

Improved zinc and iron absorption from breakfast meals containing malted oats with reduced phytate content

BY MARIE LARSSON¹, LENA ROSSANDER-HULTHÉN²,
BRITTMARIE SANDSTRÖM³ AND ANN-SOFIE SANDBERG¹

¹ Department of Food Science, Chalmers University of Technology, S-402 29 Göteborg, Sweden

² Department of Clinical Nutrition, Göteborg University, Sahlgren's Hospital, S-413 45 Göteborg, Sweden

³ Research Department of Human Nutrition, Royal Veterinary and Agricultural University, DK-1958 Fredriksberg, Denmark

(Received 8 August 1994 - Revised 12 February 1996 - Accepted 15 March 1996)

The absorption of Zn or Fe from breakfast meals containing oat porridge prepared from malted and soaked oats and a control porridge made from untreated oats was measured in human subjects. The effect on Zn and Fe absorption of reducing the phytate content of oat-porridge meals was examined in each subject by extrinsic labelling of porridge with ⁶⁵Zn and of bread rolls with ⁵⁵Fe and ⁵⁹Fe, and measuring whole-body retention and the erythrocyte uptake of isotopes. Each experiment comprised nine to ten subjects. The absorption of Zn from malted-oat porridge with a phytate (inositol hexaphosphate) content of 107 μmol was 18.3%, and significantly higher ($P < 0.05$) than from the control porridge containing 432 μmol phytate (11.8%). Fe absorption from the meal containing malted-oat porridge with 107 μmol phytate (Expt 2) was also significantly improved ($P < 0.05$) compared with that from the meal containing control porridge with 437 μmol phytate. The average increase in Fe absorption was 47%, or from 4.4 to 6.0%. In the breakfast meal containing malted porridge with 198 μmol phytate (Expt 3) the increase in Fe absorption was not significantly improved. Even though the phytate content was reduced to a greater extent in Expt 3 than Expt 2, the average increase in Fe absorption in Expt 3 was only 25% more than that from the meal containing control porridge (with 599 μmol phytate), depending on the higher absolute amount of phytate. In conclusion, an improvement in Zn and Fe absorption from oat products can be achieved by practising malting and soaking in the processing of oats. This may be of importance in the prevention of mineral deficiency in vulnerable groups.

Zinc: Iron: Phytate: Oats

Oats are considered to be a nutritious cereal grain and new applications and oat products are frequently developed. However, mineral availability can be impaired at high intakes of oat products owing to the presence of high concentrations of phytate (inositol hexaphosphate; IP-6). Rossander *et al.* (1990) studied the inhibitory effects of oat products on non-haem-Fe absorption in human subjects from single meals and found that oat bran and oat porridge markedly inhibited Fe absorption. The effect on Fe stores in young, healthy subjects after dietary changes from a diet with a relatively high fat and low fibre content to a low-fat, high-fibre and high-phytate diet was investigated in a long-term study by Sandström (1993). It was found that the ferritin levels in blood were reduced during the 8-month dietary change. The absorption of Zn from single meals based on rye, oatmeal, barley, triticale and whole wheat was also evaluated (Sandström *et al.* 1987), and the lowest Zn absorption was observed from oatmeal porridge (8.4%), which also contained the highest amounts of phytate. Sandström *et al.* (1987) concluded that when phytate-rich cereals constitute a major part of the total Zn intake of a diet, processes that reduce the

phytate content could significantly improve the absorption of Zn. The high content of phytate combined with a low phytase (*EC* 3.1.3.26) activity and heat inactivation during treatment of all commercial oat products suggests that mineral absorption from oat products is less than that from other cereals.

We recently reported (Larsson & Sandberg, 1992) that it is possible to reduce the phytate content in oats to low levels by malting and soaking under optimal conditions. The aim of the present study was to confirm that malting of oats, resulting in a lower phytate content, improves Zn and Fe absorption.

MATERIALS AND METHODS

Oats were malted in a pilot-plant process or in the laboratory according to methods previously described (Larsson & Sandberg, 1992). Malting in the pilot plant was performed at Svenska Cereallaboratoriet AB, Sweden. Oats were dehulled by an industrial process (Kungsörnen AB, Järna, Sweden) and steeped for 6.5 h at 25°. Germination was performed at 15° in rotating chests in which the temperature and moisture content were controlled precisely. To maintain adequate grain moisture during germination, air, which had been humidified (90% relative humidity), was passed through the chests. The oats were allowed to germinate for 120 h and were subsequently dried at 30° for 18 h. The material was further heat-treated in a step-wise process from 30 to 80° according to the following schedule: 2 h at 40°, 2 h at 50°, 8 h at 65° and 10 h at 80°. Malting in the laboratory was performed using a laboratory incubator (Memmert Model BKE 30; Memmert GmbH, Schwabach, Germany). Oats were dehulled with a laboratory dehuller (Nygård kvarn, Vargön, Sweden) and steeped for 7 h at 25°. The steeped oats were spread in single layers on trays and dried at 30° for 1 h in order to achieve the correct moisture conditions during germination. During the germination period the oats were moistened with water two to three times during the day. The incubator was held at 15° and the oats were germinated for 5 d, and subsequently dried overnight at 30°.

Expt 1. Zinc absorption

Preparation of malted-oat porridge. Oats malted in the pilot plant (dried at 80°) were ground to a flour in a coffee mill (Model Kenwood CG 100; Kenwood Ltd, Havant, Hants) before soaking. Samples of malted-oat flour (30 g portions of test meal) were suspended in 150 ml distilled water in plastic beakers and soaked first for 17 h at room temperature (23°) and then for 8 h at 37.8°. After soaking, the samples were kept at 4° overnight. The samples were frozen the following day and kept at -20° until used. Immediately before serving, samples were thawed at low power in a microwave oven. Each portion was prepared by cooking in a microwave oven for 2 min.

Experimental design. Zn absorption was determined using the radionuclide technique described by Arvidsson *et al.* (1978). Each individual meal was extrinsically labelled by the addition of 0.02 MBq ⁶⁵ZnCl₂ (Amersham International, Amersham, Bucks.) and the activity of each porridge was measured in the whole-body counter before serving. Each subject's background was also measured in the whole-body counter before intake of the labelled test meal. The meals were served as breakfast. No food or drink was allowed during the 12 h period before breakfast or 3 h after intake of the test meal. The whole-body retention was measured once, 10–14 d after intake of the meal to allow excretion of the unabsorbed fraction. Allowance was made for the excretion of the initially absorbed isotope during the time between intake and retention measurement, based on the mean rate of excretion of an intravenously-administered dose of ⁶⁵Zn in a similar group of subjects (Arvidsson *et al.* 1978). Each subject participated twice, and the sequence of serving the malted porridge and control porridge to the subjects was randomized. Allowance was also

made on the second occasion for residual activity from the first meal. During the first 10 d, 11 (SD 0.3)% of the absorbed dose was excreted, and the mean excretion from day 10 to day 30 was 10%. The CV due to counting statistics of a typical retention value from the administered dose was 1.5%.

The meals consisted of porridge prepared from the suspension described previously: 30 g malted-oat flour (test meal) or 30 g untreated (control meal), dehulled and ground oats (from the same batch as the malted oats). The porridge was prepared by adding 1 g table salt and cooking in a microwave oven for 2 min. The isotope was added to the porridge after heating. The porridge was served without any addition of milk or jam.

Subjects. Seven females and three males between 21 and 30 years of age volunteered to take part in the study. All ten subjects participated twice. They were all apparently healthy, not pregnant and with no known gastrointestinal disorders, and had normal serum Zn levels of 9.3–12.2 (mean 11.0) $\mu\text{mol/l}$. The subjects were given written and oral information about the aim and procedure of the study and the project was approved by the Ethics Committee and the Isotope Committee at Sahlgren's Hospital.

Expts 2 and 3. Iron absorption

Fe absorption was measured in two experiments with malted-oat porridge prepared in different ways and at two levels of phytate.

Preparation of malted-oat porridge. In Expt 2, oats malted in the pilot plant (dried at 80°) were ground to a flour in a coffee mill before soaking. Samples of malted-oat flour (30 g) were suspended in 300 ml distilled water in plastic beakers and soaked first for 16 h at room temperature and then for 7 h at 37.8°.

After soaking, 175 ml of the supernatant fraction was removed from each sample to reduce the volume of the fluid and, thus, achieve an acceptable consistency of the porridge, and the remaining 125 ml was transferred to a plastic jar. The beaker was rinsed with 25 ml of the supernatant fraction and this volume was added back to the oat-flour slurry, resulting in a sample volume of 150 ml, which was kept in the refrigerator (+4°) overnight. On the following day each portion of test porridge was prepared by cooking the sample in a microwave oven for 2 min.

The control porridge portion was prepared by mixing 150 ml distilled water with 30 g untreated, dehulled and ground oats (from the same batch as the malted oats) and 2 g salt, and then cooking in a microwave oven for 2 min.

In Expt 3, oats malted in the laboratory were dried at 30° and ground to a flour. Samples of malted-oat flour (32 g) were suspended in 150 ml distilled water and soaked for 9 h at 38°. After soaking, the samples were kept in the refrigerator overnight. On the following day each portion of test porridge was prepared by cooking the sample with 2 g salt in a microwave oven for 2 min. The control porridge was prepared by mixing 150 ml distilled water with 36 g untreated, dehulled and ground oats and 2 g salt, and then cooking in a microwave oven for 2 min. Untreated oats have a higher moisture content than oats that have been malted and dried at 80° and, therefore, different amounts of ground oats were used for the preparation of oat porridge.

Experimental design. Fe absorption was determined using the method described by Eakins & Brown (1966) with the exception that 5 mg radio-Fe is used instead of 10 mg in order to keep the administered dose of radioactivity at a lower level. The principle and basic requirements for the determination of non-haem-Fe absorption, including radioisotope measurements, calculation of absorption and sources of error in measurements of food Fe absorption, have been described previously (Hallberg, 1980).

The different portions of test porridge and control porridge (A and B) were served in breakfast meals on alternate mornings after overnight fasting, on four consecutive days

in the order ABBA or BAAB. Each control or test meal consisted of one wheat roll labelled with two different radio-Fe isotopes, ^{55}Fe and ^{59}Fe . At 2 weeks after the final breakfast a blood sample was withdrawn to determine the content of ^{55}Fe and ^{59}Fe . The total retention of ^{59}Fe was measured by whole-body counting at the same time and the total retention of ^{55}Fe was calculated from $^{55}\text{Fe}:^{59}\text{Fe}$ in erythrocytes. One oral reference dose of FeSO_4 was then given to the fasting subject as well as a second dose on the following morning. The absorption of the reference dose was then measured by whole-body counter 2 weeks later.

Oral reference dose. A solution of 10 ml 0.01 mol HCl/l containing 3 mg Fe as FeSO_4 and 30 mg ascorbic acid labelled with ^{55}Fe was used as a reference in all subjects. The 10 ml vial containing the Fe solution was rinsed twice with water and this was also consumed. Each subject received two reference doses on two consecutive mornings after overnight fasts. No food or drink was allowed for 3 h after the reference dose. Each subject received a total of 55.5 kBq ^{55}Fe .

Bread making. The rolls were baked from 40 g unfortified white wheat flour of 55% extraction, table salt, yeast, sugar and water. The native Fe content of the flour was 0.2 mg/40 g flour. FeSO_4 was added in an amount corresponding to 1.9 mg Fe/40 g flour. The dough was fermented for 1 h at 23°. It was then kneaded, and weighed amounts were transferred to small Al forms which were left standing for 10 min of further fermentation. The rolls were then baked at 250° for 15 min. During the mixing of the dough the rolls were labelled with two different radio-Fe isotopes (^{55}Fe and ^{59}Fe); each roll was labelled with 0.02 MBq (0.5 μCi) ^{55}Fe or 0.03 MBq (0.75 μCi) ^{59}Fe . The radio-Fe was added as high-specific-activity FeCl_3 in 0.01 M-HCl. Several studies have shown that it is possible to label uniformly almost all non-haem-Fe compounds in a meal by using this method (Cook *et al.* 1972; Hallberg & Björn-Rasmussen, 1972).

Subjects. Eighteen subjects, six men and twelve women, participated in the experiments. All subjects were healthy volunteers, aged 19–47 years, and each group of nine subjects included three men and six women. Some of the subjects in each group were regular blood donors, which provided a reasonable range of inter-subject variation in Fe absorption. All subjects had normal haemoglobin levels of 130–157 (mean 141) g/l. The volunteers were given oral and written information about the aims and procedures of the study. The project was approved by the Ethics Committee and the Isotope Committee at Sahlgren's Hospital.

Meal composition. Each control or test meal consisted of one wheat roll prepared as described previously. The rolls were divided into two halves and served with margarine (6 g per half) and orange marmalade (5 g per half). Coffee (150 ml) was served with the meal. The test and control porridges were made according to the preparation of porridge (Expts 1 and 2) described previously. They were boiled with 2 g table salt and served without milk. Orange juice (150 ml) was served with all meals and was prepared from a frozen concentrate, reconstituted with water. The ascorbic acid content of the meal was 70 mg and the total Fe content was between 3.4 and 3.6 mg.

Iron absorption measurements. The relative absorption of ^{55}Fe and ^{59}Fe was calculated from analyses of blood samples. The absolute absorption of the two tracers was calculated from a whole-body count of ^{59}Fe and the relative absorption of the two tracers. The analyses of ^{55}Fe and ^{59}Fe in blood was made by means of the method described by Eakins & Brown (1966). All procedures and calculations have been described previously (Björn-Rasmussen *et al.* 1974; Hallberg, 1980).

Expressing results of iron absorption measurements. By measuring absorption of ^{55}Fe in each subject both from the meals and from the reference dose, the results can be expressed as a regression line between the two absorption measurements. A difference in Fe absorption between two meals can then be assessed by comparing the slopes of the regression lines relating meal Fe absorption to absorption of the reference dose. Earlier studies indicated that such regression lines are linear and that it is reasonable to assume that the regression

line passes through the origin (Magnusson *et al.* 1981). This implies that the ratio of mean absorption from a meal to that from the reference dose is a measure of relative bioavailability of non-haem-Fe in the meal. To describe Fe absorption from a given meal, an expression is needed that is more concrete and absolute than the absorption ratio between the meal and reference dose. It is more meaningful to state the amount of Fe absorbed from meals at a specific absorption of the reference dose. In Fe balance considerations it is important to know the Fe absorption from various meals in subjects who are borderline deficient, that is subjects with absent Fe stores who have yet not developed anaemia. The absorption of the reference dose in such subjects has been assessed to be about 40%. Mean, median values and standard deviations of absorption values corresponding to a reference dose absorption of 40% can thus be calculated from groups of subjects.

Analytical methods

Duplicate samples (2.0 g) of freeze-dried porridge were analysed for their contents of Fe and Zn. All glassware was washed in 2.5 M-HCl and rinsed in deionized water before use. Fe and Zn were determined by atomic absorption spectrophotometry (Perkin Elmer Model 360; Perkin Elmer Co., Norwalk, CT, USA) after dry-ashing at 450°. After cooling, three drops HNO₃ (430 ml/l) were added, the ashing was continued, a further three drops HNO₃ were added and the ashing then continued until a white or yellow residue remained. The ash was dissolved in 5 ml 5 M-HCl and allowed to stand under cover overnight. The solutions were transferred with demineralized water to 25 ml volumetric flasks, made up to volume and left for at least 4 h before analysis. Reference standards for Zn and Fe were prepared from Titrisol (Merck, Darmstadt, Germany). Reference standard materials for Zn and Fe with concentrations representative of those found in the test meals were run simultaneously and were seen to fall within the certified range (Orchard Leaves SRM 1571 and Bovine Liver SRM 1577 (a), National Bureau of Standards, USA). The CV for control materials of Zn and Fe were 3.1 and 4.4% respectively.

Analysis of phytate and its degradation products was carried out on untreated oats, control porridge and test porridge. Duplicate samples of freeze-dried material (0.5 g) were extracted with 20 ml 0.5 M-HCl for 3 h. The inositol phosphates were separated from the crude extract by ion-exchange chromatography and determined by ion-pair C₁₈ reverse-phase HPLC using formic acid-methanol (Merck) and tetrabutyl-ammonium hydroxide (Fluka Chemie AG, Buchs, Switzerland) in the mobile phase. The mobile phase was prepared by mixing 560 ml methanol with 540 ml 0.05 M-formic acid in water. Tetrabutyl-ammonium hydroxide (15 ml) was added. The amounts of inositol phosphates (tri(IP-3)-, tetra(IP-4)-, penta(IP-5)- and hexa(IP-6)-) were determined by HPLC, according to Sandberg & Adherinne (1986) and Sandberg *et al.* (1989). The analysis was performed using a HPLC pump (Waters model 510; Waters Associates, Milford, MA, USA) equipped with a C₁₈ Chromasil (pore size 5 μm) column (150 mm, 2 mm i.d.). The inositol phosphates were detected by refractive index (ERC-7510 RI-detector; Erma Optical Works Ltd, Tokyo, Japan). Retention times and peak areas were measured using a Hewlett Packard analytical data system (HP 3350; Hewlett Packard, Palo Alto, CA, USA). Injections were made with a Poole 20 μl loop. The precision of the HPLC method was tested by repeating the extraction and analysis of the same batch of wheat bran in twelve replicate samples. The mean of wheat-bran samples was 46.9 (SD 1.5) μmol/g (dry basis). The same procedure was applied to four samples of rye (wholemeal flour) and four samples of phytase-deactivated oats (wholemeal flour). Mean values were 10.3 (SD 0.2) and 9.9 (SD 0.35) μmol inositol hexaphosphates/g (dry basis) respectively. The precision of the HPLC method was estimated from these results to be about ±3% (CV).

The phytate content of the porridge in the present study was expressed as μmol inositol

Table 1. *Expt 1. Zinc absorption from oat porridge by healthy human subjects*
(Mean values and standard deviations)

Meal	n	Zn content		Zn absorption (%)		Absorption ratio test:control		Inositol hexaphosphate ($\mu\text{mol}/\text{portion}$)
		mg/kg	$\mu\text{mol}/\text{portion}$	Mean	SD	Mean	SD	
Control	10	35.6	14.5	11.8 ^a	2.96	1.63	0.75	432
Test	10	30.8	13.6	18.3 ^b	8.12			107

^{a, b} Mean values with unlike superscript letters were significantly different ($P < 0.05$).

* For details of subjects and experimental procedures, see pp. 678–681.

hexaphosphate. For comparison of our results with previous studies, where phytate content was expressed as mg, we converted the inositol phosphate contents expressed as mg to μmol . The following molecular weights (P) were used for this conversion: IP-3 93 g/mol, IP-4 124 g/mol, IP-5 155 g/mol and IP-6 186 g/mol. In two of the studies used for comparison (Hallberg *et al.* 1989; Rossander *et al.* 1990) this was not possible since the separate inositol fractions were not analysed in these studies. The conversion of these values from mg to μmol was made by multiplying mg by the molecular weight of IP-6, these values therefore, should be regarded as estimates of the phytate content given in μmol .

Phytate hydrolysis

Phytate hydrolysis in untreated oat flour after cooking in the microwave oven for 2 min was determined by analysis of phytate content before and after soaking at 37.8° for 17 h and was compared with phytate hydrolysis in untreated oat flour, soaked without cooking. After soaking, the samples were freeze-dried before analysis of phytate (IP-6).

Statistical analysis

The significance of the differences between the absorption of the isotopes in each study was calculated by use of Student's *t* test for paired observations.

RESULTS

Zinc absorption

Zinc absorption measurements. The Zn and phytate contents of the different porridge meals and the absorption of Zn are given in Table 1. The Zn absorption from the control porridge prepared from untreated oats, with a phytate (inositol hexaphosphate) content of 432 μmol , was 11.8%. The serving of the malted-oat porridge with a phytate content of 107 μmol enhanced Zn absorption significantly ($P < 0.05$), to 18.3%.

Iron absorption

Iron absorption measurements. The Fe absorption from different breakfast meals is shown in Table 2. In Expt 2 the phytate content in the test porridge was 107 μmol and in the control porridge 437 μmol . The mean individual absorption ratio (test porridge meal: control porridge meal) was 1.47 ($P < 0.05$). In Expt 3 a tendency towards a higher absorption from the malted-porridge meal than from the control-porridge meal was observed which did not, however, reach statistical significance. The mean of the ratios calculated from the different individual Fe absorptions in this experiment was 1.25.

Phytate analysis. The inositol phosphate contents of the meals served in the Zn and Fe

Table 2. Expts 2 and 3. Iron absorption for healthy human subjects given breakfast meals containing oat porridge and radio-Fe-labelled wheat rolls and the iron and phytate contents of the meals*
(Mean values and standard deviations)

	Absorption (%) from:		A 40%		Mean of ind. abs. test/control meal		Non-haem-Fe content		Non-haem-Fe content (mg/kg)	Inositol hexaphosphate ($\mu\text{mol}/\text{portion}$)
	n	Meal	Reference dose	Mean	SD	Mean	SD	$\mu\text{mol}/\text{meal}$		
Expt 2	9									
Control		4	29.7	4.4 ^a	3.2	1.47	0.39	62.7	3.5	436
Test		5.1	29.7	6.0 ^b	3.2			60.9	3.4	107
Expt 3	9									
Control		3.2	29.3	4.8 ^a	3.2	1.25	0.35	64.5	3.6	599
Test		4.1	29.3	6.4 ^a	6.2			62.7	3.5	198

^{a, b} Mean values with different superscript letters were significantly different ($P < 0.05$).
A 40% Individual absorption (ind. abs.) corrected to 40% reference dose absorption.
* For details of subjects and experimental procedures, see pp. 678-681.

Table 3. *Content of inositol phosphates ($\mu\text{mol/g}$) in breakfast meals containing different oat porridge preparations**

(Mean values with two values from duplicate determinations shown in parentheses)

Samples	IP-3 Mean	IP-4 Mean	IP-5 Mean	IP-6 Mean	Sum of IP-3-IP-6 (μmol /portion)
Expt 1					
Control porridge	—	—	0.6 (0.62, 0.57)	16.2 (16.44, 15.96)	448
Test porridge	1.0 (0.97, 1.02)	1.6 (1.72, 1.47)	1.4 (1.48, 1.32)	3.7 (4.47, 2.93)	222
Expt 2					
Control porridge	—	—	0.6 (0.55, 0.64)	14.5 (14.88, 14.12)	452
Test porridge	1.1 (1.02, 1.18)	1.7 (1.64, 1.76)	1.5 (1.50, 1.50)	3.7 (3.85, 3.55)	231
Expt 3					
Control porridge	—	—	0.6 (0.55, 0.64)	16.6 (16.22, 15.98)	621
Test porridge	2.6 (2.56, 2.73)	2.8 (2.82, 2.78)	2.3 (2.31, 2.29)	6.0 (6.06, 5.94)	452

IP-3, IP-4, IP-5, IP-6, inositol tri-, tetra-, penta- and hexaphosphates respectively.

* For details of experimental procedures, see p. 681.

absorption experiments are shown in Table 3. In the Zn absorption experiment (Expt 1) the sum of IP-3, IP-4, IP-5 and IP-6 in the control and the test porridge was found to be similar to the values from determinations of the porridge served in Expt 2 (Fe absorption). In Expt 3 (Table 2) the phytate content was higher; the control porridge contained 599 μmol per portion and the test porridge 198 μmol per portion.

Phytate hydrolysis in oat samples after cooking in the microwave oven. The IP-6 content of untreated, dehulled and ground oats was reduced by 65% from 16.2 to 5.8 $\mu\text{mol/g}$ (3.0-1.1 mg) during soaking at 37.8° for 17 h. When the soaking was repeated with ground oats, cooked in the microwave oven for 2 min, only a small reduction was observed (8%), and there was less formation of hydrolysis products of phytate.

DISCUSSION

In the present study, malting and soaking of oats reduced the phytate (IP-6) content by 77% and doubled the amount of Zn absorbed, as compared with oat porridge prepared from untreated oats. The average individual increase in Fe absorption was 47% from oat porridge with 107 μmol phytate (Expt 2).

The absorption of Zn and Fe in the present study was estimated from measurements of the whole-body retention from radio-labelled meals. Zn absorption was measured by adding radio-Zn to porridge, and Fe absorption was measured by giving porridge with a bread roll containing the radio-Fe. The reason for this was to achieve a similar experimental design to that used in previous studies (Sandström *et al.* 1987; Rossander *et al.* 1990; Brune *et al.* 1992), and thereby facilitate comparison of the observations in our study with previous results. Extrinsic labelling of meals or diets with radioactive isotopes, followed by measurement of whole-body retention or enrichment in, for example, blood, has been

shown to give a valid and precise determination of the absorption of Zn and Fe for a range of foods (Hallberg & Björn-Rasmussen, 1972; Arvidsson *et al.* 1978). These previous studies found that, with few exceptions, a complete and rapid isotopic exchange occurs in a majority of foods items. Thus, the absorption of radio tracers can be expected to be the same from the porridge and the bread roll.

Several studies of cereal-based meals have confirmed the negative effect of phytate on Zn absorption in human subjects (Turnlund *et al.* 1984; Sandström *et al.* 1987; Kivistö *et al.* 1989).

When data obtained in these studies are evaluated together, low absorption values, less than 15%, are consistently observed when the phytate content of the test meal is above 400 μmol .

Nävert *et al.* (1985) studied Zn absorption from bread meals containing wheat bran fermented for varying lengths of time. The absorption of Zn increased from 9.6% to 19.8% when the phytate content (sum of IP-3 to IP-6) was reduced from 520 μmol (97 mg phytate-P) to 230 μmol (26 mg phytate-P) by 16 h of fermentation, thus suggesting that the Zn absorption was improved by the fermentation process. These values are almost identical to our observations. The breads in the study by Nävert *et al.* (1985) were not analysed by HPLC but by the earlier precipitation techniques that do not separate the different inositol phosphates. Since the values obtained with the Fe precipitation methods may result in overestimation of phytate, Sandström & Sandberg (1992), in a recent study, re-analysed the freeze-dried samples studied by Nävert *et al.* (1985), using the HPLC method described previously, and these data have been used for comparison with the results from our study. In another study, Sandström *et al.* (1987) measured Zn absorption from cereals prepared in the form of bread or porridge and served with 200 ml milk. The absorption of Zn was negatively correlated with the phytate content of the meal with the highest absorption, 26.8%, from a rye bread containing 126 μmol , calculated as the sum of IP-3 to IP-6 (21 mg phytate-P) and the lowest, 8.4%, from an oatmeal porridge containing 426 μmol (75 mg phytate-P), and these values are similar to the values from the studies discussed previously. However, direct comparison is not possible, as the studies of Sandström *et al.* (1987) contained different amounts of Zn and were served with milk, which may have influenced the results.

Both human (Sandström & Sandberg, 1992) and animal studies (Lönnerdal *et al.* 1989) have indicated that inositol phosphates containing less than five phosphate groups may induce lower inhibition of mineral absorption than IP-5 and IP-6. Studies with sucking rats suggest that pure fractions of inositol phosphates containing less than five phosphate groups produced via non-enzymic hydrolysis have no effect on Zn absorption (Lönnerdal *et al.* 1989). In a recent study (Sandström & Sandberg, 1992) the inhibitory effects of isolated inositol phosphates on Zn absorption in human subjects was investigated. Zn absorption was impaired when pure fractions of IP-5 and IP-6 produced by non-enzymic hydrolysis were added to a white bread, while the addition of 400 μmol IP-4 had no effect. In the present study, malting and soaking resulted in phytate hydrolysis and, as shown in Table 3, IP-3 and IP-4 constituted a considerable fraction of the total inositol phosphates in malted-oat porridge. In the Zn experiment, 13% of the inositol phosphates comprised IP-3, 21% IP-4, 18% IP-5, and 48% IP-6.

However, when phytate is hydrolysed, different isomers of inositol phosphates can be produced depending on whether the hydrolysis is non-enzymic or enzymic, and whether cereal or microbial phytases are used. As it is possible that the configuration of the phosphate groups may affect the complexing capacity of the molecule, direct comparisons between the present and previous studies such as Lönnerdal *et al.* (1989) and Sandström & Sandberg (1992) are difficult.

In the present study the Fe absorption from the bread rolls served with the porridge made from malted-oat flour was higher than the absorption from that served with the control porridge prepared from untreated oat flour. The serving of malted-oat porridge in the breakfast enhanced fractional Fe absorption by about 36%, but the percentage absorption was still relatively low, i.e. 6% v. 4.4%, or, if calculated in absolute amounts, 3.6 μmol (0.20 mg) v. 2.7 μmol (0.15 mg) absorbed Fe. A further improvement in Fe absorption may be possible by increasing the length of hydrolysis. Oat flour was soaked for only 7 h at 37.8°, resulting in a phytate reduction of about 77%. Longer periods of soaking introduce a sourish taste in the malted-oat porridge. By further modification of the preparation of malted-oat porridge it might be possible to achieve lower phytate levels and, thus, increased Fe absorption (while maintaining an acceptable taste). The inhibitory effect of phytate has been found to be counteracted by ascorbic acid, and the relative effect of ascorbic acid was more marked the more the Fe absorption was inhibited by phytate (Hallberg *et al.* 1989). Thus, a feasible way to improve further the Fe absorption from the malted-oat porridge is to increase the ascorbic acid content of the meal.

The effect of phytate on non-haem-Fe absorption has been extensively investigated. Hallberg *et al.* (1989) concluded that the inhibition of non-haem-Fe absorption by phytates in human subjects is dose-dependent and that even a small amount of phytate causes marked inhibition. The greatest decrease in Fe absorption was found at the lowest levels, from 2 to 10 mg (10–53 μmol) phytate-P. After the addition of 10–250 mg (53–1344 μmol) phytate-P the rate of decrease in Fe absorption was lower but highly significant. *In vitro* studies of soaking, germination and the addition of phytase have shown that the phytate content of cereals should be reduced to very low levels to prevent a negative effect on Fe availability (Sandberg & Svanberg, 1991; Svanberg *et al.* 1993). These results are also in agreement with recent human absorption studies (Brune *et al.* 1992).

Brune *et al.* (1992) measured Fe absorption from rye or wheat bread fermented in different ways, and having different phytate contents. The Fe absorption ratio from a bread with a phytate-P (sum of IP-3–IP-6) content of 203.5 mg (1159 μmol) was 3.2%, while serving a bread containing only 30.7 mg (266 μmol) phytate-P (sum of IP-3–IP-6) enhanced the absorption ratio to 9.2%. These values are in agreement with our observations.

Rossander *et al.* (1990) examined the effect of oat products on non-haem-Fe absorption in human subjects. Fe absorption was compared in continental-type breakfast meals consisting of wheat rolls, orange juice and coffee, and served with or without oat porridge. Except for the addition of milk to the meal, the composition was similar to that of the present study. In the study of Rossander *et al.* (1990) the phytate-P content of the breakfast containing porridge was 113 mg (608 μmol). The serving of oat porridge reduced the fractional Fe absorption by about 60%, from 12.4% to 5.2%, in comparison with reference meals served without oat porridge. In our experiment the Fe absorption from oat porridge with a phytate-P (sum of IP-3–IP-6) content of 452 μmol (83.6 mg) was 4.4% and the serving of malted-oat porridge containing 222 μmol (35.5 mg) phytate-P (sum of IP-3–IP-6) enhanced the Fe absorption ratio to 6%. It is difficult however, to compare the results of Rossander *et al.* (1990) directly with those of the present study, since in our study the phytate content of the porridge was analysed using a method that separates the different inositol phosphates, whereas in the study of Rossander *et al.* (1990) the amounts of phytate, as determined by the Association of Official Analytical Chemists' method (Harland & Oberleas, 1986), also includes partially dephosphorylated isomers of IP-6, and the contribution of these separate fractions to the total phytate-P content cannot be calculated.

The release of inorganic P during soaking under optimal conditions for phytase activity has been used by several investigators in the assay of phytase activity (Bartnik & Szafranska, 1987; Mayer & Poljakoff-Mayber, 1989). In a previous study in our laboratory

(Larsson & Sandberg, 1995), using the same material as in the present study, we showed that the release of inorganic P was related to the phytate reduction and that the release of P was much higher in malted oats than in untreated oats. In the present study phytate degradation in malted oats was very low after 2 min of cooking in the microwave oven. Rapid absorption and thermal diffusion is achieved with microwaves (Buchta & Svennebrink, 1993) owing to volumetric heating of the porridge, in contrast to surface heating, which might explain the rapid phytase inactivation (compared with conventional cooking). Studies in human subjects (Sandberg *et al.* 1987; Sandberg & Andersson, 1988) and pigs (Sandberg *et al.* 1993) have demonstrated that the cereal phytase activity plays an important role in phytate degradation in the stomach and small intestine. We do not know whether intrinsic phytase activity in malted oats would increase phytate degradation during digestion, but it is possible that processing at lower temperature, keeping phytase active, might be favourable for the total absorption of minerals, in particular as optimal conditions for oat phytase occur at 37° (Larsson & Sandberg, 1992).

In conclusion, owing to the very high phytate content of oats, which inhibits not only the absorption of Zn but also the absorption of Fe, it must be considered important to find a technological means to overcome this disadvantage. The results of our study indicate that larger amounts of Fe and Zn would be absorbed from oat products prepared from malted-oat flour than from the same products prepared from raw or commercial oats.

We would like to thank Ms Annette Almgren and Ms Elisabeth Gramatkovski for their excellent technical assistance. This work was supported by grants from the Cerealia Foundation R & D, Stockholm, Sweden.

REFERENCES

- Arvidsson, B., Cederblad, Å., Björn-Rasmussen, E. & Sandström, B. (1978). A radionuclide technique for studies of zinc absorption in man. *International Journal of Nuclear Medicine and Biology* **5**, 104–109.
- Bartnik, M. & Szafranska, I. (1987). Changes in phytate content and phytase activity during the germination of some cereals. *Journal of Cereal Science* **5**, 23–28.
- Björn-Rasmussen, E., Hallberg, L. & Isaksson, B. (1974). Food iron absorption in man. Application of the two-pool extrinsic tag method to measure heme and non-heme iron absorption from the whole diet. *Journal of Clinical Investigation* **53**, 247–255.
- Brune, M., Rossander, L., Hallberg, L., Gleerup, A. & Sandberg, A.-S. (1992). Iron absorption from bread in humans: inhibiting effects of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups. *Journal of Nutrition* **122**, 442–449.
- Buchta, R. & Svennebrink, J. (1993). Using microwave energy for rapid heating of silicon wafers. In *Microwave and High Frequency. Proceedings of the International Conference*, Göteborg, Sweden, D5 [J. Kahl, editor]. Göteborg: SIK.
- Cook, J. D., Layrisse, M., Martinez-Torres, C., Walker, R., Monsen, E. & Finch, C. A. (1972). Food iron absorption measured by an extrinsic tag. *Journal of Clinical Investigations* **51**, 805–815.
- Eakins, J. D. & Brown, D. A. (1966). An improved method for the simultaneous determination of iron-55 and iron-59 in blood by liquid scintillation counting. *International Journal of Applied Radiation Isotopes* **17**, 391–397.
- Hallberg, L. (1980). Food iron absorption. In *Methods in Hematology*, pp. 116–133 [J. D. Cook, editor]. London: Churchill Livingstone.
- Hallberg, L. & Björn-Rasmussen, E. (1972). Determination of iron absorption from whole diet. A new two-pool model using two radioiron isotopes given as haem and non-haem iron. *Scandinavian Journal of Haematology* **9**, 193–197.
- Hallberg, L., Brune, M. & Rossander, L. (1989). Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. *American Journal of Clinical Nutrition* **49**, 140–144.
- Harland, B. F. & Oberleas, D. (1986). An ion-exchange method for determination of phytate in foods: collaborative study. *Journal of Association of Official Analytical Chemists* **69**, 667–670.
- Kivistö, B., Cederblad, Å., Davidsson, L., Sandberg, A.-S. & Sandström, B. (1989). Effect of meal composition and phytate content on zinc absorption in humans from an extruded bran product. *Journal of Cereal Science* **10**, 189–197.
- Larsson, M. & Sandberg, A.-S. (1992). Phytate reduction in oats during malting. *Journal of Food Science* **57**, 994–997.

- Larsson, M. & Sandberg, A.-S. (1995). Malting of oats in a pilot-plant process. Effects of heat treatment, storage and soaking conditions on phytate reduction. *Journal of Cereal Science* **21**, 87–95.
- Lönnerdal, B., Sandberg, A.-S., Sandström, B. & Kunz, C. (1989). Inhibitory effects of phytic acid and other inositol phosphates on zinc absorption in suckling rats. *Journal of Nutrition* **119**, 211–214.
- Magnusson, B., Björn-Rasmussen, E., Hallberg, L. & Rossander, L. (1981). Iron absorption in relation to iron status. Model proposed to express results of food iron absorption measurements. *Scandinavian Journal of Haematology* **27**, 201–208.
- Mayer, A. M. & Poljakoff-Mayber, A. (1989). Dormancy, germination, inhibition and stimulation. In *The Germination of Seeds*, pp. 71–173, 4th ed. Oxford: Pergamon Press.
- Nävert, B., Sandström, B. & Cederblad, Å. (1985). Reduction of phytate content of bran by leavening in bread and its effect on zinc absorption in man. *British Journal of Nutrition* **53**, 47–53.
- Rossander, L., Gleerup, A. & Hallberg, L. (1990). Inhibitory effect of oat products on non-haem iron absorption in man. *European Journal of Clinical Nutrition* **44**, 783–791.
- Sandberg, A.-S. & Adherinne, R. (1986). HPLC method for determination of inositol tri-, tetra-, penta-, and hexaphosphates in foods and intestinal contents. *Journal of Food Science* **51**, 547–550.
- Sandberg, A.-S. & Andersson, H. (1988). Effect of dietary phytase on the digestion of phytate in the stomach and small intestine. *Journal of Nutrition* **118**, 469–473.
- Sandberg, A.-S., Andersson, H., Carlsson, N.-G. & Sandström, B. (1987). Degradation products of bran phytate formed during digestion in the human small intestine. Effect of extrusion cooking and digestibility. *Journal of Nutrition* **117**, 2061–2065.
- Sandberg, A.-S., Carlsson, N.-G. & Svanberg, U. (1989). Effects of inositol tri-, tetra-, penta-, and hexaphosphates on *in vitro* estimation of iron availability. *Journal of Food Science* **54**, 159–161.
- Sandberg, A.-S., Larsen, T. & Sandström, B. (1993). High dietary calcium levels decreases colonic phytate degradation in pigs fed a rapeseed diet. *Journal of Nutrition* **123**, 559–566.
- Sandberg, A.-S. & Svanberg, U. (1991). Phytate hydrolysis by phytase in cereals, effects on *in vitro* estimation of iron availability. *Journal of Food Science* **56**, 1330–1333.
- Sandström, B. (1993). In *Bioavailability '93, Nutritional, Chemical and Food Processing Implications of Nutrient Availability. Proceedings of the International Conference Bioavailability '93*, Ettlingen, Federal Republic of Germany pp. 159–163 [U. Schlemmer, editor]. Karlsruhe: Bundesforschungsanstalt für Ernährung.
- Sandström, B., Almgren, A., Kivistö, B. & Cederblad, Å. (1987). Zinc absorption in humans from meals based on rye, barley, oatmeal, triticale and whole-wheat. *Journal of Nutrition* **117**, 1898–1902.
- Sandström, B. & Sandberg, A.-S. (1992). Inhibitory effects of isolated inositol phosphates on zinc absorption in humans. *Journal of Trace Elements and Electrolytes in Health and Disease* **6**, 99–103.
- Svanberg, U., Lorri, W. & Sandberg, A.-S. (1993). Lactic fermentation of non-tannin and high-tannin cereals: Effects on *in vitro* estimation of iron availability and phytate hydrolysis. *Journal of Food Science* **58**, 408–412.
- Turnlund, J. R., King, J. C., Keyes, W. R., Gong, B. & Michel, M. C. (1984). A stable isotope study of zinc absorption in young men: effects of phytate and alfa-cellulose. *American Journal of Clinical Nutrition* **40**, 1071–1077.