Short communication

Wholewheat flour ensures higher mineral absorption and bioavailability than white wheat flour in rats

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(Received 8 June 1998 – Revised 30 November 1998 – Accepted 3 March 1999)

Consumption of unrefined whole flour is thought to affect mineral bioavailability because it contains high levels of fibre and phytic acid. The present experiment was designed to study the absorption of minerals from diets based on wholewheat flour and white wheat flour in rats. Two groups of male Wistar rats were fed on the diets for 3 weeks and absorption and tissue retention of minerals were studied. The rats fed on the wholewheat flour diet had significantly greater food intake, weight gain, faecal excretion and intestinal fermentation than those fed on the white flour diet. Mineral intakes, except for Ca, were significantly greater in rats fed on the wholewheat flour diet (4-fold for Mg, 2-fold for Fe and Zn). A significant rise in the apparent absorption of Fe (%) and a significant decrease in the apparent absorption of Zn (%) were observed. The amounts of minerals absorbed (mg/d) were significantly enhanced (excepted for Ca) with the wholewheat flour diet. Moreover, plasma and tibia levels of Mg and plasma, liver and tibia levels of Fe were significantly increased in rats fed on the wholewheat flour diet compared with those fed on the white flour diet. In conclusion, wholewheat flour, rich in phytic acid and minerals, did not have a negative effect on mineral absorption, but rather improved the bioavailability of some minerals. Human studies are needed to confirm these rat results before extrapolation to human nutrition.

Wholewheat flour: Bioavailability: Minerals

Over 50 % of the world's total food energy is supplied by the eight most common grains (wheat, rice, maize, oats, rye, barley, millet and sorghum). These are major sources of starch, dietary fibre, protein, minerals and vitamins (Holland *et al*. 1988). The grains consumed in industrialized countries are usually subjected to various types of processing, such as milling, heat extraction and cooking. Milling is a process that may involve separating bran and germ from the starchy endosperm in order to produce white flour. Such a refining process results in significant losses in fibre, vitamins and minerals.

Except for Ca and Na, a cereal like wheat contains sufficient minerals to fulfil most requirements (Salovaara, 1993), but wheat is a cereal rich in phytic acid (3·2 g/kg whole grain), which is known to affect mineral absorption (Morris & Ellis, 1985; Miyazawa & Yoshida, 1991). Thus, it was of interest to compare the mineral absorption in rats fed on diets in which the cereal product supplied was either white wheat flour or wholewheat flour, supplemented only with $CaCO_3$ and NaCl.

Materials and methods

Animals and diets

Twenty male Wistar rats (IFFA-CREDO, L'Arbresle, France), initial weight 150 g, were adapted to the experimental semi-purified diets for 21 d. The diets contained (g/kg): French variety (Soisson, Soft Red Winter class) wheat flour (white or whole) 875, casein 60, peanut oil 50, $CaCO_3$ 7·5, and NaCl 7. No vitamins were added because the wheat flour was considered to provide sufficient to meet essential requirements. The faeces of each rat were collected over three consecutive days.

Sampling and analytical procedures

Rats were anaesthetized with sodium pentobarbital (40 mg/kg), and blood was drawn from the abdominal aorta. After blood sampling, the caecum, with its contents, was removed and caecal contents were transferred into a microcentrifuge

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https://doi.org/10.1017/S0007114599001075 Published online by Cambridge University Press

tube for measurement of pH and short-chain fatty acids. Liver and tibia were also sampled for mineral analyses.

Phytic acid was measured by a colorimetric procedure based on the reaction between ferric ions and sulfosalicyclic acid, as described by Latta & Eskin (1980). Short-chain fatty acids were measured by GLC on a portion of the supernatant fraction of the caecal contents after centrifugation (20 000 ***g***, 10 min, 4°) (Demigné *et al*. 1980). The short-chain fatty acid and lactate caecal pools were calculated as follows: caecal pool (μmol) = caecal concentration (μmol/g) × caecal fresh content weight (g).

Mg and Ca were determined after dry mineralization of tissue, food and faeces samples (10 h at 500°). Ash was extracted with 5 M-HCl and made up to an appropriate volume with $LaCl_3$ solution (1 g/l). To determine Fe and Zn levels in tissue, food and faeces, 0·25 g dried sample was dry-ashed and extracted at 130° in HNO_3–H_2O_2, until decoloration. Final dilutions were made in 0·14 M-HNO_3. Plasma Ca and Mg concentrations were measured after a 50-fold dilution in $LaCl_3$ solution. The other trace elements were measured after a 5-fold dilution of plasma in 0·1 M-HCl. Mineral concentrations were determined by atomic absorption spectrophotometry (Perkin-Elmer 420, Norwalk, CT, USA). The apparent absorption (%) was calculated as follows: apparent absorption (%) = (daily mineral intake (mg/d) – daily faecal mineral excretion (mg/d)) × 100/daily mineral intake (mg/d). Transferrin saturation (%) was determined using a Ferrimat kit and a TIBC additive from Bio Mérieux (Lyon, France).

Statistical analysis

Values are given as means with their standard errors and Student's *t* test was used to determine the statistical significance of the means. Values were considered to be significantly different at a probability level of $P < 0·05$.

Results

Diet and caecum analyses

As shown in Table 1, the chemical analysis showed that the wholewheat flour diet, compared with the white flour diet, contained almost 4-fold greater levels of fibre and phytic acid. The mineral analysis showed, also, that the level of Ca was similar in both diets, but that the wholewheat flour diet was richer in Mg (4-fold), Zn (2-fold) and Fe (2-fold) than the white flour diet. The wholewheat flour diet met dietary mineral recommendations for rats (Reeves *et al*. 1993), whereas the white flour diet was deficient in all of the measured minerals (Table 1). The wholewheat flour diet was consumed in significantly larger amounts (23·2 (SE 0·8) *v*. 20·6 (SE 0·3) g/d, $P < 0·005$) and the weight gain of rats fed on this diet was significantly higher (5·8 (SE 0·3) *v*. 4·2 (SE 0·2) g/d, $P < 0·001$) than that of the rats fed on the white flour diet.

As bran fibre is poorly fermentable, wholewheat flour ingestion was accompanied by a significant increase in faecal weight (3-fold) compared with ingestion of white flour (3·08 (SE 0·13) *v*. 0·79 (SE 0·05) g/d, $P < 0·001$). However, the total caecal weight (4·29 (SE 0·29) *v*. 5·19 (SE 0·35) g) and the caecal pH (6·23 (SE 0·08) *v*. 6·08 (SE 0·12) were not significantly different between the wholewheat and white flour groups respectively. Finally, the short-chain fatty acid pools were significantly different (388 (SE 21) *v*. 328 (SE 15) μmol, $P < 0·05$) in rats fed on the wholewheat and white flour diets respectively.

Mineral balance (Table 2)

The Ca levels were similar in the two experimental diets. The apparent absorption (%) and the amount of Ca apparently absorbed (dietary intake – faecal excretion, mg/d) were not significantly different between the two diets.

Mg intake was 4-fold higher in rats fed on the wholewheat flour diet than in those fed on the white flour diet. The apparent absorption of Mg (%) was slightly lower in rats fed on the wholewheat flour diet than in those fed on the white flour diet. Consequently, the amount of Mg apparently absorbed (mg/d) was significantly increased in the wholewheat group.

Concerning Fe, the daily intake was doubled and its apparent absorption (%) was increased with the wholewheat flour diet compared with the white flour diet. Consequently, the amount of Fe apparently absorbed (mg/d) was more than doubled with the wholewheat flour diet.

Table 1. Starch, protein, fat, fibre, phytic acid and mineral composition of diets and mineral recommendations of the American Institute of Nutrition (AIN) (Reeves *et al*. 1993)

	Diet		
Component	White flour	Wholewheat flour	AIN recommendations for rats
Starch (g/kg)	622	522	
Protein (g/kg)	154	155	
Fat (g/kg)	62·1	73·1	
Fibre (g/kg)	20·1	92·5	
Phytic acid (g/kg)	0·235	0·821	
Calcium (g/kg)	3·10	3·50	5·0
Phosphorus (g/kg)	1·6	3·7	3·0
Magnesium (g/kg)	0·30	1·11	0·5
Iron (mg/kg)	16·0	29·1	35·0
Copper (mg/kg)	1·6	4·4	6·0
Zinc (mg/kg)	16·3	29·3	30·0

Table 2. Mineral balance values for rats fed for 3 weeks on diets based on white wheat flour or wholewheat flour*

(Mean values with their standard errors for ten rats per dietary group)

	White wheat flour		Wholewheat flour		Statistical significance of difference between means: *P*=
	Mean	SE	Mean	SE	
Calcium (mg/d)					
Intake	64·7	5·2	81·5	8·6	NS
Faecal excretion	8·2	0·5	17·8	1·1	< 0·001
Apparent absorption	56·5	2·9	63·7	2·5	NS
Apparent absorption (%)†	87	6	78	6	NS
Magnesium (mg/d)					
Intake	6·6	0·5	24·8	2·1	< 0·001
Faecal excretion	1·1	0·3	7·5	0·5	< 0·001
Apparent absorption	5·5	0·4	17·3	1·9	< 0·001
Apparent absorption (%)†	83	9	70	6	NS
Iron (μg/d)					
Intake	330	11	675	20	< 0·001
Faecal excretion	126	8	176	4	< 0·001
Apparent absorption	204	6	499	11	< 0·001
Apparent absorption (%)†	62	4	74	3	< 0·001
Zinc (μg/d)					
Intake	336	12	675	17	< 0·001
Faecal excretion	172	10	441	12	< 0·001
Apparent absorption	164	8	234	8	< 0·001
Apparent absorption (%)†	49	3	35	2	< 0·001

* For details of diets and procedures, see Table 1 and pp. 17–18.
† The percentage apparent absorption was calculated as follows: apparent absorption (%) = (mineral intake (mg/d) – faecal mineral excretion (mg/d)) × 100/mineral intake (mg/d).

Finally, the daily Zn intake was doubled with the wholewheat flour diet, compared with the white flour diet, but its apparent absorption (%) was significantly decreased (–30 %) in the first group. However, the amount of Zn apparently absorbed (mg/d) increased by about 40 % in the rats receiving the wholewheat flour diet compared with those fed on the white flour diet.

Mineral retention (Table 3)

Plasma and tibia Mg levels were significantly higher in rats fed on the wholewheat diet than in the other group (+34 % and +54 % respectively). Plasma Fe level and transferrin saturation (%) in rats fed on the wholewheat flour diet were twice as high as in the white flour group. Moreover, liver and tibia Fe levels were significantly higher in the rats fed

Table 3. Plasma, liver and bone mineral levels in rats fed for 3 weeks on diets based on white wheat flour or wholewheat flour*

(Mean values with their standard errors for ten rats per dietary group)

	White wheat flour		Wholewheat flour		Statistical significance of difference between means: *P*=
	Mean	SE	Mean	SE	
Blood plasma					
Calcium (mmol/l)	2·54	0·06	2·42	0·04	NS
Magnesium (mmol/l)	0·72	0·03	0·97	0·03	< 0·0001
Iron (μmol/l)	22·8	3·1	44·6	4·7	< 0·002
Transferrin saturation (%)	15·6	2·6	36·2	2·8	< 0·0001
Zinc (μmol/l)	14·6	0·6	16·3	0·5	NS
Liver					
Magnesium (μg/g dry wt)	805	16	829	21	NS
Iron (μg/g dry wt)	133	7	281	13	< 0·0001
Zinc (μg/g dry wt)	103	6	99	2	NS
Tibia					
Calcium (mg/g dry wt)	252	2	250	2	NS
Magnesium (mg/g dry wt)	3·48	0·11	5·35	0·05	< 0·0001
Iron (μg/g dry wt)	47·5	1·6	60·4	1·7	< 0·0001
Zinc (μg/g dry wt)	249	6	240	5	NS

* For details of diets and procedures, see Table 1 and pp. 17–18.

on the wholewheat flour diet than in the other group (+27 % and +111 % respectively). However, Ca and Zn concentrations were not significantly different between the two groups of rats in either plasma or tissue samples.

Discussion

In industrialized countries, transformations of natural products tend to deplete human food of minerals. The impact of wholewheat flour, rich in dietary fibre, on mineral bioavailability is controversial. Negative effects on mineral status have been ascribed to phytic acid (Hallberg, 1987; Torre *et al*. 1991). Thus, it is important to determine whether unrefined products, rich in minerals, can improve mineral status.

In the present experiment, the wholewheat flour allowed better animal growth than white flour. Moreover, animals exhibited greater mineral absorption than those fed on white wheat flour without any decrease in the apparent absorption of minerals except for Zn. This could be attributable to the possible extensive breakdown of phytate in rats. Phytic acid is generally consumed with various complex carbohydrates and it is likely that their fermentation, lowering the colon pH, favours phytase (*EC* 3.1.3.8) activity. Moreover, microbial fermentation of carbohydrate can increase the solubility of divalent cations and enhance their absorption in the caecum (Younès *et al*. 1996).

The fact that apparent Ca absorption was not modified in rats receiving the wholewheat flour diet was not surprising, especially because the phytic acid : Ca ratio was low (0·23). Bone Ca content confirmed that the use of the wholewheat flour diet was not accompanied by negative effects on Ca metabolism.

In the present study, the wholewheat flour rich in both phytic acid and Mg seemed to be a very good source of available Mg: 17 mg Mg was absorbed daily compared with 5·5 mg with the white flour diet and Mg status was improved. The principal route of Mg absorption is via a passive paracellular pathway in the distal part of the digestive tract, with active transcellular transport being relatively unimportant. Thus, mineral solubility and intestinal epithelial permeability are probably the principal determinants of Mg absorption (Hardwick *et al*. 1990). The fact that phytic acid inhibits Mg absorption has been reported (Morris & Ellis, 1985; Miyazawa *et al*. 1991) but is still disputed (Shah *et al*. 1990).

In cereal grains, phytic acid seems to be the major factor responsible for the low bioavailability of Fe and Zn (Brune *et al*. 1992). In contrast to Ca, the phytic acid : trace element ratios for Fe and Zn were relatively high in the present study. Thus, a lowering of the apparent absorption (%) of these minerals with the wholewheat flour diet would be expected (Hallberg *et al*. 1987; Brune *et al*. 1992).

In the present study, the wholewheat flour diet contained twice as much Fe as the white flour diet. The apparent absorption (%) of Fe (+19 %) and the amount of Fe absorbed daily (+145 %) were significantly increased with the wholewheat flour diet. Our results show also that rats fed on the wholewheat flour diet had plasma and tibia Fe concentrations twice as high as those found in rats fed on the white flour. In the liver, Fe concentration was also increased (+27 %). Because Fe homeostasis is controlled by absorption and not by modifying excretion, an increase of Fe absorption by more than 2-fold may be responsible for this rise of plasma and tissue Fe in rats fed on the wholewheat flour diet. Because Fe metabolism takes place in a closed circuit, Fe deficiency can develop only when intake is dramatically low, whereas Fe overload can occur when the daily amount of Fe absorbed in increased (Hallberg, 1981).

The present results indicate that a wholewheat flour diet induces a significant decrease in apparent Zn absorption (%) but a significant increase in the daily amount of Zn absorbed. The decrease in the apparent Zn absorption (%) is due at least partially to the higher level of Zn and other minerals in the wholewheat flour diet. Interactions between Fe and Zn absorption are well documented (Fairweather-Tait, 1995). However, Zn status was not significantly modified in rats receiving the wholewheat flour diet in comparison with rats receiving the white flour diet. It would be interesting to study the effect of such flours for a longer time.

The mineral-complexing effects of phytic acid have often been studied by adding phytic acid directly to the diets. Such protocols fail to take into account the mineral content of fibre-rich products. It is well established that rats have a high capacity to hydrolyse phytic acid, compared with human subjects who have practically no intestinal phytase activity (Iqbal *et al*. 1994). In man, the maintenance of phytase activity in cereal products may allow partial hydrolysis of dietary phytate in the stomach, improving the bioavailability of minerals. Moreover, when wheat is eaten in the form of leavened bread, more than 50 % of the phytic acid is already destroyed (Harland & Harland, 1980; Türk *et al*. 1996). Even if these results cannot be directly extrapolated to human nutrition, they show that ingestion of wholewheat flour or unrefined cereal products can contribute to improved mineral balance. Several epidemiological and clinical studies have recently stimulated interest in increasing the consumption of phytic acid-rich products in preventative nutrition (Thompson, 1994; Shamsuddin *et al*. 1997). Negative effects of such products on mineral bioavailability may be neutralized when these products are taken together with the other components of the meal (Lopez *et al*. 1998). Thus, it is necessary to reconsider the consumption of whole grains rather than of purified cereal products, to keep functionally active constituents of grains and to optimize the mineral status in man. However human studies are still needed to confirm these rat results.

Acknowledgement

Pierre Lamby is thanked for his technical assistance.

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