

# Availability of Calcium, Magnesium, Phosphorus, Iron, and Zinc in Rats Fed Oat Bran Containing Diets<sup>†</sup>

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Rats were used to study the effects of nonheated oat bran (NHOB) and baked oat bran (BOB) on apparent absorption and retention of Ca, Mg, P, Fe, and Zn. Four isonitrogenous and isoenergetic diets consisting of a control, 15% NHOB, 30% NHOB, and 30% BOB were fed for 28 days. Oat bran was baked under moisture and heat conditions simulating dough preparation and bread-making. BOB had higher insoluble fiber (mainly  $\beta$ -glucans and Klason lignin), lower total phytate, and diminished levels of highly phosphorylated inositols. NHOB feeding reduced Ca availability and impaired absorption of mineral Ca. Magnesium absorption and retention (percent of intake) were lower in the NHOB groups; due to compensation of Mg output by NHOB intake, daily Mg absorption and retention increased. Fe, Zn, and P showed higher daily absorption and retention in the NHOB groups. As compared with NHOB, ingestion of BOB resulted in lower Ca and Fe availability and had no effect on Zn and P; daily Mg absorption and retention were not affected.

## INTRODUCTION

The potentially prophylactic effects of dietary fiber sources in diseases such as colon cancer, atherosclerosis, and diverticular disease have been attributed in part to their ability to bind harmful substances in the intestinal tract. However, dietary fiber may also interact with needed mineral elements (Kelsay, 1982; Walker, 1985), possibly resulting in increased risk of development of mineral deficiencies (Reinhold et al., 1976; Donangelo and Eggum, 1986). The effects of complex carbohydrates on the absorption of minerals have been reviewed in *Complex Carbohydrates in Foods* (British Nutrition Foundation, 1990). Not only has decreased absorption of minerals been related to the ion binding capacity of fiber components but also other factors may be involved such as the intestinal transit time, the degree of bacterial digestion of fiber in the gut, and the potential for the absorption of minerals in the large intestine. Moreover, impaired mineral availability may not be due entirely to fiber, as phytate in unrefined foods may also be a causative factor (Bitar and Reinhold, 1972; Turnland et al., 1984; Sandström et al., 1987). During food processing and digestion, phytate may be degraded to inositol phosphates with a lower phosphorylation degree, reducing the adverse effect of phytic acid on mineral absorption (Sandberg et al., 1987, 1989).

In the present study oat bran was chosen as the dietary fiber source because of the growing interest in its incorporation into foods. The experiments were designed to investigate the effects of ingestion of different amounts of oat bran on the metabolic balances of Ca, Mg, P, Fe, and Zn in rats. Moreover, the heat treatment of oat bran under conditions reflecting dough preparation and bread baking was also included as an experimental variable, since food processing can possibly affect the ability of phytic acid and fiber to complex metals.

## MATERIALS AND METHODS

**Animals, Diets, and Mineral Balance Technique.** Forty male Wistar rats weighing approximately 250 g were obtained

from the University Animal Center (Leuven, Belgium) and maintained singly in stainless steel metabolic cages in quarters kept at 24 °C with a 12-h dark-light cycle. Before being transferred to the experimental diets, all rats were fed a semi-synthetic basal diet without added oat bran. Subsequently, the animals were divided in four groups and received their respective test diets for 28 days. One dietary treatment consisted of the basal diet which had also been fed during the adaptation period. The other diets contained 15 or 30% nonheated oat bran (NHOB) or 30% baked oat bran (BOB). The ingredients and chemical composition of the diets are given in Table I. All diets were approximately equal in protein content and metabolizable energy (18 MJ/kg) as calculated by a modification of the general At-water procedure (Wisker and Feldheim, 1990). Baked oat bran was obtained by simulating the processing conditions in bread-making. Deionized water (0.5 kg) was added to 1 kg of coarse oat bran, and the mixture was warmed at 35 °C for 90 min to imitate the moisture and heating conditions in dough-making. Next, the oat bran was baked at 250 °C for 35 min in an oven with forced air circulation. To equalize the moisture contents of NHOB and BOB, the baking process was followed by drying at 60 °C. NHOB and BOB were milled in a rotary knife mill. The particle size of the coarse bran and milled NHOB and BOB was measured by passing samples through graduated sieves. The major particle size (80%) of the coarse bran was between 1 and 2 mm. In both milled brans, 90% of the particles were between 0.1 and 0.3 mm.

Diets were fed as meal that entirely passed a 0.5-mm screen size. Food and deionized water were available ad libitum. Food intake, corrected for spillage, was measured every day during the 28-day experimental period. Animal weights were recorded at the start and the end of the experiment. Fecal and urine collections were made daily from day 14 to day 28 and stored at -20 °C until analysis. At the end of the feeding experiment, the excreta collected from each rat were pooled and lyophilized and moisture content was determined. Samples were ground to pass a sieve of 1 mm and analyzed per individual animal for Ca, Mg, P, Fe, and Zn. Apparent absorption of minerals was calculated as the difference between dietary intake and excretion in the feces. Mineral balance was calculated as the difference between dietary intake and fecal and urinary output, without corrections for other losses.

**Transit Time.** Whole gut transit time of the diets was measured on days 5 and 10 of the experimental period with 0.5% Fe<sub>2</sub>O<sub>3</sub> as a visible fecal marker. The marker was incorporated into the diets, and the time difference between the first intake of Fe<sub>2</sub>O<sub>3</sub> and its first appearance in the feces was measured.

**Analytical Procedures.** Determination of specific minerals (Ca, Mg, Fe, Zn, P) in diets and lyophilized feces samples was

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Table I. Composition of the Diets<sup>a</sup>

| ingredient                         | g/kg    |          |          |         |
|------------------------------------|---------|----------|----------|---------|
|                                    | control | 15% NHOB | 30% NHOB | 30% BOB |
| casein, <sup>b</sup> 91% protein   | 200     | 175      | 149      | 149     |
| beef tallow                        | 90      | 107      | 124      | 124     |
| corn oil                           | 10      | 12       | 14       | 14      |
| corn starch <sup>b</sup>           | 300     | 156      | 13       | 13      |
| sucrose <sup>b</sup>               | 350     | 350      | 350      | 350     |
| <i>dl</i> -methionine <sup>b</sup> | 3       | 3        | 3        | 3       |
| choline bitartrate <sup>b</sup>    | 2       | 2        | 2        | 2       |
| vitamin mixture <sup>b,c</sup>     | 10      | 10       | 10       | 10      |
| mineral mixture <sup>b,d</sup>     | 35      | 35       | 35       | 35      |
| oat bran                           | 0       | 150      | 300      | 300     |
| chemical composition               |         |          |          |         |
| dietary fiber                      | 6       | 30       | 56       | 57      |
| protein (N × 6.25)                 | 183     | 181      | 184      | 182     |
| Ca                                 | 4.96    | 5.23     | 5.48     | 5.45    |
| Mg                                 | 0.54    | 0.85     | 1.18     | 1.15    |
| P                                  | 5.37    | 6.20     | 7.07     | 7.04    |
| Fe                                 | 0.04    | 0.06     | 0.07     | 0.07    |
| Zn                                 | 0.03    | 0.04     | 0.05     | 0.05    |

<sup>a</sup> NHOB, nonheated oat bran; BOB, baked oat bran (see Materials and Methods). <sup>b</sup> Ingredients were obtained from ICN Biomedicals, Costa Mesa, CA. <sup>c</sup> The vitamin mixture (AIN-76 vitamin mix) contained the following per kg of mix: thiamin hydrochloride, 0.6 g; riboflavin, 0.6 g; pyridoxin hydrochloride, 0.7 g; nicotinic acid, 3 g; calcium pantothenate, 1.6 g; folic acid, 0.2 g; biotin, 0.02 g; cyanocobalamin, 1 mg; retinyl palmitate (250 000 IU/g), 1.6 g; cholecalciferol (400 000 IU/g), 0.25 g; menaquinone, 5 mg; sucrose, 972.9 g. <sup>d</sup> The mineral mixture (AIN-76 mineral mix) contained the following per kg of mix: CaHPO<sub>4</sub>, 500 g; NaCl, 74 g; potassium citrate hydrate, 220 g; K<sub>2</sub>SO<sub>4</sub>, 52 g; MgO, 24 g; MnCO<sub>3</sub>, 3.5 g; ferric citrate, 6 g; ZnCO<sub>3</sub>, 1.6 g; CuCO<sub>3</sub>, 0.3 g; KIO<sub>3</sub>, 0.01 g; Na<sub>2</sub>SeO<sub>3</sub>, 0.01 g; CrK(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O, 0.55 g; sucrose, 118 g.

performed according to the method of McGary and Young (1976). Briefly, the procedure involved dry ashing at 525 °C and dissolving the ash in 6 M HCl followed by heating to near dryness. After appropriate dilution with 0.1 M HCl and filtration, Ca, Mg, Fe, and Zn were measured by atomic absorption spectrophotometry (Varian-Techtron AAG, Brussels, Belgium). Ca and Mg were determined in solutions containing lanthanum chloride. Phosphorus was analyzed colorimetrically after reaction with sodium molybdate. Urinary Ca, Mg, Fe, and Zn were measured by direct aspiration of appropriately diluted and undigested samples into the atomic absorption flame after being passed through a sintered glass filter. Phosphorus in the urine was analyzed directly using the sodium molybdate reagent method. The contents of total, soluble, and insoluble dietary fiber were determined according to the method of Asp et al. (1983). Neutral sugars and Klason lignin were measured according to the procedure of Shinick et al. (1988), uronic acids by the method of Blumenkrantz and Asboe-Hansen (1973), and  $\beta$ -glucans in starch-free residue by the method of Aman and Graham (1987). Inositol phosphates in oat bran were determined by HPLC (Sandberg et al., 1987).

**Statistical Analysis.** Statistical analyses were performed using PROC GLM software by SAS. Data were analyzed by ANOVA, using the least significant difference procedure to evaluate differences between groups. As mineral contents were different across diets, data on mineral absorption, retention, and excretion were analyzed with mineral intake as the covariate. Differences were considered significant at  $P < 0.05$ .

## RESULTS

**Body Weight and Food Intake.** During the 28-day experimental period, average daily food intakes in the groups fed the control diet and the 15% NHOB, 30% NHOB, or 30% BOB diet were  $13.6 \pm 1.3$ ,  $13.8 \pm 1.0$ ,  $13.5 \pm 1.1$ , and  $13.9 \pm 1.3$  g, respectively; daily weight gains averaged  $1.7 \pm 0.2$ ,  $1.8 \pm 0.2$ ,  $1.9 \pm 0.3$ , and  $1.7 \pm 0.2$  g, respectively. Significant differences in body weight increase or food intake among the four experimental groups were not detected.

Table II. Composition of Oat Bran

| component                          | g/kg                    |                         |
|------------------------------------|-------------------------|-------------------------|
|                                    | unheated oat bran       | baked oat bran          |
| water                              | 95                      | 97                      |
| protein (N × 6.25)                 | 155                     | 150                     |
| lipid                              | 103                     | 98                      |
| ash                                | 35                      | 35                      |
| total dietary fiber                | 168                     | 169                     |
| soluble fiber                      | 92                      | 73                      |
| total neutral sugars <sup>a</sup>  | 22 (13.1%) <sup>d</sup> | 19 (11.8%) <sup>d</sup> |
| $\beta$ -glucans                   | 69 (41.1%)              | 53 (31.4%)              |
| uronic acids                       | 1 (0.6%)                | 1 (0.6%)                |
| Klason lignin                      |                         |                         |
| insoluble fiber                    | 76 (45.2%)              | 97 (56.8%)              |
| total neutral sugars <sup>b</sup>  | 34 (20.2%)              | 32 (18.9%)              |
| $\beta$ -glucans                   | 14 (8.3%)               | 28 (16.6%)              |
| uronic acids                       | 2 (1.2%)                | 3 (1.8%)                |
| Klason lignin                      | 25 (14.9%)              | 34 (20.1%)              |
| nonfiber carbohydrate <sup>c</sup> | 444                     | 451                     |
| Ca                                 | 1.7                     | 1.7                     |
| Mg                                 | 2.0                     | 2.1                     |
| P                                  | 5.5                     | 5.4                     |
| Fe                                 | 0.09                    | 0.09                    |
| Zn                                 | 0.08                    | 0.07                    |

<sup>a</sup> Consisted of 87% glucose, 6% arabinose, 4% xylose, 1% galactose, 0.5% rhamnose, and 1.5% mannose; baking did not influence monosaccharide composition. <sup>b</sup> Consisted of 40% glucose, 21% arabinose, 32% xylose, 5% galactose, 0.5% rhamnose, and 1.5% mannose; baking did not influence monosaccharide composition. <sup>c</sup> Calculated as the difference between total and the sum of water, protein, lipid, ash, and dietary fiber. <sup>d</sup> % of total dietary fiber.

Table III. Effect of Heat Treatment on Inositol Phosphate Content in Oat Bran<sup>a</sup>

| heat treatment <sup>b</sup> | $\mu\text{mol/g}$            |                 |                 |                 |            |
|-----------------------------|------------------------------|-----------------|-----------------|-----------------|------------|
|                             | IP <sub>3</sub> <sup>c</sup> | IP <sub>4</sub> | IP <sub>5</sub> | IP <sub>6</sub> | total      |
| A                           |                              | 0.1 ± 0.1       | 1.6 ± 0.3       | 22.0 ± 2.4      | 23.6 ± 2.6 |
| B                           |                              | 1.3 ± 0.3       | 0.9 ± 0.2       | 20.8 ± 2.0      | 23.0 ± 2.3 |
| C                           | 0.2                          | 0.5 ± 0.2       | 3.1 ± 0.4       | 17.1 ± 2.9      | 20.9 ± 3.0 |

<sup>a</sup> Mean ± SD. Four replicate samples of each bran were analyzed. <sup>b</sup> A, raw oat bran; B, 35 °C, 90 min, 38% moisture; C, treatment B followed by baking at 250 °C for 35 min. <sup>c</sup> IP<sub>*x*</sub>, inositol phosphates containing *x* (=3–6) phosphate groups.

**Effect of Processing on Fiber and Phytate in Oat Bran.** Baking the oat bran/water mixture at 250 °C for 35 min had no effect on total fiber content, but approximately 21% of the originally soluble fiber fraction was rendered insoluble (Table II). This was mainly due to conversion of soluble into insoluble  $\beta$ -glucans and to increased Klason lignin content. Neutral sugars contents remained practically unchanged by baking. Only small amounts of pectins, determined as uronic acids, were determined.

In unprocessed oat bran, hexaphosphate was the main inositol phosphate (Table III). Warming the oat bran/water mixture at 35 °C for 90 min did not change total phytate content; however, more inositol tetrakisphosphate appeared to be formed at the expense of the penta- and hexaphosphates, suggesting a slight phytase activity. During the baking process an additional breakdown of total phytate occurred, which was reflected in lower contents of inositol hexaphosphate; concomitantly, more phytate derivatives containing fewer phosphate units were formed. A similar observation was reported by Sandberg et al. (1987) when extrusion cooking at 120 °C on wheat bran was performed.

**Transit Time of Ingested Diets.** A significant reduction of food passage time was observed when oat bran was incorporated in the diet: control group,  $21.3 \pm 2.4$  h; 15% NHOB group,  $16.5 \pm 1.3$  h; 30% NHOB group,  $16.7 \pm 2.4$  h; and 30% BOB group,  $15.7 \pm 2.2$  h. There were

Table IV. Intake, Excretion, Apparent Absorption, and Retention of Calcium and Magnesium<sup>a</sup>

| measure                       | dietary regimens <sup>b</sup>    |                                   |                                  |                                  |
|-------------------------------|----------------------------------|-----------------------------------|----------------------------------|----------------------------------|
|                               | control                          | 15% NHOB                          | 30% NHOB                         | 30% BOB                          |
|                               |                                  | Calcium                           |                                  |                                  |
| intake, mg/day                | 68.0 ± 3.0 <sup>c</sup><br>(100) | 71.3 ± 3.8 <sup>cd</sup><br>(105) | 75.4 ± 3.1 <sup>d</sup><br>(111) | 74.7 ± 3.6 <sup>d</sup><br>(110) |
| fecal output, mg/day          | 14.9 ± 1.6 <sup>c</sup><br>(100) | 27.7 ± 2.5 <sup>d</sup><br>(186)  | 33.9 ± 4.0 <sup>d</sup><br>(228) | 50.3 ± 8.1 <sup>e</sup><br>(338) |
| urinary output, mg/day        | 0.5 ± 0.2 <sup>c</sup>           | 0.4 ± 0.1 <sup>c</sup>            | 0.4 ± 0.1 <sup>c</sup>           | 0.4 ± 0.1 <sup>c</sup>           |
| apparent absorption<br>mg/day | 53.1 ± 2.8 <sup>e</sup><br>(100) | 43.6 ± 3.1 <sup>d</sup><br>(82)   | 41.5 ± 3.8 <sup>d</sup><br>(78)  | 24.0 ± 4.2 <sup>c</sup><br>(46)  |
| % of intake                   | 78.1 ± 8.7 <sup>e</sup>          | 61.2 ± 8.6 <sup>d</sup>           | 55.1 ± 6.1 <sup>d</sup>          | 32.6 ± 7.7 <sup>c</sup>          |
| apparent retention<br>mg/day  | 52.6 ± 2.8 <sup>e</sup><br>(100) | 43.2 ± 3.0 <sup>d</sup><br>(82)   | 41.1 ± 3.7 <sup>d</sup><br>(78)  | 24.0 ± 4.1 <sup>c</sup><br>(46)  |
| % of intake                   | 77.4 ± 8.6 <sup>e</sup>          | 60.6 ± 9.8 <sup>d</sup>           | 54.7 ± 6.3 <sup>d</sup>          | 32.0 ± 7.9 <sup>c</sup>          |
|                               |                                  | Magnesium                         |                                  |                                  |
| intake, mg/day                | 7.4 ± 0.6 <sup>c</sup><br>(100)  | 11.6 ± 0.9 <sup>d</sup><br>(157)  | 16.2 ± 1.2 <sup>e</sup><br>(219) | 15.8 ± 1.2 <sup>e</sup><br>(213) |
| fecal output, mg/day          | 3.1 ± 0.4 <sup>c</sup><br>(100)  | 6.3 ± 0.5 <sup>d</sup><br>(203)   | 8.9 ± 0.7 <sup>e</sup><br>(287)  | 9.7 ± 1.0 <sup>e</sup><br>(313)  |
| urinary output, mg/day        | 0.9 ± 0.3 <sup>c</sup><br>(100)  | 1.4 ± 0.3 <sup>d</sup><br>(156)   | 2.1 ± 0.4 <sup>e</sup><br>(233)  | 1.8 ± 0.4 <sup>e</sup><br>(200)  |
| apparent absorption<br>mg/day | 4.3 ± 0.5 <sup>c</sup><br>(100)  | 5.0 ± 0.4 <sup>cd</sup><br>(116)  | 7.3 ± 0.7 <sup>e</sup><br>(170)  | 6.1 ± 1.1 <sup>de</sup><br>(142) |
| % of intake                   | 57.5 ± 5.3 <sup>e</sup>          | 46.0 ± 5.3 <sup>d</sup>           | 45.2 ± 3.3 <sup>d</sup>          | 38.4 ± 6.0 <sup>c</sup>          |
| apparent retention<br>mg/day  | 3.4 ± 0.5 <sup>c</sup><br>(100)  | 3.9 ± 0.4 <sup>cd</sup><br>(115)  | 5.2 ± 0.7 <sup>e</sup><br>(153)  | 4.3 ± 0.8 <sup>de</sup><br>(126) |
| % of intake                   | 45.0 ± 4.8 <sup>e</sup>          | 34.2 ± 7.7 <sup>d</sup>           | 32.4 ± 5.3 <sup>cd</sup>         | 27.0 ± 5.1 <sup>c</sup>          |

<sup>a</sup> Means ± SD (*n* = 10) in a row with common superscripts are not significantly different at *P* > 0.05, based on ANOVA followed by Fisher's LSD test. Values within parentheses represent percentages vs control group. <sup>b</sup> NHOB, diets containing 15 or 30% nonheated oat bran; BOB, diet containing 30% baked oat bran.

no significant differences among groups fed either of the oat bran containing diets.

**Calcium and Magnesium Availability (Table IV).** Fecal excretion of both Ca and Mg was substantially higher when the NHOB containing diets were fed. This was related not only to higher Ca and Mg intake, which was entirely due to incorporation of oat bran in the diets, but clearly also to interferences between oat bran and dietary Ca or Mg. Relative to the control group, the daily intakes of Ca in the 15 and 30% NHOB groups were only 5 and 11% higher, whereas fecal excretion increased 86 and 128%. For Mg, the intakes in the 15 and 30% NHOB groups were 57 and 119% higher than in the control group, while fecal excretions rose to 103 and 187% higher values. Daily apparent absorption and retention of Ca were negatively influenced by NHOB consumption; however, for Mg, the higher fecal and urinary output was compensated by higher intake, resulting in a positive effect from the NHOB diets on daily Mg absorption and retention. When expressed as a percentage of intake, apparent absorption and retention of both Ca and Mg were significantly lower in the rats fed NHOB. The lower apparent Ca absorption upon feeding of the NHOB diets was not reflected in urinary output, which remained practically unchanged. On the other hand, urinary loss of Mg increased with oat bran intake, corresponding with the substantially higher daily intake and higher daily absorption of Mg upon feeding of the NHOB diets. Heat processing of oat bran resulted in significantly higher fecal output of Ca and lower retention. A similar effect was observed for Mg, although the effect was less pronounced and only statistically significant when apparent absorption was expressed as a percentage of intake.

**Iron and Zinc Availability (Table V).** As opposed to Ca and Mg, the higher intake of Fe and Zn in the 15 and 30% NHOB groups was paralleled by similarly

increased fecal excretion of both minerals. Relative to the ingested amount, urinary output of Fe was very small but still significantly affected by dietary treatment: 0.1% in the control group and 0.2% in the NHOB groups. Unlike Fe, the urinary excretion of Zn was not affected by oat bran feeding and averaged 0.9% of intake. Daily apparent absorption and retention of Fe and Zn were higher when unprocessed oat bran was supplemented, indicating that the increased daily fecal output was largely offset by Fe and Zn from oat bran. Expressed on the basis of Fe and Zn intake, apparent absorption and retention of both minerals were not significantly affected by oat bran. Baking of oat bran significantly decreased Fe absorption and retention; the same tendency was found for Zn, however, not statistically significant.

**Phosphorus Availability (Table VI).** Apparent absorption and retention of P, when expressed as a function of intake, were not influenced by dietary supplementation with unprocessed or baked oat bran. However, on a daily basis, more P was absorbed as well as excreted in the urine when oat bran intake was higher, still resulting in elevated apparent daily retention. Contrary to the other mineral elements studied, baking of oat bran had no apparent effect on P utilization in this study.

## DISCUSSION

Unlike Fe, Zn, and P, apparent absorption and retention (expressed as percent of intake) of Ca and Mg were significantly diminished with increasing NHOB consumption. Thus, the availability of Ca and Mg originating from oat bran was apparently lower than from the inorganic sources (CaHPO<sub>4</sub> and MgO) in the mineral mixture. This was not true for Fe, Zn, and P, in spite of the high contribution of plant minerals to total mineral content in the 15 and 30% NHOB diets: Fe, 23 and 39%; Zn, 30 and

Table V. Intake, Excretion, Apparent Absorption, and Retention of Iron and Zinc<sup>a</sup>

| measure   | dietary regimens <sup>b</sup>       |                                     |                                     |                                     |
|---|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
|   | control                             | 15% NHOB                            | 30% NHOB                            | 30% BOB                             |
|   | Iron                                |                                     |                                     |                                     |
| intake, $\mu\text{g}/\text{day}$                | 548 $\pm$ 27 <sup>c</sup><br>(100)  | 811 $\pm$ 49 <sup>d</sup><br>(148)  | 959 $\pm$ 90 <sup>e</sup><br>(175)  | 948 $\pm$ 85 <sup>e</sup><br>(173)  |
| fecal output, $\mu\text{g}/\text{day}$          | 340 $\pm$ 38 <sup>c</sup><br>(100)  | 478 $\pm$ 54 <sup>d</sup><br>(141)  | 619 $\pm$ 75 <sup>e</sup><br>(182)  | 688 $\pm$ 69 <sup>e</sup><br>(202)  |
| urinary output, $\mu\text{g}/\text{day}$        | 0.5 $\pm$ 0.2 <sup>c</sup><br>(100) | 1.6 $\pm$ 0.5 <sup>d</sup><br>(320) | 1.9 $\pm$ 0.4 <sup>d</sup><br>(380) | 1.3 $\pm$ 0.4 <sup>d</sup><br>(260) |
| apparent absorption<br>$\mu\text{g}/\text{day}$ | 208 $\pm$ 30 <sup>c</sup><br>(100)  | 333 $\pm$ 50 <sup>e</sup><br>(160)  | 340 $\pm$ 81 <sup>e</sup><br>(163)  | 260 $\pm$ 72 <sup>d</sup><br>(125)  |
| % of intake                                     | 37.9 $\pm$ 3.5 <sup>d</sup>         | 41.0 $\pm$ 6.1 <sup>d</sup>         | 35.5 $\pm$ 4.2 <sup>d</sup>         | 27.4 $\pm$ 3.6 <sup>c</sup>         |
| apparent retention<br>$\mu\text{g}/\text{day}$  | 208 $\pm$ 30 <sup>c</sup><br>(100)  | 331 $\pm$ 49 <sup>e</sup><br>(159)  | 338 $\pm$ 80 <sup>e</sup><br>(163)  | 259 $\pm$ 72 <sup>d</sup><br>(125)  |
| % of intake                                     | 37.8 $\pm$ 3.6 <sup>d</sup>         | 39.8 $\pm$ 6.0 <sup>d</sup>         | 35.3 $\pm$ 4.3 <sup>d</sup>         | 27.3 $\pm$ 3.8 <sup>c</sup>         |
|   | Zinc                                |                                     |                                     |                                     |
| intake, $\mu\text{g}/\text{day}$                | 411 $\pm$ 29 <sup>c</sup><br>(100)  | 547 $\pm$ 31 <sup>d</sup><br>(133)  | 695 $\pm$ 42 <sup>e</sup><br>(169)  | 678 $\pm$ 43 <sup>e</sup><br>(165)  |
| fecal output, $\mu\text{g}/\text{day}$          | 289 $\pm$ 25 <sup>c</sup><br>(100)  | 407 $\pm$ 49 <sup>d</sup><br>(141)  | 501 $\pm$ 36 <sup>e</sup><br>(173)  | 530 $\pm$ 49 <sup>e</sup><br>(183)  |
| urinary output, $\mu\text{g}/\text{day}$        | 4.5 $\pm$ 0.8 <sup>c</sup>          | 4.9 $\pm$ 1.3 <sup>c</sup>          | 4.9 $\pm$ 0.9 <sup>c</sup>          | 6.8 $\pm$ 1.7 <sup>c</sup>          |
| apparent absorption<br>$\mu\text{g}/\text{day}$ | 122 $\pm$ 26 <sup>c</sup><br>(100)  | 140 $\pm$ 49 <sup>cd</sup><br>(115) | 194 $\pm$ 46 <sup>d</sup><br>(159)  | 148 $\pm$ 50 <sup>cd</sup><br>(121) |
| % of intake                                     | 29.8 $\pm$ 5.1 <sup>d</sup>         | 25.6 $\pm$ 3.1 <sup>cd</sup>        | 27.9 $\pm$ 2.1 <sup>cd</sup>        | 21.8 $\pm$ 2.6 <sup>c</sup>         |
| apparent retention<br>$\mu\text{g}/\text{day}$  | 118 $\pm$ 27 <sup>c</sup><br>(100)  | 135 $\pm$ 46 <sup>c</sup><br>(114)  | 189 $\pm$ 44 <sup>d</sup><br>(160)  | 141 $\pm$ 45 <sup>cd</sup><br>(119) |
| % of intake                                     | 28.7 $\pm$ 5.2 <sup>d</sup>         | 24.7 $\pm$ 3.3 <sup>cd</sup>        | 27.2 $\pm$ 2.2 <sup>cd</sup>        | 20.8 $\pm$ 2.7 <sup>c</sup>         |

<sup>a</sup> Means  $\pm$  SD ( $n = 10$ ) in a row with common superscripts are not significantly different at  $P > 0.05$ , based on ANOVA followed by Fisher's LSD test. Values within parentheses represent percentages vs control group. <sup>b</sup> NHOB, diets containing 15 or 30% nonheated oat bran; BOB, diet containing 30% baked oat bran.

Table VI. Intake, Excretion, Apparent Absorption, and Retention of Phosphorus<sup>a</sup>

| measure                                       | dietary regimens <sup>b</sup>        |                                      |                                       |                                       |
|---|--------------------------------------|--------------------------------------|---------------------------------------|---------------------------------------|
|   | control                              | 15% NHOB                             | 30% NHOB                              | 30% BOB                               |
| intake, $\text{mg}/\text{day}$                | 73.6 $\pm$ 3.1 <sup>c</sup><br>(100) | 85.4 $\pm$ 4.0 <sup>d</sup><br>(116) | 97.2 $\pm$ 3.9 <sup>e</sup><br>(132)  | 96.4 $\pm$ 4.0 <sup>e</sup><br>(131)  |
| fecal output, $\text{mg}/\text{day}$          | 23.3 $\pm$ 3.5 <sup>c</sup><br>(100) | 28.8 $\pm$ 3.9 <sup>d</sup><br>(124) | 26.4 $\pm$ 3.0 <sup>cd</sup><br>(113) | 27.6 $\pm$ 4.1 <sup>cd</sup><br>(118) |
| urinary output, $\text{mg}/\text{day}$        | 12.8 $\pm$ 2.2 <sup>c</sup><br>(100) | 19.0 $\pm$ 3.9 <sup>d</sup><br>(148) | 19.8 $\pm$ 4.0 <sup>d</sup><br>(155)  | 21.5 $\pm$ 3.9 <sup>d</sup><br>(168)  |
| apparent absorption<br>$\text{mg}/\text{day}$ | 50.3 $\pm$ 3.2 <sup>c</sup><br>(100) | 56.6 $\pm$ 3.8 <sup>d</sup><br>(113) | 70.8 $\pm$ 3.6 <sup>e</sup><br>(141)  | 68.8 $\pm$ 4.0 <sup>e</sup><br>(137)  |
| % of intake                                   | 68.3 $\pm$ 4.5 <sup>c</sup>          | 66.2 $\pm$ 6.1 <sup>c</sup>          | 72.8 $\pm$ 6.1 <sup>c</sup>           | 71.4 $\pm$ 6.1 <sup>c</sup>           |
| apparent retention<br>$\text{mg}/\text{day}$  | 37.5 $\pm$ 3.1 <sup>c</sup><br>(100) | 37.6 $\pm$ 3.5 <sup>c</sup><br>(100) | 51.0 $\pm$ 3.3 <sup>d</sup><br>(136)  | 47.3 $\pm$ 4.0 <sup>d</sup><br>(126)  |
| % of intake                                   | 50.9 $\pm$ 8.4 <sup>c</sup>          | 43.7 $\pm$ 6.9 <sup>c</sup>          | 52.4 $\pm$ 6.0 <sup>c</sup>           | 49.1 $\pm$ 9.2 <sup>c</sup>           |

<sup>a</sup> Means  $\pm$  SD ( $n = 10$ ) in a row with common superscripts are not significantly different at  $P > 0.05$ , based on ANOVA followed by Fisher's LSD test. Values within parentheses represent percentages vs control group. <sup>b</sup> NHOB, diets containing 15 or 30% nonheated oat bran; BOB, diet containing 30% baked oat bran.

48%; P, 13 and 23%. Although 80% of total P in oat bran was present as phytate P, the apparent absorption of oat bran P was not different from that of P in  $\text{CaH}_2\text{PO}_4$ . Taking into account that the absorption measurements were obscured by fecal excretion of metabolic and microflora P, the observation of the high apparent absorption of P in the NHOB groups still implied that considerable enzymatic dephosphorylation of phytic acid took place in the digestive tract to render P in phytate available. This would also substantiate the observation that apparent Fe and Zn absorption was not significantly reduced in the rats fed NHOB, as in the presence of nondegraded phytic acid inverse relationships between phytic acid content and Fe (Hallberg et al., 1987) or Zn (Torre et al., 1991) should be found. In this regard, Kivistö et al. (1986) reported

that undigested phytate in the ileal contents of humans resulted in decreased absorption of Zn, Mg, and P.

What caused the inhibition of Ca and Mg absorption in the rats fed NHOB? It is unclear whether the significant shorter intestinal transit time in the rats fed NHOB impaired Ca and Mg absorption, since the other minerals studied showed no reduced availability. A possible interaction of dietary lipid with Ca and Mg, causing the formation of insoluble soaps, could be excluded since apparent lipid digestibility was not significantly altered by oat bran supplementation (De Schrijver et al., unpublished data). Dietary supplementation with NHOB resulted in 3.6% more fecal excretion of nitrogen ( $P < 0.05$ ; De Schrijver et al., unpublished data), which could help to explain the higher fecal Ca and Mg output by binding

of these minerals to the nitrogenous matrix. Phytate is often considered to be an important factor in mineral malabsorption, but Torre et al. (1991) reported in their review that there is no conclusive evidence that Ca is bound by phytate in *in vivo* conditions; relevant studies on *in vivo* binding of Mg by phytic acid are lacking. In our experiments, preferential binding of Ca and Mg to phytic acid could be postulated as a causative factor due to the much higher dietary concentration of Ca and Mg as compared with that of Fe and Zn. This would correspond with the nonreduced availability of Fe and Zn in the NHOB groups, but elevated excretion of phytate contradicts the unaltered apparent P absorption in these rats. There are no literature data on the binding of minerals to oat bran fiber, but several minerals have been shown to interact with fiber in other plant materials or isolated fiber fractions (Southgate, 1987; Laszlo, 1987; Bertin et al., 1988). Hemicellulose, pectin, and  $\beta$ -glucan may possibly retain minerals either by real chemical binding or just by physical retention, although the interferences from these fiber components will disappear with their digestion. Less degradable fibers such as cellulose may interact more with mineral absorption (Jiang, 1986). Lignin is known to retain minerals in *in vitro* systems (Platt and Clydesdale, 1984, 1987) and thus perhaps also *in vivo*. Our data regarding the differences in mineral absorption correspond with the finding that indigestible remnants of bran from wheat or corn associate preferentially with Ca as compared with Fe, Zn and Cu; moreover, phytate did not seem necessary for these effects to occur (Dintzis et al., 1985).

Unlike Mg, daily apparent absorption and retention of Ca were significantly lower in the NHOB groups. In the 15 and 30% NHOB diets, total Mg consisted for 35 and 51% of oat bran Mg, while oat bran Ca contributed only 4.8 and 9.3% to total dietary Ca. Whereas additional Mg intake compensated for the inhibition of Mg absorption by NHOB feeding, the inhibition of Ca absorption by NHOB feeding was not compensated by higher Ca intake, which resulted in a less positive daily Ca balance as compared with that of the control group. This became even more evident when apparent absorptions of oat bran minerals were calculated by subtracting the average control group data from the average data obtained with the NHOB groups. Relative to intake, the absorptions of oat bran minerals in the respective 15 and 30% NHOB groups were as follows: Mg, 24 and 34%; Fe, 48 and 32%; Zn, 13 and 25%; P, 53 and 87%. Ca intakes from oat bran were 3.3 and 7.4 mg/day, while the corresponding fecal excretions were 12.8 and 19 mg/day, respectively. Consequently, oat bran feeding impaired absorption of Ca from other dietary sources. Except for Ca, the present findings are supported by rat experiments with wheat bran, indicating that this fiber source does not inhibit absorption of mineral salts added to the diet (Bagheri and Guéguen, 1982). Apparently, brans have different capacities for immobilization of Ca in particular.

The present data indicate that baking of oat bran may change its content and chemical composition of the fiber and phytate fraction. Baking caused a 27% relative increase of the fiber that was analyzed as being insoluble. This was mainly related to a shift from soluble to insoluble  $\beta$ -glucans and formation of more Klason lignin. The effect on phytate was predominantly associated with a lower content of inositol hexaphosphate and higher amounts of less phosphorylated inositols. Did these major changes in oat bran fiber and phytate influence mineral bioavailability? Our data clearly showed that the effect of dietary supplementation with BOB was dependent on the mineral

element itself. From a comparison of the 30% BOB group with the 30% NHOB group, the following reductions in apparent absorption were obtained: Ca, 41% ( $P < 0.01$ ); Mg, 16% ( $P < 0.1$ ); Fe, 23% ( $P < 0.05$ ); and Zn, 22% ( $P < 0.1$ ). Apparent absorption of P was not significantly reduced by baking ( $P > 0.1$ ). Evidently, utilization of mineral cations was not positively influenced by disruption of plant cell wall or by partial removal of phytate and highly phosphorylated inositol derivatives, although phytic acid is generally believed to be a major binding agent of minerals (Davies, 1982). Experiments on Zn and Ca absorption in suckling rat pups (Lönnerdahl et al., 1987) and Zn absorption in Japanese quail (Tao et al., 1986) indicated that inositols with less phosphate groups did not inhibit absorption. In the current study, the changes that occurred in the phytate fraction were perhaps too little to result in a beneficial effect on mineral utilization. Moreover, any positive effect from decomposition of phytate was apparently greatly counteracted by negative influences. In this regard the question arises whether the depressed absorption of cations upon feeding of BOB could be related to inactivation of vegetable phytase during baking or to less intestinal activity from phytases from microbial or animal origin, resulting in less release of mineral cations from phytates in the digestive tract. Although this hypothesis was not investigated in the present study, it would anyhow be inconsistent with the finding that P absorption was not significantly reduced by feeding of BOB, while the opposite was observed for mineral cations. Regarding the involvement of fiber in the reduction of cation availability in the rats fed BOB, it is logical to admit that the soluble fiber fraction had a small inhibition on mineral absorption, since this fiber fraction was decreased by baking and easily degraded in the intestinal tract. On the other hand, less breakdown of insoluble fiber may occur, although the physiological behavior of fiber fractions in the intestine is not necessarily fully predicted by their solubility in laboratory conditions (Lund et al., 1989). In addition, since the insoluble fiber fraction was substantially higher in BOB, there is the strong possibility that those insoluble fiber components that increase during baking were responsible for more mineral immobilization and subsequently lowered absorption of mineral cations. This would support the suggestion of Reinhold et al. (1986) that Fe retention by fiber in the rat intestine occurs by formation of insoluble polymers. In addition to dietary fiber and phytate, oat bran contains other compounds such as starch, protein, and lipid which may be modified by baking, and as a consequence, their interactions with minerals within the food matrix and in the gastrointestinal tract may be changed. Metal ions are known to influence the Maillard reaction, but the effect of Maillard products on mineral availability is not well understood (Andrieux and Sacquet, 1984; O'Brien and Morrissey, 1989; Rendlemann and Inglett, 1990). The apparent protein digestibilities were  $92.1 \pm 0.4\%$  in the rats fed the 30% NHOB diet and  $91.0 \pm 1.7\%$  in the rats fed the 30% BOB diet (De Schrijver et al., unpublished data). Thus, there was no parallelism between protein digestion and the reduction of mineral cation absorption as influenced by dietary BOB.

Since fiber, phytate, and other oat bran substances occurred together in the experimental diets, it was not possible to clearly separate their relative roles in the reduced availability of metallic ions as influenced by supplementation of the diet with NHOB and BOB. Nevertheless, this study demonstrated that oat bran supplementation to diets may give rise to Ca malabsorp-

tion as also other Ca sources in the diet may be rendered less available. Such negative effects were not observed for Fe, Zn, and P. Oat bran ingestion decreased apparent absorption of total dietary Mg, since the absorption of Mg from oat bran was inhibited. This negative effect on overall Mg utilization was apparently alleviated with increased intakes of Mg provided by oat bran containing diets. Baking of oat bran greatly enhanced the negative effect on Ca utilization, while also apparent absorption (expressed as percent of intake) of Mg, Fe, and Zn was diminished.

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Registry No. Ca, 7440-70-2; Mg, 7439-95-4; Fe, 7439-89-6; Zn, 7440-66-6; P, 7723-14-0; lignin, 9005-53-2; phytate, 83-86-3; inositol, 87-89-8.