Eur J Nutr (2005) 44 : 334–340
DOI 10.1007/s00394-004-0529-4

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# A decrease in iron status in young healthy women after long-term daily consumption of the recommended intake of fibre-rich wheat bread

Received: 12 February 2004
Accepted: 29 June 2004
Published online: 3 September 2004

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**Summary** *Background* Fibre-rich bread and cereals are included in the recommendations of a healthy diet. *Objective* To measure the effects of long-term consumption of the recommended intake of fibre-rich wheat bread on the iron status of young healthy women with adequate iron stores. *Design* Four-months intervention study including healthy female subjects assigned into two groups provided daily with 300 g of fibre-rich wheat bread, prepared with or without phytase as a supplement to their habitual diet. *Subjects* Forty-one women aged 24.8 ± 3.8 years (mean ± SD) and an average BMI of 22.0 ± 2.9 kg/m$^2$ participated. Baseline values for serum ferritin were 45 µg/L, 22–83 (geometric mean, range) and for haemoglobin 132 g/L, 119–148 (arithmetic mean, range), respectively. *Results* Distribution of energy intake from protein, fat and carbohydrate, and daily intake of dietary fibre and iron were similar in the two groups and within the recommended levels. There was no effect of the phytase added to the wheat bread on the iron status of the subjects, but an effect of the intervention period. Serum ferritin and haemoglobin levels were significantly reduced by 12 ± 1.1 µg/L (27 %) ($P < 0.001$) and 2 ± 0.8 g/l (1.5 %) (mean ± SE) ($P < 0.05$) respectively, after four months of intervention. *Conclusions* The present long-term study indicates that consumption of the recommended daily intake of fibre-rich wheat bread results in an impairment of iron status in women with initially sufficient iron stores. Reduction of the phytic acid concentration in the bread was not sufficient to maintain iron status.

**Key words** haemoglobin – serum ferritin – phytic acid – dietary fibre – wheat bran

## List of abbreviations

NNR Nordic Nutrition Recommendations
PTU Phytase unit
$IP_6$ Inositol hexaphosphates
$IP_5$ Inositol pentaphosphates
$IP_4$ Inositol tetraphosphates
$IP_3$ Inositol triphosphates

EJN 529

## Introduction

Epidemiological studies have identified correlations between habitual consumption of whole grain cereals and reductions in prevalence of certain lifestyle diseases [1–3]. Whole grain cereals are universally recommended as part of a healthy diet since they are valuable sources of nutrients due to their contents of dietary fibre, vitamins, minerals and phytochemicals. The Nordic Nutrition Recommendations (NNR) recommend an intake of approximately 3 g of dietary fibre/MJ for adults [4], the Danish Veterinary and Food Administration advises a daily intake of 250–300 g of fibre-rich bread and cereals daily [5] and "Dietary guidelines for Americans, 2000" advise an intake of several servings of whole grain foods daily [6]. Despite an extensive search for the identification of health-related mechanisms of a diet rich in whole grains, very few studies have investigated possible adverse health effects when following the dietary recommendations.

Sandström (1993) evaluated the effects on iron stores in healthy male and female subjects of an eight-month, completely controlled, low-fat, high-fibre diet, composed in accordance with the official recommendations of the NNR. The intervention diet resulted in a decrease in total iron intake and an increase in vitamin C intake. The long-term intervention study resulted in a reduction in serum ferritin in the majority of the subjects, with a median decrease of 30 μg/L in men and 11 μg/L in women.

Iron deficiency and iron deficiency anaemia affects more than 3.5 billion people in the developing world [7]. In industrialised countries especially women of childbearing age have a low iron status and a relatively high prevalence of iron deficiency and iron deficiency anaemia [8, 9], whereas males in general have a satisfactory iron status with a low frequency of iron deficiency [10]. Two recently published studies have found associations between body iron stores and the risk of developing type 2 diabetes in a prospective nested case-control study, Nurses' Health Study [11] and possible relations between iron stores and cardiovascular diseases in a population-based study, Study of Health in Pomerania [12].

Approximately 40 % of premenopausal Danish women have serum ferritin levels < 30 μg/L, indicating absent stainable bone marrow haemosiderin iron and thus low iron stores [9, 13, 14].

Wheat bran and whole grain wheat flour have a higher iron content compared with flour from refined grains, but concomitantly whole grains have a high concentration of phytic acid. Even small concentrations of phytic acid have been shown to reduce non-haeme iron absorption from the diet [15, 16], whereas an intake of vitamin C enhances non-haeme iron absorption [17]. It is therefore the composition of the diet as a whole that influences the bioavailability and utilisation of the iron present in the diet.

Diets rich in whole grains and dietary fibre result in a reduced bioavailability of dietary non-heme iron mainly due to the concomitant increased intake of phytic acid. Knowledge of the inhibitory effect of phytic acid on iron absorption is primarily based on single meal studies where the iron absorption outcome is assumed to reflect long-term effects on iron status [17–20]. However, the difference in iron absorbed from a diet rich in inhibitors or enhancers is smaller when measured from the whole diet compared to a single meal [21–23]. Thus long-term studies seem to a greater extent to reflect possible adjustable mechanisms of iron absorption.

It has been suggested that increasing the bioavailability of iron from cereals and whole wheat may be achieved by a reduction of the phytic acid content [20, 24], which may be obtained by fermentation of the dough with sourdough or by addition of phytase during the preparation of the dough.

Therefore the aim of the present study was to measure the effects of long-term consumption of the recommended intake of fibre-rich wheat bread on the iron status of healthy young women with adequate iron stores. An objective was to study a possible effect of addition of phytase, produced from *Aspergillus niger*, to the dough during bread processing in improving the iron status of the subjects.

## Subjects and methods

### Subjects

A total of 107 women were screened and 47 were selected after having provided a screening blood sample showing serum ferritin > 20 μg/L and haemoglobin > 120 g/L. Six subjects dropped out of the study, five from the wheat bran bread group and one from the wheat bran bread + phytase group, all stating that the daily bread intake was too high. The 41 women who completed the study (wheat bran bread group, n = 18, and wheat bran bread + phytase group, n = 23) had an average age of 24.8 ± 3.8 years (19–37) (mean ± SD, range) and an average BMI of 22.0 ± 2.9 kg/m$^2$ (17–32). All were non-smokers, not pregnant or lactating nor exercising heavily. The subjects did not take any medication or vitamin, mineral or other supplements, were not diagnosed with any type of chronic inflammation and hat not donated blood during or at least two months prior to the study. Twenty-two subjects took oral contraceptives throughout the study, 12 in the phytase group and 10 in the control group. The subjects were randomised into the two groups, according to their screening values of serum ferritin and use of contracep-

tives to ensure that the two groups were as homogeneous as possible.

All participants were informed, orally and in writing, about the study. Written consent of participation, stating that the subject participated voluntarily and was free to withdraw from the study at any time, was obtained before enrolment. The study protocol was approved by the Ethics Committee of Copenhagen and Frederiksberg (authorisation number (KF) 01-387/96).

## Study design

Subjects were provided daily with 300 g of fibre-rich wheat bread as a substitute for part of their habitual diet for a period of 16 weeks and received either bread prepared without phytase (wheat bran bread group) or the same bread prepared with phytase (wheat bran bread + phytase group). The subjects collected bread once a week at the Department of Human Nutrition.

After nine weeks of intervention, the subjects carried out a three day weighed food record. The subjects were provided with a digital weighing scale and were instructed to weigh and record all foods and drinks during two predetermined weekdays and one weekend day. Nutrient values were calculated using the Dankost 2000® dietary assessment software (National Food Agency of Denmark, Soeborg, Denmark).

Compliance was evaluated by feedback from participants during their weekly visit to the department for collecting bread. All subjects were encouraged to inform the staff of any problems or discomfort due to the large quantity of bread.

Subjects donated three blood samples both at baseline and at the end of the study and all three were taken within one week. After eight weeks of intervention, one blood sample was taken. All blood samples were drawn from the cubital vein after 12 hours of fasting and an additional 10 minutes rest in the supine position. Haemoglobin and serum ferritin levels were determined in all samples. In order to prevent artificially high serum ferritin values due to infections, the subjects were told to report any sign of infection at the time of blood sampling. In the case of infection, blood sampling was postponed until 14 days after the end of the infection as judged by the subject. Subjects' individual weight was recorded at baseline and at the end of the intervention period, using the same digital weight.

## Composition and preparation of the bread

All wheat bread was prepared at Schulstad Bread A/S (Hvidovre, Denmark) and stored at –18 °C until collected by the subjects. The bread consisted of 32 % white wheat flour, 11 % whole wheat flour, 8 % wheat bran (particle size: 1.2 mm – 0.6 mm), 3 % specially formulated flour, 1.6 % sugar, 1.1 % salt, 1.9 % yeast, 1.8 % sunflower oil, 40 % water, with or without 2500 phytase unit (PTU)/100 g (*Aspergillus Niger* phytase produced from Aspergillus Oryzae NOVO-L (Novo Nordisk A/S, Bagsvaerd, Denmark) added together with the other ingredients. The dough was prepared in 73 minutes (mixing time 6 minutes, rising time 12 minutes at 22 °C and 55 minutes at 36 °C) and baked for 32 minutes (the oven temperature reached 70 °C after 16 minutes and 220 °C as final baking temperature). Bread pH was 5.6 and optimum pH value for the phytase was between 5.0 and 5.5 (activity range of pH 2.5–6.0), and optimum temperature was between 50 and 55 °C (activity range 25–70 °C). Each type of bread was baked in 3 batches.

## Chemical analyses

A sample from each batch was collected in acid-washed polyethylene containers, freeze-dried, homogenised and analysed in duplicate for phytic acid, iron and calcium. A HPLC method was used to determine both total and subclasses of phytic acid [25]. Iron and calcium were analysed in duplicates by atomic absorption spectrophotometry (SpectrAA-200, Jonathan Mofft, VARIAN, Techtron pty-Limited, Schweiz Mulgrave, Victoria, Australia) after wet washing with nitric acid. Reference materials (Iron BCR 184 lyophilised Bovine Muscle and Calcium total diet NIST Standard Reference Material 1548) were analysed in the same run as the bread samples. An iron content of 79.0 µg/g (certified value: 79 µg/g) and a Ca content of 2.0 mg/g was found (certified value: 1.7 mg/g).

At each blood sampling, 5 ml blood was drawn in EDTA coated glass for haemoglobin analysis and 5 ml in dry glass for ferritin analysis. Haemoglobin was determined on Cobas Minos (Roche, ABX, Park Euromedicine, Montpellier, France). Blood samples were centrifuged at room temperature for 15 min at 3000 x g for ferritin analysis. Serum was frozen at –20 °C before analysis. Serum ferritin was determined by nephelometery using the N Latex Ferritin™ kit (Dade Behring Marburg GmbH, Marburg, Germany). The kit was calibrated according to the World Health Organisation (WHO) International Human Ferritin Standard 80/578. In the concentration range 36–620 µg/L, the within assay reproducibility (coefficient of variation) was 1.0–4.6 % and the between assay variation (total assay variation) 1.6–5.1 %.

## Statistics

Sample size of the wheat bran bread group and the wheat bran bread + phytase group was calculated from

results of serum ferritin concentrations measured at the Institute of Human Nutrition (unpublished data). To obtain a power of 80 %, 18 subjects are needed to detect a difference in serum ferritin of 10 µg/L at a SD of 10, since the original hypothesis was to observe a difference between the wheat bran bread group and the wheat bran bread + phytase group. Differences in iron status from baseline and after two and four months of intervention for the two groups individually and for the two groups as one, were examined by a repeated measurement analysis of variance using the Statistical Analysing System (SAS) software package, version 8.2 (SAS institute, Cary NC). The SAS procedure MIXED was used with treatment, time and treatment × time interaction as dependent fixed variables and a covariance structure of compound symmetry. An average of the three blood samples was used at baseline and after four months of intervention. Significance was set at $P < 0.05$. Homogeneity of variance and normal distribution were investigated by plots and histograms of residuals. Shapiro-Wilk's test for normal distribution was performed and showed that it was necessary to log-transform values for serum ferritin, but not for haemoglobin. Least square means and standard errors were estimated and transformed back where necessary before presentation.

## Results

All subjects remained weight stable throughout the four-month study period. Compliance to the dietary intervention, judged by the feedback by the subjects during their visits to the department, was high. The content of inositol tri-, tetra-, penta- and hexaphosphates ($\sum IP_{3-6}$), ($\sum IP_{5-6}$), iron and calcium and the molar ratio of phytic acid:iron are listed in Table 1. Values of the three batches of bread did not differ. The content of $\sum IP_{3-6}$ and $\sum IP_{5-6}$ was significantly reduced by 23 % and 18 %, respectively, in the wheat bran + phytase bread compared to the wheat bran bread.

Results from the three-day weighed food record showed that the average daily nutrient intakes in the two intervention groups were similar (Table 2). The distribution of energy intake from protein, fat and carbohydrates, and the average intakes of dietary fibre and iron, were within the recommended levels. The average intake of vitamin C was 50 % higher than the recommended intake. The daily portion of bread provided 33 % of the energy intake, 66 % of fibre intake and 43 % of the daily intake of iron. On average the female subjects consumed 475 ± 290 g of fruit and vegetables daily.

Serum ferritin and haemoglobin concentrations at baseline and after two and four months are shown in Table 3 for the two groups individually. No difference in baseline values of serum ferritin and haemoglobin was observed between the two groups. There was no effect of the phytase added to the wheat bread on the iron status

**Table 1** Concentration of inositol phosphates, iron, molar ratio of phytic acid:iron, and calcium in the wheat bran bread with or without added phytate of the three batches prepared (median, range)

| | Wheat bran bread | Wheat bran + phytase bread |
|---|---|---|
| $\sum IP_{3-6}$ (µmol/100 g bread) | 308[a] (301, 313) | 240[b] (227, 241) |
| $\sum IP_{5-6}$ (µmol/100 g bread) | 277 (272, 280) | 231 (226, 235) |
| Iron (mg/100 g bread) | 2.0 (1.9, 2.1) | 2.0 (1.9, 2.1) |
| Molar ratio (phytic acid:iron) | 8.5:1 | 6.7:1 |
| Calcium (mg/100 g bread) | 30 (28, 32) | 27 (27, 28) |

Numbers with different letter superscripts within the same row are significantly different ($p < 0.001$)

**Table 2** Intake of energy, distribution of energy intake and intake of fibre, iron, calcium and vitamin C, (mean ± SD) for the wheat bran bread group and wheat bran bread + phytase group, based on a three days weighed food records compared to recommended intake by the Nordic Nutrition Recommendations (NNR)

| | Wheat bran bread group (n = 18) | Wheat bran bread + phytase group (n = 23) | NNR[1] |
|---|---|---|---|
| Energy (MJ/day) | 9.2 ± 2.3 (2200 ± 550 kcal) | 9.3 ± 1.7 (2220 ± 400 kcal) | – |
| Protein (E-%) | 13.7 ± 2.0 | 14.7 ± 2.4 | 10–15 |
| Fat (E-%) | 25.7 ± 7.8 | 26.0 ± 8.2 | < 30 |
| Carbohydrate (E-%) | 58.0 ± 7.4 | 55.6 ± 7.5 | 55–60 |
| Dietary fibre (g/d) | 32.6 ± 10.1 | 33.6 ± 9.1 | 25–35 |
| Iron (mg/d) | 13.6 ± 2.9 | 14.7 ± 3.4 | 12–18 |
| Calcium (mg/d) | 846 ± 298 | 870 ± 269 | 800 |
| Vitamin C (mg/d) | 92.7 ± 52.7 | 85.5 ± 60.0 | 60 |

[1] (Sandström et al., 1996)

**Table 3** Serum ferritin (μg/L) and haemoglobin (g/L) concentrations at baseline and after two and four months intervention in young female subjects daily receiving 300 g of wheat bran bread or wheat bran bread baked with phytase during a four-month intervention

| | n | Baseline | $Q_{25}$ | 2 month | $Q_{25}$ | 4 month | $Q_{25}$ | Δ0–4 month | Effect of phytase | Effect of time 0–2 month | Effect of time 2–4 month | Effect of time 0–4 month |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Serum ferritin (μg/L)[a] | | | | | | | | | | | | |
| Wheat bran bread group | 18 | 45±1.1 | 34 | 33±1.1 | 26 | 32±1.1 | 23 | 13 | | P < 0.001 | N. S. | P < 0.001 |
| Wheat bran bread + phytase group | 23 | 44±1.1 | 37 | 34±1.1 | 28 | 34±1.1 | 25 | 11 | | P < 0.001 | N. S. | P < 0.001 |
| Total | 41 | 45±1.1 | 34 | 34±1.1 | 24 | 33±1.1 | 24 | 12 | N. S. | P < 0.001 | N. S. | P < 0.001 |
| Haemoglobin (g/L)[b] | | | | | | | | | | | | |
| Wheat bran bread group | 18 | 132±1.7 | 127 | 131±1.8 | 127 | 130±1.7 | 124 | 2 | | N. S. | N. S. | N. S. |
| Wheat bran bread + phytase group | 23 | 132±1.5 | 127 | 134±1.5 | 127 | 130±1.5 | 124 | 2 | | P < 0.05 | P < 0.001 | N. S. |
| Total | 41 | 132±1.1 | 126 | 133±1.3 | 126 | 130±1.1 | 123 | 2 | N. S. | N. S. | P < 0.001 | P < 0.05 |

[a] Geometric mean ± SE; [b] Arithmetic mean ± SE; *N. S.* Non-significant

of the subjects in the wheat bran bread + phytase group compared to the wheat bran bread group, but an effect of the intervention period on the serum ferritin values was observed for the two groups individually. Serum ferritin decreased significantly in both groups after 2 months intervention and did not decrease further the remaining two months. Since no difference was observed of the addition of phytase, all statistical calculations on the effect of the intervention period are therefore performed as one group.

Baseline values (average of all subjects) of serum ferritin was 45 μg/L, 22–83 (geometric mean, range) and for haemoglobin 132 g/L, 119–148 (arithmetic mean, range). Following two months of intervention, geometric mean of serum ferritin concentrations had decreased significantly by 11 ± 1.1 (SE) μg/L ($P < 0.001$) and there was no further decrease after additional two months intervention. After four months intervention the serum ferritin values had decreased by 27 %. Haemoglobin concentrations decreased after four months of intervention by an arithmetic mean of 2 ± 0.8 g/L (SE) ($P < 0.05$) by 1.5 % (Table 3).

## Discussion

The subjects' self-selected diet during the four months study was in accordance with the Nordic recommended dietary guidelines [4] as regards dietary fibre, iron and calcium contents, but contained a 50 % higher level of vitamin C than the recommended diet. The relatively low average energy intake of 9.3 ± 2.0 MJ/day of the study population taken into consideration, the daily portion of fruit and vegetables reflects the recommendations of a daily intake of 600 g/day. During the intervention period 300 g of fibre-rich wheat bread was provided as a substitute for the habitual intake of breakfast cereals, bread for lunch, and afternoon snacks, thus the amount corresponded to the intake of 250–300 g/day of fibre-rich bread and cereals recommended by the Danish Veterinary and Food Administration [5].

Despite the compliance with the current dietary recommendations, the four-month intervention diet resulted in a decreased iron status for the subjects, who all had a sufficient iron status at the beginning of the study as judged by their initial serum ferritin and haemoglobin levels. The present long-term study therefore indicates an adverse effect of the recommended diets with a high content of fibre-rich bread on iron status in young women who otherwise follow the current dietary recommendations.

Iron stores, measured as serum ferritin, decreased within the first two months of the intervention period and reached a steady state during the remaining two months, whereas the haemoglobin concentration decreased within the last two months of intervention, as also seen in a previous study by Sandström (1993) [26]. It is well established that the fractional absorption of iron is affected by the iron status of the individual and is higher in iron deplete subjects compared to subjects with adequate iron stores [27]. In the present study the obtained steady state in serum ferritin in the last two months of intervention could be due to the decreased iron status of the subjects after the first two, resulting in a higher absorption rate of the non-haeme iron present in the diet. Haemoglobin levels decreased after the four months intervention, which could be due to a shifted equilibrium between menstrual losses and the reduced serum ferritin status of the subjects.

The phytic acid content was significantly reduced in the wheat bran bread + phytase compared to the wheat bran bread, but only by 23 % compared to 18 % in the wheat bran bread. The reduction of phytic acid in the wheat bran bread + phytase was low considering the high concentration of PTU added to the dough. The particle size of the bran was relatively large (1.2 mm –

0.6 mm) and it is reasonable to believe that the added phytase did not have sufficient time or the particles were too large to more effectively break down the phytic acid present in the bran. Using sourdough is normally an effective means of decreasing the phytic acid content [28], but was in the present situation not an efficient remedy in reducing the phytic acid content of the bread to very low values.

Sandberg (1989) has stated that the molar ratio phytic acid:iron should not exceed 0.18:1, and the total concentration of $IP_5$ and $IP_6$ should not exceed 50 µmol/100 g, based on *in vitro* studies, if an increased iron absorption is to be obtained [15]. A long-term intervention with a reduced phytate content in weaning cereals with a molar ratio of phytic acid and iron ranging from 0.14:1–0.83:1 did not result in improved iron status in infants [23]. The molar ratio of phytic acid and iron and the total concentration of $IP_5$ and $IP_6$ in the daily portion of bread provided in the present study, by far exceeded the mentioned values. The daily intake of bread contributed, on average, with 33 % of the subjects' daily energy intake and 43 % of the daily intake of iron. It is therefore reasonable to assume that the high phytic acid concentration of the bread has influenced the iron bioavailability of the subjects' diet as a whole. The reduction of phytic acid in the wheat bran bread + phytase was insufficient to increase the bioavailability of the iron in the wheat bran bread + phytase compared to the wheat bran bread.

The net absorption of total iron from the diet is a balance between the effects of the enhancers and inhibitors present. The reduced serum ferritin levels in the subjects in the present study were probably caused by a low bioavailability of iron from the diet. The relatively high intake of vitamin C (50 % above recommended level) was not sufficient to counteract the negative effects of a concomitant intake of phytic acid. The lack of effect of a high intake of vitamin C is in accordance with results obtained by Cook and Reddy (2001) investigating iron absorption from complete diets [22]. The meat intake of the present study population was low and only contributed with 7.7 ± 1.1 % (mean ± SE) of the total intake of iron (results not shown). Apart from being an excellent source of haeme iron, meat is an enhancer of non-haeme iron absorption in a phytic acid rich-meal [19]. The low intake of meat has most likely not been sufficient to affect the iron status, neither as a source of haeme iron nor as an enhancer of non-haeme iron absorption. The combination of a low intake of meat and a frequent intake of bread with a high content of phytic acid throughout the day in the present study is possibly the main reason for the reduced iron status observed during the first two months of the intervention period.

Research indicates that a diet rich in whole grains can have substantial beneficial effects on certain lifestyle diseases [1–3] and subsequently, efforts have been made in public health information to promote the consumption of whole grains [29]. In these procedures, it should be taken into consideration that a high intake of fibre-rich wheat bread can result in a decreased iron status of women with otherwise sufficient iron stores. The post-treatment concentrations of serum ferritin in the young women in the present study were close to the levels indicating small or reduced values of serum ferritin (serum ferritin < 30 µg/L) [30]. The implication of the present study would therefore be to advise, especially young women, not to consume whole grains, rich in phytic acid, to all main meals.

The present long-term study indicates that consumption of the recommended daily intake of fibre-rich bread results in a decreased iron status in women with an initially sufficient iron status although the diet, as a whole, was in line with current dietary recommendations. Reduction of the phytic acid concentration in the bread, by addition of phytase to the dough, was not sufficient to increase or maintain the iron status of the subjects.

**Acknowledgements** Schulstad Brød A/S is thanked for donating bread for the intervention. Also the authors wish to acknowledge Hanne Lysdal Petersen, Ella Jessen and Lone Berggren for taking the blood samples and performing chemical analyses.

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