zinc and ^{67}Zn in subjects fed a nonextruded mixture and an extruded mixture of wheat bran and flour together with 5.13 mg ^{67}Zn as zinc chloride*

	Nonextruded cereal		Extruded cereal	
	Men	Women	Men	Women
Total Zn (mg)	58.96 ± 6.46	58.85 ± 10.55	54.58 ± 7.03	63.22 ± 8.81
^{67}Zn from oral dose (mg)†	4.25 ± 0.08	4.06 ± 0.16	4.14 ± 0.12	4.26 ± 0.11
^{67}Zn retention (% of administered dose)	17.4 ± 1.5	20.8 ± 3.2	19.3 ± 2.3	17.0 ± 2.1

* $\bar{x}$ ± SEM.
† Calculated from FABMS as follows:

$$^{67}Zn = \frac{^{67}Zn_e \cdot ^{67}Zn_a \cdot W_e}{^{*}Zn_a}$$

Where $^{67}Zn_e$ is the abundance of ^{67}Zn in enriched isotope (93.11 atom %), $^{67}Zn_a$ is the atomic weight of ^{67}Zn (66.9271), $^{*}Zn_a$ is the atomic weight of enriched isotope (66.8600), and W_e is the weight of enriched isotope in feces.

tomy patients given an extruded cereal compared with a nonextruded product may be indirectly due to differences in phytate levels. They suggest that extrusion cooking may deactivate the phytase that is naturally present in bran thereby leading to higher intestinal phytate levels and hence greater Zn binding. This suggestion was supported by the fact that the ileostomy output of subjects fed the extruded cereal contained more phytate than those fed the nonextruded cereal. It is clear, however, that the small reduction in phytate in the present study had no bearing on Zn availability from the mixture of cereal and milk because there was no significant difference in Zn retention from the stable ^{67}Zn solution given with either the nonextruded porridge or the extruded bran-flour mixture.

Another factor that needs to be considered is the modifying influence of milk on Fe and Zn availability. Although any effects will be similar in the two types of meal administered, it was demonstrated in vitro that milk generates more soluble Fe from cereals than a solution of water of similar pH containing Ca (17). Milk also exerts a protective effect on mineral precipitation by sodium phytate (18). It could be argued that any changes in Fe or Zn availability caused by extrusion cooking may have been counteracted by the milk. Kivisto et al (1) prepared an extruded cereal product similar to bread that was not given with milk. Thus, the fact that they observed a reduction in Zn availability whereas we found no difference may be because the cereal in our study was given with milk, which masked any adverse effects of extrusion cooking.

Maillard products were shown to adversely affect Zn metabolism in humans (19) and both Zn and Ca metabolism in rats (20). Although not analyzed, it is likely that the extruded bran-flour mixture contained some Maillard fractions (11) but if present they apparently had no influence on Fe or Zn retention.

To conclude, the percent retention of both ^{58}Fe (1.253 mg) and ^{67}Zn (5.13 mg) was similar in men and women whether taken with a nonextruded bran-flour porridge or with the same ingredients after extrusion cooking. The lack of effect on Zn compared with a similar study with ileostomy patients (1) may be due to differences in the composition of the foodstuffs. However, undoubtedly there were major differences in the intestinal physiology of the normal healthy adults in our study and the ileostomy patients used for the Swedish study, which may be responsible for the different findings. It appears, therefore, that extrusion cooking has no detrimental effect on Fe or Zn retention from a bran-flour mixture with milk as measured with stable isotopes given with a single meal.

TABLE 6
Fecal sample collected 1–2 d before second test meal*

	Men	Women
^{64}Zn:^{67}Zn sample ratio	11.6311 ± 0.2199	11.4262 ± 0.3880
^{64}Zn:^{67}Zn ratio of unenriched Zn standard†	11.4723 ± 0.2471	11.0681 ± 0.3197
Sample/standard	1.0143 ± 0.0111	1.0322 ± 0.0182

* $\bar{x}$ ± SEM.
† Calculated ^{64}Zn:^{67}Zn ratio of unenriched Zn is 11.8954.

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