The effect of wheat bran on the absorption of minerals in the small intestine

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1. Studies on mineral absorption were carried out in ileostomy patients using the metabolic balance technique. The effect of wheat bran on the absorption of phosphorus, calcium, magnesium, zinc and iron was studied. The extent of digestion of bran phytate in the stomach and small intestine was also investigated.

2. Eight patients with well established conventional ileostomies were studied during two periods while on a constant low-fibre diet. In the second period, 16 g wheat bran/d (American Association of Cereal Chemists) was added to the diet. The amount of phytate-P, non-phytate-P, Ca, Mg, Zn and Fe was determined in the ileostomy contents and in duplicate portions of the diet.

3. Of the added bran phytate-P 24–61% was recovered in the ileostomy contents. In the bran period a significantly decreased amount of Zn was absorbed, while the apparent absorption of Fe and phytate-P increased and that of non-phytate-P, Ca and Mg remained constant. Due to the mineral content of bran, the relative absorption differed in some respects from the absolute absorption, being decreased for Zn, Mg and phytate-P but unchanged for Ca, Fe and non-phytate-P.

4. It is concluded that phytate is partly digested in the stomach and small intestine or possibly absorbed. Addition of 16 g bran/d to the diet does not seem to impair the mineral absorption from the small intestine except that of Zn.

Wheat bran has been suggested as having an inhibitory effect on mineral absorption (McCance & Widdowson, 1942*a*, *b*; Ismail-Beige *et al.* 1977; Cummings, 1978; Davies, 1979). The supposed impairment of mineral absorption is ascribed to the high content of phytate or fibre in bran; components which have a capacity to bind metals in vitro. Studies on zinc and iron absorption, both those utilizing the radionucleide technique (Björn-Rasmussen, 1974; Dobbs & Baird, 1977; Simpson *et al.* 1981; B. Sandström, unpublished results) and those performed with conventional metabolic-balance technique (McCance & Widdowson, 1942*a*, *b*; Reinhold *et al.* 1973, 1976*a*, *b*) have pointed in that direction. However, the studies measuring the uptake of radioactive Fe and Zn isotopes have been carried out as single-meal tests and it does not give a measure of the total absorption from all the meals during the day. The metabolic-balance technique has serious limitations as regards trace elements since even a minor contamination may affect the results since the level of absorption usually is relatively low (Sandström, 1980). Small analytical errors and practical problems in demarcating faeces in portions that correspond to the period of intake can cause large errors in the final evaluation of the calculated absorption (Isaksson & Sjögren, 1967).

The fate of ingested phytin-phosphorus in the human body has been investigated by McCance & Widdowson (1935), who concluded that 20–60% is excreted unchanged in the faeces. Whether or not digestion and absorption of phytate from bran occurs in the stomach and small intestine or colon is unknown.

The aim of the present investigation was to study the effect of wheat bran on the absorption of Fe, Zn, magnesium, calcium and P. To avoid the practical problems and errors

associated with collecting and demarcating faeces the study was confined to well established conventional ileostomy patients. Balance studies in this group of patients, collecting the ileostomy contents in external appliances, would be greatly facilitated and would also allow for an estimation to what extent phytate in bran is digested in the stomach and small intestine.

The Ethical Committee of Sahlgren's Hospital approved the study.

SUBJECTS AND METHODS

Subjects. Eight patients (six men, mean age 38 years; two women aged 52 and 67 years) 7 months to 2 years previously proctocolectomized for ulcerative colitis and with well-established ileostomies volunteered to take part in the study. Only a few centimetres of the terminal ileum had been removed. The ileostomies functioned properly and the volumes of excreta were within normal range without the use of drugs. The patients were hospitalized for local treatment of persisting perineal sinuses but were otherwise healthy and in excellent general condition, having completely recovered after surgery. General drug therapy was given only to one patient who took Seloken (Hässle, β-adrenergic receptor antagonist).

Experimental model. Five patients were studied for two consecutive weeks at hospital. In the first week (period 1), starting on Monday at lunch and ending on Friday morning after breakfast, the patients were given a constant low-fibre diet. They spent the weekend at home. The next week (period 2) the same regimen was followed except that the constant low-fibre diet was now supplemented with 16 g wheat bran/d. The other three patients were studied during ten consecutive days on a low-fibre diet supplemented with bran on days 5, 6 and 7.

Diets. The low-fibre diet consisted mainly of rice, fish or meat, white bread and ice-cream.

American Association of Cereal Chemists (AACC) certified food grade wheat bran was used in the study, except for patient no. 1, who was given a Swedish commercial bran. In period 2, 16 g raw bran was taken in separate doses during the day, mixed in rice (lunch, dinner) or on the sandwiches.

Duplicate portions of the diet were collected on the third day in each period and homogenized. Minerals and phytate-P were analysed in diets, and bran was analysed separately. Details of diets, collection and analytical methods for determination of fibre components and starch have been given elsewhere (Sandberg *et al.* 1981).

Collection of ileostomy contents. Ileostomy contents were collected the last 3 d in each period. For patients studied during 10 d, ileostomy contents were collected during the last 9 d.

Analytical methods. The ileostomy contents of each 24 h period and duplicate portions of diet were analysed. All mineral and phytate-P analyses were performed in acid-washed glassware and only demineralized water was used in analyses. Duplicate samples for analyses of Fe, Zn and Mg were prepared by dry-ashing of freeze-dried material (0·3 g ileostomy contents or 0·6 g diet) at 450° overnight. After cooling, three drops of nitric acid (2:1, v/v) were added, the ashing was continued, another three drops of HNO_3 were added and then ashing continued until a white or yellow residue remained. The ash was dissolved in 5 ml 5 M-hydrochloric acid and allowed to stand under cover overnight. The solutions were transferred with demineralized water to 25 ml flasks (diet samples) and 100 ml flasks (ileostomy contents) respectively and made to volume. Triple samples for Ca and P analyses were prepared by the wet-ashing of 0·1 g freeze-dried material in 1 ml sulphuric acid and 3 ml hydrogen peroxide for 15 min at 295°. (If the digest was not colourless the ashing was continued after adding another 2 ml H_2O_2.) The digests were diluted with demineralized water to 75 ml.

Fe, Zn, Mg and Ca were determined against their blanks in an atomic absorption

Table 1. *Intake, recovery in ileostomy fluid, and apparent absorption of phytate-P and non-phytate-P*

(Mean values with their standard errors)

	Phytate-P (mmol/24 h)		Non-phytate-P (mmol/24 h)	
	Mean	SE	Mean	SE
Intake:				
Diet	1·2	0·14	48·4	3·3
Swedish bran	4·2	—	2·0	—
AACC* bran	3·9	—	1·7	—
Recovery in ileostomy fluid:				
Period 1†	0·29	0·03	12·8	2·1
Period 2†	1·9	0·16	15·5	2·7
Period 3‡	0·28	0·03	15·2	4·7
Statistical significance of difference between periods 1 and 2 (*P*)	< 0·01	—	NS	—
Amounts absorbed:				
Period 1	0·88	0·13	35·5	2·1
Period 2	3·22	0·29	34·6	2·3
Period 3	1·20	0·19	32·5	3·0
Statistical significance of difference between periods 1 and 2 (*P*)	< 0·01	—	NS	—
Relative absorption:				
Period 1	0·74	0·03	0·74	0·03
Period 2	0·62	0·04	0·69	0·04
Period 3	0·80	0·05	0·69	0·04
Statistical significance of difference between periods 1 and 2 (*P*)	< 0·02	—	NS	—

* American Association of Cereal Chemists.

† Mean values of 3 d collection of ileostomy contents when the subjects were given a constant low-fibre diet (period 1) or a diet supplemented with 16 g wheat bran/d (period 2).

‡ For the three patients studied during ten consecutive days; mean values of the last 3 d collection of ileostomy contents when the subjects took a constant low-fibre diet.

spectrophotometer (Perkin Elmer Model 360). P was determined by a colorimetric method according to Fiske & Subbarow (1925).

Phytate was determined by an Fe-precipitation method according to Ellis *et al.* (1977) modified by Hasselblad & Sandberg (unpublished results) and analysis of phytate-P. Samples of 0·5 g freeze-dried diet or ileostomy contents were extracted with 20 ml 0·5 M-HCl containing sodium sulphate (50 g/l), filtrated through a Munktell OOH filter, frozen overnight and filtrated through an MF-Millipore filter (0·10 μm pore size) under pressure. Each sample was then precipitated with ferric ion by adding 0·25 ml ferric chloride (4 g/l) in 0·5 M-HCl containing Na_2SO_4 (50 g/l) to 5 ml of the filtrate. The mixtures were kept in a 95° water-bath for 20 min, cooled and centrifuged at 2300 rev./min for 20 min at 20°. The ferric precipitates were washed three times with 5 ml 0·5 M-HCl and then dissolved in 1 ml concentrated H_2SO_4. From each tube was withdrawn 0·75 ml for wet-ashing at 295° for 15 min, 0·25 ml H_2O_2 (300 ml/l) was added, and then the wet-ashing was continued for another 15 min. The digests were quantitatively transferred to 10 ml flasks, diluted with approximately 8 ml demineralized water, kept in a boiling water-bath for 15 min, then cooled and made up to volume. The flasks were allowed to stand at room temperature overnight and then analysed for P according to Fiske & Subbarow (1925). Addition of calcium hydrogen phosphate to samples of diets and ileostomy contents verified that no coprecipitation of inorganic phosphate occurred when phytate was precipitated with Fe^{3+} as described previously.

Table 2. *Intake, recoveries in ileostomy fluid, and 'apparent absorption' of minerals*
(Mean values with their standard errors)

	Calcium (mmol/24 h)		Magnesium (mmol/24 h)		Zinc (mg/24 h)		Iron (mg/24 h)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Intake:								
Diet	31·8	2·6	9·0	0·5	12·9	1·2	10·6	0·9
Swedish bran	0·57	—	2·8	—	1·0	—	1·7	—
AACC* bran	0·60	—	3·0	—	1·3	—	2·2	—
Recovery in ileostomy fluid:								
Period 1†	19·6	1·8	5·0	0·31	10·9	1·2	8·2	0·74
Period 2†	21·7	2·3	7·3	0·36	12·7	1·2	9·1	0·61
Period 3‡	19·0	2·7	4·8	0·44	10·4	1·6	7·6	0·46
Statistical significance of difference between periods 1 and 2 (*P*)	< 0·02	—	< 0·01	—	< 0·01	—	< 0·01	—
Amounts absorbed:								
Period 1	12·2	1·5	4·1	0·34	2·0	0·30	2·5	0·37
Period 2	10·8	1·3	4·5	0·39	1·2	0·31	3·3	0·44
Period 3	15·2	3·3	4·0	0·10	1·9	0·64	2·2	0·23
Statistical significance of difference between periods 1 and 2 (*P*)	NS	—	NS	—	< 0·01	—	< 0·02	—
Relative absorption:								
Period 1	0·38	0·029	0·45	0·025	0·16	0·023	0·23	0·032
Period 2	0·34	0·034	0·38	0·025	0·076	0·020	0·26	0·031
Period 3	0·44	0·029	0·45	0·028	0·16	0·066	0·23	0·021
Statistical significance of difference between periods 1 and 2 (*P*)	NS	—	< 0·01	—	< 0·01	—	NS	—

* American Association of Cereal Chemists.

† Mean values of 3 d collection of ileostomy contents when the subjects were given a constant low-fibre diet (period 1) or a low-fibre diet supplemented with 16 g wheat bran/d (period 2).

‡ For the three patients studied during ten consecutive days; mean values of the last 3 d collection of ileostomy contents when the subjects took a constant low-fibre diet.

Calculations and statistical methods. The amount of undigested bran phytate was estimated as the difference between analytical values of ileostomy contents of periods 1 and 2 for each patient. The value for non-phytate-P was calculated as the difference between total phosphorus and phytate-P and apparent absorption of minerals as the difference between dietary intake and recovery in ileostomy contents. For statistical comparison of values from the two periods Wilcoxon's matched-pairs signed-ranks test was used.

RESULTS

Recovery of unabsorbed phytate-P and non-phytate P in the ileostomy contents. Daily intake of phytate-P and non-phytate-P from the low-fibre diet and bran and recovery in ileostomy contents are summarized in Table 1. Phytate-P constituted only a small part of the total phosphorus intake, especially during the low-fibre diet period. Between 24 and 61% (mean ± SE, 41 ± 4·3%) of phytate-P from ingested bran was found in the ileostomy contents. There was no significant correlation between recovery of phytate-P in ileostomy fluid and that of Zn, Fe, Mg, Ca and non-phytate-P during the low-fibre diet or bran period.

Apparent absorption of minerals. Intake, recoveries in ileostomy fluid and apparent

absorption of minerals are given in Tables 1 and 2. In the bran period a significantly decreased amount of Zn was absorbed ($P < 0{\cdot}01$) while that of phytate-P ($P < 0{\cdot}01$) and Fe ($P < 0{\cdot}02$) increased. No significant difference in absolute absorption of non-phytate-P, Ca and Mg between the two periods was found. Due to the mineral content of bran the relative absorption differed in some respect from the absolute absorption. There was a significant decrease in relative absorption of Zn, Mg and phytate-P after consumption of bran while that of Ca, Fe and non-phytate-P did not differ significantly between the periods.

DISCUSSION

The transit time of the small intestine is short and consequently the difficulties in demarcating faeces in portions that correspond to the period of intake are avoided by studying ileostomy patients. The undigested bran components are excreted on the day of consumption, as reported earlier (Sandberg *et al.* 1981). Phytate is partly digested in the stomach and small intestine or possibly absorbed. Our results correspond well to that of McCance and Widdowson (1935), who found 20–60% of ingested phytin-P from wheat products in the faeces of normal subjects. The bacterial degradation in the colon thus seems less important. This is in agreement with studies on dogs which indicate that the degradation of phytate occurs chiefly in the first two-thirds of the small intestine and to a minor extent in the colon (Jenkins & Philips, 1960).

It is a matter of discussion whether or not there is any difference between normal subjects and ileostomy patients concerning mineral absorption. It is likely that a slight absorption takes place in the colon but the principal absorption should occur in the small intestine. The formation of mineral–organic complexes is highly pH-dependent.

The pH value of the ileostomy contents was not measured in the study but in other ileostomy patients we have found pH values between 6 and 8 corresponding to that of the ileum contents of normal subjects. Thus, in this respect ileostomy patients should not differ from normal subjects and the result would reflect the absorption in the human small intestine. In the present study the endogenous excretion of minerals was not measured and was supposed to be the same in both periods.

The amounts of absorbed Mg and non-phytate-P did not seem to be affected by the consumption of 16 g wheat bran. The absorption of Ca was slightly decreased in seven of the eight patients but not significantly. It appears as if phytate-P is almost as effectively utilized as non-phytate-P. Bran seems to decrease the absorption of Zn. That the reduced absorption of Zn during period 2 was due to the bran intake is supported by the fact that the three patients studied during ten consecutive days showed an increase in Zn absorption during the last 3 d i.e. after the bran period. The reduced Zn absorption might be due to the formation of a Zn–phytate complex; however, there was no correlation during the bran period between the daily excretion of phytate and Zn in ileostomy contents. It has been demonstrated that a dietary intake of phytate causes a depression in growth rate in young rats if the phytate : Zn molar ratios are large (Oberleas & Prasad, 1976; Davies & Olpin, 1979; Morris & Ellis, 1980*a*, *b*). The effect on Zn absorption in humans of 2·5 g phytic acid added daily to the diet was studied by Reinhold, Nasr *et al.* (1973). Balance studies on subjects with intake of leavened or unleavened whole-meal bread were also performed (Reinhold, Hedayati *et al.* 1973). In both studies the conclusion drawn was that phytic acid decreases the availability and intestinal absorption of Zn. The importance of phytic acid was later questioned by Reinhold *et al.* (1976*a*). This new view was supported by negative Zn balances observed in two subjects who had a high intake of whole-meal bread, with a moderate phytate content (Reinhold *et al.* 1976*b*) and in vitro studies on the Zn-binding capability of various fibre fractions (Ismail-Beige *et al.* 1977). When comparing Zn availability to rats from diets supplemented with bran, bran fibre or phytate, Davies *et al.*

(1977) concluded that phytate rather than fibre is the main determinant of Zn availability. The fibre content was not found to affect Zn balances in a 30 d study period during which 26 g of various fibres were added to a normal American diet (Sandstead *et al.* 1979). Bioavailability to rats of Zn in raw wheat bran and low-phytate brans containing almost the same amounts of neutral detergent fibre was investigated by Morris & Ellis (1980*b*). They found no evidence that the fibre of wheat bran diminished the bioavailability of Zn to rats. In a study on humans (B. Nävert, Å. Cederblad and B. Sandström, unpublished results) bran-containing breads with low content of phytate, obtained by long leavening time (15 h), was compared with high-phytate breads made with short leavening time (1 h). The amount of dietary fibre was equal in both types of bread. The Zn absorption was approximately twice as high from the low-phytate bread. Most of the recent literature indicates an effect of phytate. The present study, however, does not determine whether phytate or some other component in bran is responsible for the reduced Zn absorption.

The apparent absorption of Fe was high in both periods. A diet rich in meat products should give good Fe and Zn absorption, but does not explain the very high Fe absorption found in some patients. The Fe status was assessed by measuring haemoglobin, serum-Fe and total iron binding capacity. These were within normal range, except for three patients, who had slightly lowered serum-Fe. Fe-deficiency anaemia frequently occurs in patients with ulcerative colitis before they are proctocolectomized but the high Fe absorption in some of the patients indicated low Fe stores 7 months–2 years after the operation. The Fe status should, however, be the same in the two consecutive periods. In balance studies only apparent absorption is measured. A prolonged radio iron excretion has been demonstrated in normal subjects after oral intake, indicating a temporary mucosal uptake and storage of iron (Björn-Rasmussen *et al.* 1980). The apparent absorption might therefore be higher than the true absorption of iron.

The apparent absorption of Fe in absolute amounts increased when bran was consumed. More than 60% of the Fe in wheat bran is present in the form of monoferric phytate (Morris & Ellis, 1976). Studies on rats (Ellis & Morris 1979), dogs (Lipschitz *et al.* 1979) and humans (Simpson *et al.* 1981) demonstrate that Fe from monoferric phytate can be absorbed. Of great importance is the extent to which Fe is solubilized in the upper duodenum, where the main absorption takes place (Schulerud, 1980). Studies on anaemic rats (Schulerud, 1980) and pigs (Frölich & Lysö, 1980) have shown that Fe from bran is utilized just as well as or even better than ferrous sulphate. In this respect our results conflict with those of Björn-Rasmussen (1974), Dobbs & Baird (1977) and Simpson *et al.* (1981), who found a decrease in relative Fe absorption when a single meal with baked bread or muffins containing wheat bran or whole meal was compared with bread of low bran content, white bread or plain muffins. Simpson *et al.* (1981) held the Fe content constant by the addition of $FeCl_3$, but in the studies of Björn-Rasmussen (1978) and Dobbs & Baird (1977) there was no reduction of absorption when calculating in absolute amounts since bran is rich in Fe. Sandstead *et al.* (1979) found no effect of bran on Fe balances. In the studies on humans referred to, the bran was given in baked bread in which Fe might no longer be present as monoferric phytate. Ferric phytate with 2–4 mol Fe has poor bioavailability, at least in the rat (Ellis & Morris, 1979). Formation of such compounds may take place during baking. Another possible explanation is that a single meal test does not give the same results as a study of the total daily absorption as a reduced absorption from one meal might be compensated by another. The protein level in the meals may also be of importance.

It is concluded that phytate is partly digested in the stomach and small intestine or possibly absorbed. A supplement of 16 g wheat bran to the diet does not negatively affect the absorption of minerals from the small intestine, other than Zn.

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