Iron Absorption from Bread in Humans: Inhibiting Effects of Cereal Fiber, Phytate and Inositol Phosphates with Different Numbers of Phosphate Groups

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ABSTRACT Iron absorption was measured from five kinds of bread made from various types of flour and fermented in different ways in order to obtain a wide variation in the content of fiber, phytate (inositol hexaphosphate) and its degradation products, inorganic phosphate and inositol phosphates with fewer numbers of phosphate groups (inositol pentaphosphate through monophosphate). Each experiment had 9–10 subjects and, in each subject, iron absorption was measured from control rolls made from low extraction wheat flour and one kind of test roll using two different radioiron tracers: $^{55}$Fe and $^{59}$Fe. The inhibition of iron absorption was closely related to the content of phytate-phosphorous as determined using the AOAC method, and to the sum of the tri- through hexaphosphate groups as determined using the HPLC method. As an example, prolonged fermentation of whole-rye bread reduced total inositol phosphates to the same amount as in the control rolls and increased fractional iron absorption to the same high level, in spite of a fiber content five times as great. The results strongly suggest that the inhibitory effect of bran on iron absorption is due to its content of phytate and other inositol phosphates present after fermentation, rather than to its content of fiber or other constituents. Thus, effective fermentation will increase the bioavailability of iron in whole-meal bread. J. Nutr. 122: 442-449, 1992.

INDEXING KEY WORDS:
- iron absorption
- phytate
- inositol phosphates
- fiber
- humans

The primary dietary factors determining the amount of iron absorbed in humans are the contents of heme and nonheme iron and the balance between factors influencing the bioavailability of nonheme iron in particular [1]. Some dietary factors enhance iron absorption in humans, e.g., ascorbic acid and meat or fish, whereas others inhibit the absorption of nonheme iron, e.g., iron-binding phenolic compounds [2], calcium [3] and, notably, phytate [4].

Bread is a staple food in many countries and is an important source of both iron and the inhibiting phytate. The chemical composition of flour depends on the proportion of the cereal grain removed by the milling process. Low extraction white flour mainly originates from the endosperm, whereas flours with higher extraction also contain increasing amounts of bran. Whole flour has a low native iron content and is therefore enriched with iron in several countries. The higher the extraction of the flour, the higher the content of iron and phytate that originates from the bran.

Iron absorption from bread baked from high extraction flour is often poor in spite of its higher iron content. The lower absorption has been ascribed to its content of bran. The inhibitory effect of bran on iron absorption in humans has been clearly demonstrated using both the chemical balance method [5] and the extrinsic radioiron technique [6]. However, the negative effect of bran on iron absorption is not seen in all species. Results from a careful comparative study [7] on humans and rats explain why the inhibitory effect of bran is not observed in a rat model [8, 9].


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It was suggested early that the inhibitory effect of bran on iron absorption in humans was due to the phytate content of bran [5]. This was not confirmed in a study comparing the effect on iron absorption of native bran and dephytinized bran [10]. The dephytinization, however, was not complete in that study. Further studies on the effect of removing phytate from bran [11] and studies showing that even small amounts of phytate have a marked inhibitory effect on iron absorption [4] may well explain why seemingly contradictory results were obtained. There is thus much evidence that phytate is one of the key nutritional factors influencing the bioavailability of dietary nonheme iron.

The high content of fiber in bran has also been suggested as an explanation of its inhibitory effect on the absorption of iron. This effect, however, has never been established.

The content of phytate-P in flour can vary from a few milligrams to several hundred milligrams per 100 g of flour [12]. In unprocessed flours and bran the inositol phosphates occur mainly in the hexaphosphate form [phytate] and to a small extent in the pentaphosphate form [13, 14]. During bread fermentation these inositol phosphates are partly or completely degraded to inorganic phosphates and to inositol phosphates with fewer numbers of phosphate groups, mono- through pentaphosphate [14, 15]. The contents of the different inositol phosphates remaining in bread after fermentation and baking depend on 1) the choice of cereal (e.g., wheat, rye, oats), 2) the extent of extraction during milling of the grain, 3) the freshness of the flour (influencing the phytase activity), and 4) the techniques employed during fermentation and baking (e.g., leavening time, temperature, amounts and properties of the yeast used).

It currently is possible to determine the inositol phosphates remaining in bread after fermentation and baking by employing a recently described HPLC method [16, 17]. Using this method, Larsson and Sandberg [14] demonstrated that it is possible to reduce the phytate content of bread containing rye bran by 97% by means of rye sourdough fermentation or, in rye bread, by scalding with 70°C water and adjusting the pH to 4.5 with lactic acid.

In vitro studies suggest that inositol phosphates with fewer numbers of phosphate groups (the tri- through pentaphosphate groups) may induce a smaller inhibitory effect on iron absorption than inositol hexaphosphate [16], and that complete reduction of phytate by soaking, germination and fermentation leads to a marked increase in the bioavailability of iron [14, 18]. It is not known, however, to what extent different inositol phosphates inhibit the absorption of iron in humans and hence it is not known to what extent variations in bread-making techniques may influence iron absorption in humans.

The main purpose of the present study was to determine the amount of different inositol phosphates in bread prepared in different ways and to relate these amounts to the bioavailability of iron in humans. The use of two readily measurable radioiron isotopes makes it possible to compare iron absorption from differently prepared breads by relating the absorption in each subject to a standard white wheat bread with almost no remaining inositol phosphates. Another purpose of the study was to compare the amounts of phytate in bread and flour as determined by the anion-exchange resin method [AOAC method] [19] and an HPLC method [16, 17] determining individual inositol phosphates in the triphosphate to hexaphosphate range. Moreover, we examined the possibility of reducing the inhibiting effect of inositol phosphates on the absorption of iron from bread by more effective fermentation.

**MATERIALS AND METHODS**

**Experimental design.** Each experiment had 9–10 subjects and iron absorption was measured in each subject using two different radioiron tracers, from one control meal and one kind of test meal. Thus, each subject served as his or her own control. The control meal (two rolls), served in each experiment, consisted of white wheat bread, butter and water. The test meals in the five experiments also consisted of two rolls, butter and water. The test rolls, however, were made from different kinds of flours, or flour mixtures, and fermented in different ways in order to obtain different contents of inositol phosphates. The control rolls were prepared in a standardized way from low extraction (55%) wheat flour to produce rolls with a very low content of inositol phosphate).

Test rolls and control rolls (a and b) were served on alternate mornings after an overnight fast on four consecutive days in the order abba or baab. The rolls were labeled with two different radioiron isotopes, 55Fe and 59Fe. A blood sample was drawn 2 wk after serving the last roll to determine the content of 55Fe and 59Fe. The total retention of 59Fe was measured by whole-body counting at the same time. The total retention of 55Fe was calculated from the ratio of 55Fe to 59Fe in red cells. An oral reference dose (see below) was then given to the fasting subject and a second dose was given on the following morning. The absorption of the reference doses were then measured by whole-body counting 2 wk later (see the expression of results below). For a review of the extrinsic tag method, see reference [1].

**Subjects.** Forty-nine subjects, 20 men and 29 women, participated in the five experiments. All subjects were healthy volunteers, aged 19–47 y, and each group of 9–10 subjects included both men and women. Some of the subjects in each group were...
TABLE 1
Iron absorption from different breads

<table>
<thead>
<tr>
<th>Experimental Meals&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Subjects&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Nonheme iron content&lt;sup&gt;4&lt;/sup&gt;</th>
<th>Meal Reference dose</th>
<th>Individual absorption ratios&lt;sup&gt;5&lt;/sup&gt;</th>
<th>Mean of individual absorption test rolls:control rolls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rolls</td>
<td>10[1M,9F[2]]</td>
<td>0mg</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Whole rye + W</td>
<td>4.0(3.7)</td>
<td>18.8</td>
<td>33.2</td>
<td>0.61 ± 0.13</td>
<td>24.4 ± 5.2</td>
</tr>
<tr>
<td>Control rolls</td>
<td>3.7(2.0)</td>
<td>18.2</td>
<td>33.2</td>
<td>0.59 ± 0.14</td>
<td>23.6 ± 5.6</td>
</tr>
<tr>
<td>Whole wheat + W</td>
<td>4.0(3.7)</td>
<td>19.8</td>
<td>27.8</td>
<td>0.68 ± 0.06</td>
<td>27.2 ± 2.4</td>
</tr>
<tr>
<td>Control rolls</td>
<td>3.7(2.3)</td>
<td>15.6</td>
<td>27.8</td>
<td>0.58 ± 0.06</td>
<td>23.2 ± 2.4</td>
</tr>
<tr>
<td>Wheat 85% extr.</td>
<td>4.0(3.7)</td>
<td>22.2</td>
<td>37.4</td>
<td>0.57 ± 0.07</td>
<td>22.8 ± 2.8</td>
</tr>
<tr>
<td>Control rolls</td>
<td>3.4(2.0)</td>
<td>8.9</td>
<td>37.4</td>
<td>0.23 ± 0.05</td>
<td>9.2 ± 2.0</td>
</tr>
<tr>
<td>Whole rye + Wheat 85% extraction</td>
<td>4.0(3.7)</td>
<td>16.2</td>
<td>25.0</td>
<td>0.64 ± 0.10</td>
<td>25.6 ± 4.0</td>
</tr>
<tr>
<td>Control rolls</td>
<td>3.4(1.0)</td>
<td>6.1</td>
<td>25.0</td>
<td>0.23 ± 0.07</td>
<td>9.2 ± 2.8</td>
</tr>
<tr>
<td>Whole wheat + W</td>
<td>4.0(3.7)</td>
<td>28.0</td>
<td>42.0</td>
<td>0.55 ± 0.06</td>
<td>22.0 ± 2.4</td>
</tr>
<tr>
<td>Wheat bran + W</td>
<td>3.0</td>
<td>3.8</td>
<td>42.0</td>
<td>0.08 ± 0.08</td>
<td>3.2 ± 0.8</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are means ± SEM, n = 9-10.
<sup>2</sup>W = white wheat flour.
<sup>3</sup>M = male and F = female subjects. Number in parentheses designates blood donors.
<sup>4</sup>The amount of fortification iron in parentheses.
<sup>5</sup>The mean value of the individual absorption ratios (meal:reference dose) were multiplied by 40 to obtain the percentage absorption of iron corresponding to a 40% reference dose absorption.

regular blood donors (Table 1), which provided a reasonable range of intersubject variation in iron absorption. The volunteers were given oral and written information about the aims and procedures of the study. The project was approved by the Ethical Committee of the Medical Faculty of the University of Göteborg.

Meals, bread-making and labeling of meals. Each control or test meal consisted of two rolls made from 80 g of unfortified flour or flour mixture. In all experiments the rolls were served with 20 g of butter and 150 mL of water. The calculated energy content of the meals was 1.8 MJ. Iron as ferrous sulfate was added to the dough of all rolls except the bran rolls, to obtain a similar iron content in all rolls (Table 1).

Control rolls were made from 40 g of unfortified white wheat flour (55% extraction), yeast, sugar, table salt and water. The amounts of yeast, sugar and salt per kg of flour were 65 g, 35 g and 10 g, respectively. The dough was kneaded and weighed amounts transferred to small aluminum forms, which were left standing for 20 min for further fermentation. The bread was then baked at 250°C for 15 min.

In Experiment 1, test rolls were made from whole rye flour and low extraction (55%) wheat flour. Whole-meal rye (500 g), yeast (25 g) and water (750 mL: 40°C) were mixed and fermented at 23°C for 1 h. The dough was then made in exactly the same way as the control rolls.

In Experiment 2 test rolls were made from whole wheat flour and low extraction (55%) wheat flour. Whole wheat flour (500 g), yeast (25 g) and water (750 mL: 40°C) were mixed and fermented for 48 h at 23°C. The dough was then made in exactly the same way as the rye rolls in Experiment 1.

In Experiment 3 test rolls were made from wheat flour (85% extraction). The amounts and proportions of flour, yeast, sugar, table salt and water were the same as in the control rolls. The dough was fermented for 1 h at 23°C. The rolls were then made exactly as the control rolls.

In Experiment 4 test rolls were made from whole rye flour and 85% extraction wheat flour. Whole rye flour (500 g), yeast (25 g) and water (750 mL: 40°C) were mixed and fermented at 23°C for 48 h. The dough was then made in exactly the same way as the control rolls.

In Experiment 5 test rolls were made from wheat bran and low extraction (55%) wheat flour. Wheat flour (675 g) was mixed with wheat bran (325 g). Water, yeast, salt and sugar were added in the same proportions as in the other experiments. The dough was fermented for 1 h at 23°C. The rolls were then made exactly as the control rolls.
made exactly as the control rolls. The bran used was a Swedish commercial wheat-bran product containing 20% whole grain and a dietary fiber content of 50%.

In all studies, each meal was labeled with 46.2 kBq of $^{59}$Fe or $^{55}$Fe. High specific activity radioiron (Amersham International, Amersham, Buckinghamshire, U.K.) was added to the dough fluid as ferric chloride in 0.01 mol/L hydrochloric acid. In Experiments 1–4 the test rolls were labeled with $^{55}$Fe and the control rolls with $^{59}$Fe, whereas in Experiment 5 the control rolls were labeled with $^{55}$Fe and the test rolls with $^{59}$Fe.

**Oral reference doses.** A solution of 10 mL of 0.01 mol/L hydrochloric acid containing 3 mg of Fe as FeSO$_4$ and 30 mg of ascorbic acid labeled with $^{59}$Fe was used as a reference in all 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 an overnight fast. No food or drink was allowed for 3 h after the reference dose. Each subject received a total of 55.5 kBq of $^{59}$Fe.

**Chemical measurements.** Aliquots of rolls were freeze-dried and ground to a powder in a porcelain mortar. Weighed amounts of this powder were analyzed for total iron (20) and phytic acid phosphorus. The latter analyses were made in duplicate using the AOAC method (19). In rolls with a low content of phytate, 6 mL of the original HCl extract was used instead of 1 mL (original method), and the amount of NaOH-EDTA was increased proportionally. This modification markedly increased the lower limit of detection of phytate in the extract. The precision of the method and the recovery of added sodium phytate were unchanged. Samples of flour and freeze-dried phytate were also analyzed by an HPLC method to identify and quantify inositol tri-, tetra-, penta- and hexaphosphates (16, 17). Several complete analyses were made from most samples (Table 2).

The AOAC and the HPLC methods were carefully compared and proved to give the same results for pure sodium phytate standards.

Total fiber content in the rolls was analyzed in duplicate (21, 22).

**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 $^{59}$Fe and the relative absorption of the two tracers. Analyses of $^{55}$Fe and $^{59}$Fe in blood were made by a modification of the method described by Eakins and Brown (23), using a liquid scintillation spectrometer (Tri-Carb, Packard Instruments, San Antonio, TX). All procedures and methods of calculation have been described previously (24).

**Expressing results of iron absorption measurements.** The mean of the individual absorption ratios, test meal:control meal, is an expression of the difference in iron bioavailability between the two meals. In the present experiments, a standardized control meal was served on alternate days with the test meal. In the presentation of the results, the mean of the individual absorption ratios is considered to be the most accurate basis for comparisons between the different experiments.

The ratio of the absorption of nonheme iron from a meal and from a reference dose is an expression of the bioavailability of nonheme iron in the meal. In each experiment the mean value and the SEM of the individual absorption ratios (meal:reference dose) were multiplied by 40 to obtain the percentage absorption of iron that corresponds to a 40% reference dose absorption. Absorption values adjusted to a 40% absorption from reference doses were chosen because they roughly correspond to the absorption expected in subjects who are borderline iron-deficient (25). This adjusted absorption value is the basis for comparisons between the amounts of iron absorbed from different meals, served to different groups.

**Statistical methods.** All statistical analyses were made using a Statview II computer program (Abacus Concepts, Berkeley, CA). For statistical comparisons, the mean and SEM of the individual absorption ratios (test meal:control meal) in each experiment were used. The possible statistical significance of the differences between the mean absorption and 1 was in each experiment examined by a t test.

**RESULTS**

**Iron absorption experiments.** In Experiment 1, iron absorption from the rolls baked from whole rye flour and low extraction wheat flour was not statistically different from the absorption from the control rolls. The mean of the individual iron absorption ratios (test rolls:control rolls) was 0.94, (not significant) (Table 1). In Experiment 2, iron absorption from the whole wheat rolls was lower than from the control rolls. The difference was statistically significant (absorption ratio 0.79; $P < 0.05$). In Experiments 3–5 the differences between iron absorption from the test rolls and the control rolls were all statistically significant. The mean individual iron absorption ratios (test rolls:control rolls) were 0.39 ($P < 0.01$), 0.32 ($P < 0.01$) and 0.13 ($P < 0.01$), respectively.

**Analysis of phytate and inositol-phosphates.** Values for the content of phytate (AOAC method) and inositol phosphates (sum of the tri-, tetra-, penta- and hexaphosphate groups; HPLC method) in the different flours and rolls are given in Table 2. Using the HPLC method only inositol hexaphosphate and small amounts of inositol pentaphosphate could be detected in the flours and bran. In the rolls, after fermentation and baking, there were different amounts of various inositol phosphates (the tri-, tetra-, penta- and hexaphosphate groups). The greatest reduction of inositol...
TABLE 2

<table>
<thead>
<tr>
<th>Type of flour</th>
<th>Fermentation time</th>
<th>Series 1</th>
<th>Series 2</th>
<th>Series 3</th>
<th>Series 4</th>
<th>Series 5</th>
<th>Control rolls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole rye + low extraction wheat flour</td>
<td>2 d + 1 h + 20 min</td>
<td>Whole wheat + low extraction wheat flour</td>
<td>2 d + 1 h + 20 min</td>
<td>Wheat 85% extraction</td>
<td>1 h + 20 min</td>
<td>Wheat 85% extraction</td>
<td>Low extraction wheat (range)</td>
</tr>
<tr>
<td>AOAC-method</td>
<td>Rolls, mg P/80 g flour</td>
<td>95</td>
<td>101</td>
<td>87</td>
<td>157</td>
<td>379</td>
<td>14.2–12.3</td>
</tr>
<tr>
<td></td>
<td>Difference, mg</td>
<td>89.3</td>
<td>92.8</td>
<td>72</td>
<td>122.9</td>
<td>152</td>
<td>5.8–8.0</td>
</tr>
<tr>
<td>HPLC-method</td>
<td>Flour, mg P/80 g</td>
<td>83.4 (2.77)</td>
<td>92.9 (2.4)</td>
<td>66.3 (2.2)</td>
<td>137.1 (2.9)</td>
<td>334.4 (7.29)</td>
<td>7.0–8.5</td>
</tr>
<tr>
<td></td>
<td>Rolls, mg P/roll</td>
<td>6.0 (0.24)</td>
<td>4.6 (0.11)</td>
<td>3.4 (0.12)</td>
<td>5.5 (0.15)</td>
<td>20.8 (0.56)</td>
<td>0.4–0.5</td>
</tr>
<tr>
<td></td>
<td>Difference, mg</td>
<td>89.4</td>
<td>97.5</td>
<td>69.7</td>
<td>142.6</td>
<td>355.2</td>
<td>7.6–9.3</td>
</tr>
<tr>
<td></td>
<td>Rolls, samples</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>IP6, mg/80 g</td>
<td>0.21 (0.06)</td>
<td>0.17 (0.04)</td>
<td>3.95 (0.22)</td>
<td>2.3 (0.36)</td>
<td>155.6 (1.55)</td>
<td>0.28–1.01</td>
</tr>
<tr>
<td></td>
<td>IP5, mg</td>
<td>0.04 (0.01)</td>
<td>0.14 (0.01)</td>
<td>0.65 (0.05)</td>
<td>2.6 (0.19)</td>
<td>8.5 (0.19)</td>
<td>0.07–0.43</td>
</tr>
<tr>
<td></td>
<td>IP4, mg</td>
<td>0.23 (0.08)</td>
<td>0.26 (0.04)</td>
<td>0.87 (0.02)</td>
<td>14.9 (0.83)</td>
<td>14.0 (0.32)</td>
<td>0.05–0.15</td>
</tr>
<tr>
<td></td>
<td>IP3, mg</td>
<td>0.82 (0.19)</td>
<td>1.64 (0.21)</td>
<td>3.17 (0.40)</td>
<td>10.9 (0.55)</td>
<td>24.4 (1.2)</td>
<td>0–0.05</td>
</tr>
<tr>
<td></td>
<td>Σ IP, mg</td>
<td>1.30</td>
<td>2.21</td>
<td>8.64</td>
<td>30.7</td>
<td>203.5</td>
<td>0.4–1.6</td>
</tr>
<tr>
<td>Difference (flour-rolls)</td>
<td>Σ IP, mg</td>
<td>88.1</td>
<td>95.3</td>
<td>61.0</td>
<td>111.9</td>
<td>151.5</td>
<td>7.7–7.2</td>
</tr>
<tr>
<td>Fiber content, g/roll</td>
<td>6.2</td>
<td>5.2</td>
<td>3.8</td>
<td>9.9</td>
<td>17.8</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Absorption</td>
<td>0.94 ± 0.05</td>
<td>0.79 ± 0.03</td>
<td>0.39 ± 0.02</td>
<td>0.32 ± 0.03</td>
<td>0.13 ± 0.02</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

1Values are means (sd).
2Abbreviations used: IP, inositol phosphate; IP3, inositol triphosphate; IP4, inositol tetraphosphate; IP5, inositol pentaphosphate; IP6, inositol hexaphosphate.
3Values are means ± SEM, n = 9–10.

Phosphate content in the rolls compared with the flour (99%) was in Experiment 1.

The agreement between the results obtained with the two methods was good. However, the values for phytate-P as determined with the AOAC method were consistently slightly higher than the sum of the inositol phosphates determined using the HPLC method. This was noted for both flours and rolls. The control rolls contained on average 6 mg of phytate-P per portion (80 g of flour), using the AOAC method, in contrast to the finding of at most 1.6 mg of remaining inositol phosphates using the HPLC method. The difference (6 - 1.6 = 4.4 mg) observed was almost exactly the same in the control rolls and the white wheat flour used. Moreover, the differences were of the same order of magnitude (range 3.4–6.4 mg phosphorus/portion) as in the test rolls used in Experiments 1–4. In Experiment 5 (bran rolls), however, the difference was greater (24 mg phosphorus/portion).

Iron absorption and phytate content. Figure 1 shows the absorption of iron from the five different test rolls in relation to the iron absorption from the control rolls, graphed against the content of remaining inositol phosphates (sum of tri-, tetra-, penta- and hexaphosphates; HPLC method) and phytate (AOAC method) in the rolls. The results from both analytical methods are expressed as milligrams of phosphorus per portion. The solid lines in the graph describe results from a previous study (4) illustrating the effect of adding known amounts of sodium phytate to the same kind of control rolls as used in the present study. It should be noted that in Figure 1 (but not in Table 2) the AOAC values for phytate content in the experimental rolls have, as a correction, been reduced by 4.4 mg phosphorus.

Analysis of fiber content. The fiber (nonstarch polysaccharides including lignin) contents of the rolls are shown in Table 2. There was a wide range in fiber content from 1.1 g per portion in the control rolls to 17.8 g per portion in the bran rolls.

DISCUSSION

In the present study, iron absorption from five kinds of rye or wheat bread, fermented in different
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The iron absorption from the whole wheat bread (Experiment 2), fermented in the same way as the whole rye bread, was also high (absorption ratio 0.79), but slightly lower than from the whole rye bread. As shown in Table 2, the content of inositol phosphate, as analyzed with both analytical methods used, differed between the two kind of breads; this fact may explain the observed difference in iron absorption. The cause of the difference in phytate content between the rye and the wheat bread may be the usually higher phytase activity in rye flour (26).

The inhibition of iron absorption by adding sodium phytate to wheat rolls observed in a previous study is graphed in Figure 1 (4). As seen in the same graph, the extent of inhibition was about the same per milligram of phosphorus from the mixtures of various inositol phosphates in the presently used rolls. It should be emphasized that inositol triphosphate and inositol tetraphosphate constituted a considerable fraction of the total inositol phosphates in all breads (19–84%). The relationship between the inhibition of iron absorption and the content of inositol phosphate would have been weak, if for example, only inositol hexaphosphate and possibly inositol pentaphosphate act as inhibitors. An illustration of this is the bread baked from whole rye flour and 85% extraction wheat flour (Experiment 4). The iron absorption from this bread was reduced by ~70%, in spite of the fact that there was only 4.5 mg of inositol hexaphosphate and inositol pentaphosphate remaining after fermentation and baking. Our findings thus strongly suggest that all inositol phosphates, at least the inositol hexaphosphates to inositol triphosphates analyzed, in relation to their phosphorus content, inhibit iron absorption to about the same extent.

The fiber content was quite high (6.2 g) in the whole rye rolls. The control rolls, however, had a very low fiber content (1.1 g). In spite of this difference, the fractional, as well as the absolute iron absorption, was almost identical. This finding indicates that the rye fibers per se had no inhibitory effect on iron absorption. In fact, in the present study there seems to be no relationship between the fiber content in rye or wheat rolls and the degree of inhibition of iron absorption (Table 2). This result is in accordance with earlier studies in which completely dephytinized bran (11) or cellulose (27) have been found to have no effect on iron absorption.

Complex chemical processes take place during sourdough fermentation and some of those may affect iron absorption. Still, the most likely explanation for the increased bioavailability of iron observed is the degradation of phytate, because there is a close inverse relationship between iron absorption and remaining inositol phosphates. It has previously been shown that sourdough fermentation influenced neither the content of dietary fiber nor the distribution of soluble and insoluble fiber components (28).
Lactic acid is the main organic acid formed during sourdough fermentation. No effect on iron absorption was seen when very large amounts of this acid were added to different meals [29, 30]. Thus, the reduction of the inhibition of iron absorption observed in Experiments 1, 2 and 4 is not likely due to changes in the fiber content or the formation of lactic acid during prolonged fermentation.

From Table 2 it can be calculated that during the degradation of phytate in the test rolls, rather large amounts of inorganic phosphates were formed (about 60–150 mg phosphorus/portion). These inorganic phosphates had no detectable inhibitory effect on the absorption of iron, even in this sensitive experimental model.

Taken together, the findings clearly indicate that the inhibition of iron absorption by wheat and rye bran could be ascribed to its content of phytate, or after baking to its degradation products (inositol phosphates), and not to fiber components or other constituents of bran.

The AOAC method for phytate determination is based on the separation of inorganic phosphates and phytate by means of an ion-exchange resin column. As can be seen in Table 2, the agreement between the values for phytate-P (AOAC method) and the sum of the tri-, tetra-, penta- and hexaphosphates (HPLC method) were good. This indicates that the AOAC method determines not only phytate but also, at least, inositol tri-, tetra- and pentaphosphates. However, slightly higher values for phytate-P were consistently found using the AOAC method compared with the sum of the tri-, tetra-, penta- and hexaphosphates obtained by the HPLC method (Table 2). This difference is probably due to several factors. In bread, it is possible that some of the phosphorus determined using the AOAC method might actually be inositol mono- and diphosphates, which cannot be determined by the HPLC method. This contribution to the AOAC values is probably negligible in flour because inositol phosphates in flour occur almost exclusively as inositol pentaphosphates and inositol hexaphosphates [13, 14, 31].

A source of overestimation of the true content of inositol phosphates using the AOAC method is that, besides inositol phosphates, certain other phosphates with also be measured. This is the case with nucleotide-phosphorous compounds [31] and adenosine-triphosphate (unpublished observations). The consistent finding of small amounts of phytate-P (AOAC method) in the control rolls (Table 2) is probably mainly due to such a systematic error. This conclusion is supported by the finding of negligible amounts of inositol phosphate in the control rolls [up to 1.6 mg] using the HPLC method, and by the fact that even after prolonged and repeated fermentation the content of phytate-P in the control rolls remained unchanged (values not shown). Thus a phosphate-containing fraction exists in the control rolls that is determined as phytate-P but is actually not inositol phosphate. As was pointed out in Results, this fraction seems to be of the same order of magnitude in all test rolls except the bran rolls. In Figure 1, the AOAC values for phytate-P content of all rolls have been arbitrarily reduced by 4.4 mg to correct for this source of error.

One conclusion of the present comparisons between the two methods is that it is only at very low inositol phosphate levels that the overestimation of the phytate values, using the AOAC method, is of any practical significance. Knowledge of this source of error, however, is important considering the significant inhibitory effect on iron absorption even of very small amounts of phytate [4]. Moreover, it should be emphasized that very low phytate-P contents cannot be detected using the original AOAC method, but only by the presently used modification of the method (see Materials and Methods).

The present findings have important nutritional implications. One is the demonstration that not only phytate inositol hexaphosphate, but also its inositol phosphate-degradation products, seem to be potent inhibitors of iron absorption. An even more practical implication is the finding that effective fermentation of whole-meal bread to very low inositol phosphate levels will markedly improve the bioavailability of iron and that the effect of fiber per se on iron absorption can be disregarded. Consequently, the amounts of iron absorbed from bread baked from whole wheat flour, with its high content of iron, would be greater than from white bread, provided the fermentation procedures are optimized.

It is well known that phytate interferes with the absorption of other minerals, e.g., zinc and calcium. It is possible that many of the findings and implications of the present study may also be valid for these elements.

LITERATURE CITED


