Dietary Aspergillus niger Phytase Increases Iron Absorption in Humans1,2

ANN-SOFIE SANDBERG,*3 LENA ROSSANDER HULTHÉN1 AND MARIA TÜRK*

*Chalmers University of Technology, Department of Food Science, S-402 29 Göteborg and
1Sahlgren’s Hospital, Department of Clinical Nutrition, Göteborg University, S-413 45
Göteborg, Sweden

ABSTRACT Phytate is an inhibitor of iron absorption that can be removed before the intestinal site of absorption by microbial phytase, thereby increasing iron absorption from a meal. The effects of two kinds of dietary phytase, cereal phytase and microbial phytase from Aspergillus niger, on iron absorption were investigated. Iron absorption was measured from single meals containing white wheat rolls supplemented with wheat bran with or without phytase activity (expt. 1) and phytase-deactivated wheat bran with or without addition of microbial phytase from A. niger (expt. 2). Each experiment had 10 subjects and two different radio iron tracers: 55Fe and 59Fe were used for comparison of the absorption from the test meals in each experiment. No differences in iron absorption were found between meals containing wheat bran with or without phytase activity. Addition of microbial phytase to the meal containing phytase-deactivated wheat bran increased iron absorption from 14.3 ± 2.6 to 26.1 ± 3.8% (P < 0.0001). Two pH optima, one at pH 2.0 and one at pH 6.0, were found for A. niger phytase at 37°C, but activity occurred at all pH values between 1.0 and 7.5. The results suggest that effective and complete degradation of phytate occurred in the stomach when A. niger phytase was given with the meal. This may be explained by high activity of microbial phytase at physiological pH conditions of the stomach, whereas wheat phytase has a different pH optimum. J. Nutr. 126: 476–480, 1996.

INDEXING KEY WORDS:
• phytase • phytate • iron absorption • humans

Wheat bran and whole grain products contain considerable amounts of phytate. Phytate possesses the ability to form insoluble complexes with minerals, e.g., calcium, iron and zinc, which lowers the bioavailability of these minerals (Hallberg et al. 1987, Heaney et al. 1991, McCance and Widdowson 1942, Nävert et al. 1985). The removal of phytate substantially increases iron absorption from these foods (Brune et al. 1992, Hurrell et al. 1992). Hydrolysis of phytate may also occur in the gastrointestinal tract before the intestinal site of absorption and could thereby influence absorption.

Ileostomy studies demonstrate that 60% of phytate from raw wheat bran (containing phytase activity) was hydrolyzed during passage through the stomach and small intestine, but this did not occur when phytase-deactivated bran was fed (Sandberg and Andersson 1988, Sandberg et al. 1987). The studies suggest that there is a negligible human endogenous phytase activity but that dietary phytase is an important factor for intestinal phytate hydrolysis. Recent studies also demonstrate that the level of phytase activity in human small intestine is low (Iqbal et al. 1994).

The consequences of the intestinal phytate hydrolysis by dietary phytase for nonheme iron absorption is, however, not known. In the present study this was investigated by measurement of iron absorption from single meals with a white wheat roll not containing phytate to which was added raw wheat bran or phytase-deactivated bran.

Nonheme iron absorption was also measured from the same kind of rolls supplemented with phytase-deactivated wheat bran and with added microbial phytase from Aspergillus niger (A. niger)4 to the rolls. Microbial phytases work in a broader pH area than cereal phy-
MATERIALS AND METHODS

Subjects. Twenty subjects, 9 men and 11 women, participated in the experiments. All subjects were healthy volunteers aged 20–52 y. Each group included both men and women. Some of the subjects in each group were regular blood donors, which provided a reasonable range of intersubject variation in Fe absorption. Subjects were given written information about aims and procedures of the studies. The project was approved by the ethical committee of the Medical Faculty of the University of Göteborg, Sweden.

Experimental design. The effect of dietary phytase was studied by comparing in the same subject Fe absorption from wheat rolls containing no phytate but added 1 g of raw wheat bran with intrinsic phytase activity or 1 g of phytase-deactivated wheat bran (Study 1). The amount of bran added, contained 9 mg of phytate phosphorus. Using this amount of phytate, a small reduction in phytate content could be expected to result in a marked increase in iron absorption (Hallberg et al. 1989), as estimated from the previously found relationship between amount of phytate in a meal and the inhibition of iron absorption. In Study 2 the effect on nonheme Fe absorption of adding microbial phytase to the same kind of rolls with phytase-deactivated bran was investigated. The two types of rolls (A and B) in each study were given on alternate mornings after an overnight fast on four consecutive days in the order ABBA or BAAB. Water (150 mL) was consumed with the rolls. The A and B rolls were labeled with two different Fe isotopes, $^{55}$Fe and $^{59}$Fe. A blood sample was drawn 2 wks after the last roll was served to determine the content of $^{55}$Fe and $^{59}$Fe. The total retention of $^{55}$Fe was measured by whole body counting at the same time, and the total retention of $^{59}$Fe was calculated from the ratio of $^{55}$Fe and $^{59}$Fe in red cells. One oral reference dose 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 wk later.

Oral reference doses. A solution of 10 mL of 0.01 mol/L HCl containing 3 mg of Fe as FeSO$_4$ and 30 mg of ascorbic acid labeled with $^{59}$Fe was used as a reference in the two studies. The 10-mL vials containing the Fe solution were 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 $^{59}$Fe.

Meal composition and iron isotope labeling of meals. All meals contained two wheat rolls, each prepared from 40 g unfortified white flour, (60% extraction) yeast, sugar, table salt and water. The dough was kneaded in a machine and allowed to rise for 1 h at 23°C. Weighed amounts were transformed to small aluminum forms, which were left standing for another rise of 30 min. The bread was baked at 250°C for 15 min. The flour was fortified with 3.7 mg Fe as FeSO$_4$ per 80 g flour. Native Fe content was 0.4 mg. Two rolls were served with 20 g of margarine and 150 mL water.

The commercial microbial phytase used in Study 2 was added dropwise to the rolls before spreading the margarine and without getting in contact with the bran before the rolls were eaten.

The dough for the wheat rolls was labeled by mixing Fe isotope with water and yeast before adding the flour. The Fe isotope was added as ferric chloride in 0.01 mol HCl/L. Each meal was labeled with 46.3 kBq $^{59}$Fe or 55.5 kBq $^{59}$Fe.

Wheat bran was delivered from AB Tre Lejon (Göteborg, Sweden). Preparation of phytase-deactivated bran was performed as follows. Samples of 100 g wheat bran were spread out in a thin layer and heat treated in an autoclave for 6 min at 120°C (to deactivate phytase). The deactivated samples were checked for phytase activity by analysis of inositol hexaphosphate (IP$_6$) before and after incubation at optimal conditions for wheat phytase activity (pH 5, 55°C). A check was also made that hydrolysis of IP$_6$ was minimal during heat treatment in the autoclave.

A phytase preparation from A. niger Finase S 40 (lot no. 532034) was provided by ALKO Biotechnology Ltd., Rajamäki, Finland. Finase S40 contains a declared phytase activity of 4×10$^3$ phytase unit (PU)/L. Five milliliters of Finase was added to the test meal (2.5 mL to each roll).

Wheat rolls were freeze-dried and ground to a powder in a porcelain mortar. Weighed amounts of this powder were analyzed for total Fe (Björn-Rasmussen et al. 1974). Inositol tri- to hexaphosphates in wheat rolls and bran were determined according to Sandberg and Ahderinne (1986) and Sandberg et al. (1989).

Incubation of sodium phytate with A. niger phytase at different pH values. To 10 mL of sodium phytate solution (1.5 mmol/L) 0.5 mL of A. niger 4.76×10$^5$ PU/L phytase was added. No buffer was used because we have found the pH in these systems to be stable throughout incubations. The pH was adjusted with 0.1 mol/L HCl to 7.5, 7.0, 6.5, 6.0, 5.5, 5.0, 4.5, 4.0, 3.5 and with 0.5 mol/L HCl to 3.0, 2.5, 1.5 and 1.0, and test tubes were incubated at 37°C for 3 h. The pH was measured again after incubation and had not changed. The reaction was stopped by adding 5 mL 2 mol/L HCl and IP$_6$ were analyzed as previously described.
Iron absorption measurements. Relative absorption of $^{55}$Fe and $^{59}$Fe was calculated from analyses of blood samples. Absolute absorption of the two tracers was calculated from whole body counting of $^{55}$Fe and the relative absorption of the two tracers. Analysis of $^{55}$Fe and $^{59}$Fe in blood was made with a modification of the method described by Eakins and Brown (1966), using a liquid scintillation spectrometer (Tri-Carb, Packard Instruments, San Antonio, TX). All procedures and methods of calculation have been described previously (Björn-Rasmussen et al. 1974, Hallberg 1980).

Statistical methods. All statistical analyses were made using a Statview II computer program (Abacus Concepts, Inc., Berkeley, CA). For statistical comparisons the means with their standard errors of the individual absorption ratios in each experiment were used. The possible statistical significance of the difference between the mean absorption and 1 was examined by an unpaired, two-sided t-test.

RESULTS

Determination of phytate. The wheat rolls contained no detectable amounts of inositol phosphates. The concentration of inositol hexa-, penta- and tetraphosphates in the raw wheat bran was 46.9 $\mu$mol/g, 1.0 $\mu$mol/g and 0.8 $\mu$mol/g, respectively (corresponding to 8.9 mg phytate phosphorus) and in the phytase-deactivated wheat bran 43.2 $\mu$mol/g, 4.3 $\mu$mol/g and 0.7 $\mu$mol/g, respectively (corresponding to 8.8 mg phytate phosphorus). Only traces of inositol triphosphates were present.

pH optima at 37°C for A. niger phytase activity. Figure 1 demonstrates the effect of pH variation on A. niger activity at 37°C. Studying IP$_{3}$, reduction in the same samples, maximal reduction (43.5%) was found at pH 6.0, with a high activity again at pH 2.0. Thus, two pH optima at 37°C were found, one at pH 6.0 and one at pH 2.0, but there was a considerable activity at all pH values between 1.5 and 7.0.

Iron absorption measurements. Iron absorption from wheat rolls given with either raw wheat bran or phytase-deactivated wheat bran, was almost the same, 10.4 and 10.3%, respectively (Table 1). The absorption ratio was 1.12 $\pm$ 0.11 which was not different from 1 ($t = 1.09$). In Study 2, the addition of phytase to wheat rolls with phytase-deactivated bran significantly increased iron absorption from 14.3 to 26.1% (Table 2). The absorption ratio was 1.98 $\pm$ 0.18, which was significantly different from 1 ($t = 5.44; P < 0.0001$).

DISCUSSION

Hydrolysis of phytate may occur in the gastrointestinal tract before the intestinal site of absorption. Because iron and most of the other essential minerals and trace elements are absorbed in the duodenal or jejunal part of the small intestine, the site and degree of phytate degradation can affect the nutritional value of a high phytate diet.

The effect of intrinsic wheat bran phytase and a microbial A. niger phytase on iron absorption was investigated in this study. The addition of A. niger phytase to the phytate-containing meal just before consumption was found to markedly increase the iron absorption (from 14 to 26%). The absorption of iron from this meal containing a white wheat roll with added phytase-deac-
Absorption of absorption et ach. in the stomach, and soaking of the raw bran, we found that 60% of phytate from raw bran was hydrolyzed during passage through the stomach and small intestine, but this did not occur when they were fed phytase-deactivated bran [Sandberg and Andersson 1988, Sandberg et al. 1987]. Mineral balances performed in the ileostomy studies indicated no differences in apparent iron absorption when raw or phytase-deactivated bran were fed, whereas apparent zinc absorption was increased in the period with the raw bran [Kivistö et al. 1986]. The use of radioisotope labeling of a meal, however, gives a more sensitive measurement of inhibitory effects on iron absorption, but with this technique no differences were found in this study. Apparently, the phytate hydrolysis by cereal phytase in the stomach and small intestine is not sufficient to influence iron absorption. From previous investigations it is known that for iron the absorption-depressing effect is pronounced even at low levels of phytate [Hallberg et al. 1989].

Two kinds of phytase (3-phytase and 6-phytase) have been recognized, where 3-phytase is found in microorganisms and 6-phytase in plants. The optimum pH for wheat phytase has been shown to be 5.15 (Peers 1951). The activities of plant phytases are considered to diminish markedly as the pH is varied from its optimal value [Irving 1980]. It is likely that some phytate hydrolysis occurs in the stomach but that wheat phytase is inactivated when the pH is reduced to pH 2–3. Some degradation of phytate could also take place in the duodenum before a neutral pH is obtained. Microbial phytases seem to be active over a wider pH range than plant phytases (Ullah 1987, 1988, Wang et al. 1980). Phytase produced by A. niger has two pH optima at 55°C, one at 2.5–3.0 (possibly because of additional acid phosphatase activity) and one at pH 5.0 (Simell et al. 1990). We found at 37°C two pH optima; one at pH 2.0 and one at pH 6.0. Activity occurred at all pH values between pH 1.0 and 7.5. It is thus likely that a high activity would occur in the stomach and also in the small intestine of humans. The effect of deactivated microbial phytase has not been investigated. It is, however, not likely that a deactivated microbial phytase has an effect on iron absorption because it does not contain any known enhancers of iron absorption. The differences in optima for the two kinds of phytase enzymes studied explain why addition of A. niger phytase to a phytate-containing meal had a marked influence on iron absorption, whereas no effect of intrinsic wheat bran phytase was found.

**LITERATURE CITED**


of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups. J. Nutr. 122: 442–449.


