Effect of Dietary Cereal Brans on the Metabolism of Trace Elements in a Long-Term Rat Study[1]

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ABSTRACT

To determine the effect of cereal brans on the metabolism of iron, zinc, copper, and manganese, a seven-month study was conducted using male and female Sprague-Dawley rats. They were fed diets containing cellulose, oat bran, hard red spring wheat bran, soft white wheat bran, corn bran, or rodent chow at 4 or 14% total dietary fiber. During week 7 (phase 1) and week 24 (phase 2), mineral balance studies were conducted. The diet, urine, feces, liver, heart, muscle, kidney, and femur were analyzed for the trace elements. Apparent absorption of the minerals decreased from phase 1 to 2, but tissue levels generally increased with age. Tissue levels were generally higher in females than males. Dietary fiber or phytate did not adversely affect apparent mineral absorption or tissue level. In phase 2, liver iron of the male rats fed diets containing higher endogenous iron levels was approximately 50% higher than that of the males fed diets with lower iron levels. The femur zinc levels in rats fed 14% wheat bran fiber were about 15% lower than those of the other rats, despite higher dietary zinc, but this may not be physiologically meaningful.

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In another article (Shah et al 1990), we reported on the long-term effects of feeding brans of corn, oats, and wheat on the metabolism of calcium, phosphorus, magnesium, and phytate by male and female rats. Here we describe the effects of these cereal brans on the metabolism of iron, zinc, copper, and manganese.

The adverse effects of a diet consisting predominantly of whole wheat bread (unleavened) on the zinc status of rural Iranians and Egyptians was well recognized (Prasad 1984), but there was some controversy about whether the phytate or fiber in whole wheat was responsible (Ismail-Beigi et al 1977, Davies et al 1977). However, more recent work has shown that a reduction of phytate in bread by leavening improved the availability of zinc in humans (Nävert et al 1985). Similarly, a soluble phosphate-rich fraction of bran was reported to inhibit iron absorption in humans (Simpson et al 1981). Nevertheless, white wheat bran or corn bran added to the normal diet of men in the United States did not affect the retention of iron and zinc. The copper balance was actually improved by the ingestion of soft white wheat bran, probably because of the increased intake of copper (Sanstead et al 1978).

In young rats, dietary sodium phytate reduced whole-body retention of iron, zinc, copper, and manganese (Davies and Nightingale 1975). However, when rice bran was included in the diet of weanling male and female rats fed for three weeks, no effect on the liver levels of zinc and copper or on the zinc in the tibia was noted. Interestingly, the females had higher serum ceruloplasmin activities and liver concentrations of copper and zinc than did the males (Ballam et al 1984).

In view of the inconsistent observations on the effect of dietary bran on trace element metabolism in short-term experiments, a long-term rat experiment was performed (using both males and females) to determine if any adaptation occurred in trace element metabolism as reported for calcium metabolism (Davies 1982).

MATERIALS AND METHODS

Since complete details were provided elsewhere (Shah et al 1990), only a brief description is given here.

Animals and Diets

Weanling male and female Sprague-Dawley rats (10 of each sex per diet) were fed one of seven diets containing the following: 1) wood cellulose 4% (total dietary fiber; denoted as diet CEL-4); 2) oat bran 4% (OAT-4); 3) hard red wheat bran 4% (H-WHT-4); 4) hard red wheat bran 14% (H-WHT-14); 5) soft white wheat bran 14% (S-WHT-14); 6) corn bran 14% (CORN-14); and 7) rodent chow (CHOW) (Rodent Laboratory Chow 5001, Purina Mills Inc., Richmond, IN).

The basal diet contained the following, in grams per 100 g: casein, 25; DL-methionine, 0.3; choline bitartrate, 0.2; AIN-76 salt mix (AIN 1980), 3.5; AIN-76A vitamin mix (AIN 1980), 1.0; and corn oil, 10. Corn starch and a fiber source were added to obtain the dietary fiber levels indicated above. These purified diets were not isocaloric but "fiber adjusted" according to the National Research Council (NAS 1978).

Protocol

The animals were fed for 29 weeks. During week 7 (phase 1), a four-day balance study was performed on five randomly chosen rats from each group. The animals were killed the following week by exsanguination under anesthesia (Somnotol, MTC Pharmaceuticals, Mississauga, ON); their organs and femurs were collected and stored at −20°C for mineral analysis. A second metabolic study was undertaken during week 24. The animals were killed during week 29 (phase 2) after colonic function tests during weeks 25–28. Tissues were taken from the rats as before.

Methods

Diets, feces, and tissues were dry-ashed in a programmable furnace (Fisher Scientific, Model 497) at 450°C using concentrated nitric acid as an oxidizing agent. The ash was dissolved in 2.9*N* hydrochloric acid, and the solution was analyzed for iron, zinc, copper, and manganese by flame atomic absorption spectrometry (Perkin Elmer AA 5000). Urine samples were acidified with 6*N* hydrochloric acid and analyzed only for zinc because the levels of iron, copper, and manganese were too low for measurement by flame atomic absorption spectrometry. This method for the determination of iron, zinc, copper, and manganese was verified with the National Bureau of Standards Standard Reference Material No. 1577, Bovine Liver Powder, and gave results within the specified ranges. Phytate in the diets and feces was analyzed by the method of Harland and Oberleas (1986).

Statistics

Data were analyzed separately for each variable using analysis of variance (ANOVA) procedures with factors phase (phase 1, week 8; phase 2, week 29), sex, and diet. ANOVA residuals were screened for outliers and for normality using the Shapiro-Wilk test (Shapiro and Wilk 1965). Data were analyzed on the original scale or were ranked, ignoring phase, sex, and diet, and the ranks were analyzed as deemed appropriate after screening ANOVA residuals. Ranking was done to transform the data into numbers

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that more nearly fit the assumptions of the parametric model for the ANOVA and to retain all the ordinal information in the data (Conover and Iman 1976). Data were then tested for homogeneity of variance using Barlett's test (Snedecor and Cochran 1967).

Pairwise comparisons for significant effects were made using *t* tests on least square means (SAS Institute 1987). Differences were not considered significant if the *P* values exceeded 0.01.

RESULTS AND DISCUSSION

The trace element contribution of cellulose to the diet was minimal, but oat bran and wheat bran provided 13–20% of all minerals at the 4% dietary fiber level and 32–44% at the 14% level (Table I). Interestingly, corn bran provided only 4–7% of the dietary trace elements. It was also low in phytate, with which most of the minerals in brans are associated (Frølich and Asp 1985). The levels of iron, zinc, and copper in the CHOW were much higher than they were in the other diets, and some of the minerals were present as ferrous carbonate, zinc sulfate and oxide, copper sulfate, and manganese oxide. The total amount of each of the trace elements in every diet exceeded the AIN-76 recommendations (AIN 1980), so that tissue levels would show adverse effects only if the fiber source had an inhibitory effect on the absorption of minerals from the salt mixture and the minerals bound to it were not available.

The molar ratios of phytate to zinc were less than 17, but the ratios of phytate × calcium to zinc (moles per kilogram) for the diets containing bran ranged from about 0.2 (CORN-14) to 2.3 (S-WHT-14) and was about 2.3 for the CHOW. From the molar ratios of phytate to zinc, an adverse effect on zinc bioavailability was not likely since zinc status was jeopardized by a ratio above 20 (Oberleas and Harland 1981). Also, based on the molar ratio of phytate × calcium to zinc, a deleterious effect on zinc availability would not be expected because all the ratios were less than the proposed maximum of 3.5 mol per kilogram of diet (Fordyce et al 1987).

Apparent Absorption

Since the results showed wide variations for the fractional absorption of the minerals, the data for each element were ranked, ignoring phase, sex, and diet, and the ranks were analyzed as described before. Some diet or sex effects were significant but not consistent. The only important observation was that from phase 1 to 2, a decrease occurred in iron absorption (11.7 versus −21.6%, $P < 0.001$) and zinc (6.0 versus −13.7%). A similar effect of age on the absorption of zinc by rats was reported by others (Jiang 1986, Khan and Weaver 1989). The negative absorption in phase 2 indicated a loss of body iron even without considering urinary and other losses, but the tissue levels actually increased. This was also true for zinc, copper, and manganese. It was appropriate, therefore, to depend on the tissue levels for the interpretation of the effects of diet in each phase or sex. Tables II–IV show levels of the elements in the liver, an important storage site for many trace elements, in the femur and muscle, important storage sites for zinc (Newberne 1981), and in the heart and kidney.

TABLE I
Mineral Elements in Rat Diets by Analysis[a]

Diet[b]	Elements (mg/kg of diet)				Phytate (g/kg of diet)
	Fe	Zn	Cu	Mn	
CEL-4	61	45	7	63	0.15
From CEL, %	5.8	0.0	0.4	0.1	...
OAT-4	72	56	9	79	3.77
From OAT, %	19.5	17.7	13.8	16.6	...
H-WHT-4	68	54	8	75	2.66
From H-WHT,%	14.0	14.3	12.9	15.5	...
H-WHT-14	92	68	11	98	9.53
From H-WHT,%	38.1	42.0	37.4	43.9	...
S-WHT-14	82	64	10	81	10.93
From S-WHT,%	34.8	37.5	34.1	32.4	...
CORN-14	56	44	7	59	0.72
From CORN, %	5.5	6.8	7.2	3.8	...
CHOW	271	88	16	72	6.92

[a] Fifteen samples were collected during the experiment and analyzed separately. The coefficients of variation (%) for the purified diets were: Fe, 2.1–4.5; Zn, 1.4–3.3; Cu, 1.2–2.5; Mn, 2.5–10.0; phytate, 7.2–9.8. The coefficient of variation for the CHOW ranged from 11.5 for Mn to 15.9 for phytate.

[b] CEL-4 = 4% total dietary fiber (TDF) from wood cellulose; OAT-4 = 4% TDF from oat bran; H-WHT-4 = 4% TDF from hard red wheat bran; H-WHT-14 = 14% TDF from hard red wheat bran; S-WHT-14 = 14% TDF from soft white wheat bran; CORN-14 = 14% TDF from corn bran; CHOW = 14% TDF from rat chow.

Liver Weight

Before considering the mineral levels in the livers, one must determine whether the diets had any effect on liver weight, although no significant effect on body weight was noted at 29 weeks (Shah et al 1990). Liver weights per 100 g of body weight at 8 and 29 weeks were: males, 3.8 ± 0.4 g (SD, n = 35); females, 3.7 ± 0.3 g; and males, 2.8 ± 0.2 g; females, 3.1 ± 0.4 g, respectively. Diet had no effect on liver weight in either phase, and no difference was noted between the two sexes in phase 1. Liver weights, corrected for body weight, were less in phase 2 than in phase 1, and in phase 2, the livers of the females were about 10% heavier than those of the males. These results were comparable to those reported earlier (Shah et al 1981) for rats fed casein, rapeseed protein concentrates, or rat chow for 16 weeks. Burch and Hahn (1982) reported that the liver weight of Fischer-344 male rats was about 3.8 g per 100 g of body weight at 8 weeks, but at 28 weeks the corresponding value was about 18% greater than the average of 2.8 observed in the present study. This may be attributed to a difference in strain.

Tissue Iron

As diet did not have any remarkable, consistent effect on the concentration of iron in the tissues, data for all the diets were averaged (Table II). Liver iron content increased 60–70% from phase 1 to 2 in both sexes. The liver iron level was about three times higher in females than in males. Similar but smaller differences associated with sex and age were seen in the iron content of muscle and kidneys. Changes in the heart and femur were minimal, though significant. Sherman et al (1985), who studied the concentrations of trace elements in the tissues of male Fischer rats at different ages also observed increases in liver and kidney iron levels with age. The large differences in the iron content of tissues associated with sex may be unreported because male rats are used in almost all experiments.

Reports in the literature are inconsistent regarding the effect of dietary brans on iron metabolism. Several short-term human studies (Simpson et al 1981, Hallberg 1987, Rossander 1987, Brune et al 1989) reported that phytate in wheat bran inhibits iron absorption. Moreover, Brune et al (1989) also observed that men and women who were long-term vegetarians showed no adaptation to the adverse effect of phytate on iron absorption. On the other hand, results of two rat experiments (Morris and Ellis 1980,

TABLE II
Iron in Tissues (μg/g of dry weight) of Rats[a]

Tissue	Phase 1 (Week 8)		Phase 2 (Week 29)	
	Males	Females	Males	Females
Liver[b,c]	292 (56)[d]	887 (154)	499 (168)	1,420 (365)
Muscle[b,c]	56 (6)	70 (9)	70 (7)	84 (8)
Kidney[c]	242 (35)	324 (66)	483 (134)	813 (249)[e]
Heart[b,c]	373 (26)	400 (49)	400 (27)	411 (22)
Femur[c]	93 (12)	108 (16)	95 (28)	114 (29)

[a] For diet effects see text.
[b] Phase 2 > phase 1 ($P \leq 0.01$) for both sexes.
[c] Females > males ($P \leq 0.01$).
[d] Mean (SD), n = 5.
[e] Phase 2 > phase 1 ($P \leq 0.01$) for females.

Fairweather-Tait 1982) and two human studies (Anderson et al 1983, Morris et al 1988) indicated no adverse effects of wheat bran on iron metabolism. Moreover, iron from wheat bran was found to be as good as that from ferrous ammonium sulfate in a rat bioassay (Morris and Ellis 1980). Our results for male rats showed that the iron from hard and soft wheat bran at the 14% dietary fiber level resulted in higher liver iron levels than did the AIN-76 reference diet (0.6 versus 0.3 mg per gram of dry weight). Thus, the high fiber and phytate did not impair the bioavailability of iron from ferrous sulfate in the salt mixture, and some of the iron from the bran was also utilized, as reported by others (Morris and Ellis 1980). When pure phytate was added at a 1% level in a rat diet, whole-body retention of iron was reduced at three weeks (Davies and Nightingale 1975). This could be attributed to lack of phytase, which is present in cereal brans (Sandberg and Andersson 1988).

Tissue Zinc

The zinc levels (Table III) in all tissues except the heart were 10–40% higher in female than in male rats at 8 and 29 weeks. The increase in zinc associated with age was seen only in the muscle and femur of both sexes, although the level in male rats decreased slightly from 8 to 29 weeks. The interesting effects of diet were on the femur zinc in the animals fed H-WHT-14 and S-WHT-14 (220 and 215 μg per gram of dry weight, respectively, for both sexes and phases)—significantly less than the corresponding levels for all the other groups (247–283 μg per gram of dry weight).

It was also interesting that the femur zinc for the rats fed the OAT-4 diet was the highest (283 μg per gram of dry weight), although the zinc content of the diet was not the highest. Since bone zinc is used as a parameter for determining the bioavailability of zinc in a rat bioassay (Morris and Ellis 1980), these differences probably represent the lower availability of zinc in diets H-WHT-14 and S-WHT-14 than in diet OAT-4, in spite of the higher digestibility of phytate in wheat bran (Shah et al 1990). The molar ratio of phytate to zinc was 14:17 for diets H-WHT-14 and S-WHT-14, respectively, compared with 7 for diet OAT-4. Since the molar ratio of phytate to zinc in diet H-WHT-4 was only 5, the rats in this group had higher femur zinc (256 μg per gram of dry weight) than those in the H-WHT-14 group (220 μg per gram of dry weight). This is in agreement with the observation of Morris and Ellis (1980) that with a calcium content of 0.75% and a phytate-to-zinc molar ratio of 8 or less, there was no adverse effect on the femur zinc level in young rats. It should be noted, however, that although diet OAT-4 had a phytate-to-zinc molar ratio of 6.7, higher than that in H-WHT-4, it resulted in a much higher femur zinc level (283 μg per gram of dry weight). Thus, the phytate or its zinc binding in oat bran may be qualitatively different from that in wheat bran. Since the calcium contents of all purified diets were lower than 0.75%, they would not have caused these differences in femur zinc.

Another factor in favor of oat bran could be its low insoluble fiber content (8%), compared with the 41 and 46% in soft and hard wheat bran, respectively (Shah et al 1990). In addition, the insoluble fiber in diet OAT-4 was more extensively fermented in the lower gut than that in H-WHT-4 (76 and 45%, respectively, *unpublished data*) or in other wheat bran diets. Above all, although the level of femur zinc indicates an adverse effect of the excess of dietary wheat bran, it may not be physiologically meaningful.

Although apparent zinc absorption decreased from week 7 (6%) to week 24 (−14%), femur zinc levels increased, and liver zinc did not decrease. A decrease in zinc absorption with age was reported for rats by several workers (Jiang 1986, Khan and Weaver 1989) but the tissue levels were not determined. Higher liver zinc levels in females than in males were also observed in Wistar rats (Ballam et al 1984) but not in Fischer-344 rats (Burch and Hahn 1982). However, Ballam et al (1984) thought the sex difference was anomalous. Our data indicate that this may not be so, because the zinc levels in Sprague-Dawley female rats were higher than those in males—not only in the liver but also in the muscle, kidney, and femur.

The effect of age on zinc levels in the bone of male Fischer-344 rats (Burch and Hahn 1982) was similar to the increase in the femur zinc observed in the present study, but our data did not confirm the increase in liver and kidney levels from two to six months.

TABLE III
Zinc in Tissues (μg/g of dry weight) of Rats[a]

	Phase 1 (Week 8)		Phase 2 (Week 29)	
Tissue	Males	Females	Males	Females
Liver[b]	81.3 (9.0)[c]	95.7 (5.4)	84.6 (9.2)	93.9 (6.0)
Muscle[b,d]	44.4 (7.6)[b]	47.8 (4.6)	49.5 (7.4)	50.7 (4.8)
Kidney[b]	92.5 (3.5)	134.6 (34.5)	94.6 (7.0)	131.9 (22.1)
Heart[e]	74.5 (3.2)	73.7 (5.3)	70.3 (3.6)	72.4 (4.7)
Femur[b,d]	209 (22)	237 (28)	245 (24)	300 (35)

[a] For diet effects see text.
[b] Females > males ($P \leq 0.01$).
[c] Mean (SD), n = 5.
[d] Phase 2 > phase 1 ($P \leq 0.01$).
[e] Phase 2 < phase 1 in males ($P \leq 0.01$).

TABLE IV
Copper in Tissues (μg/g of dry weight) or Rats[a]

	Phase 1 (Week 8)		Phase 2 (Week 29)	
Tissue	Males	Females	Males	Females
Liver[b,c]	12.2 (1.3)[d]	17.1 (2.7)	13.0 (1.4)	21.5 (10.5)
Muscle[b,e]	4.97 (0.63)	5.41 (0.91)	4.77 (1.09)	4.89 (0.93)
Kidney[b,c]	39.5 (7.7)	52.3 (14.3)	40.7 (10.1)	72.6 (19.7)
Heart[b,c]	23.2 (0.9)	23.8 (1.3)	24.5 (1.8)	26.1 (2.1)

[a] For diet effects see text.
[b] Females > males ($P \leq 0.01$).
[c] Phase 2 > phase 1 only in females ($P \leq 0.01$).
[d] Mean (SD), n = 5.
[e] Phase 2 < phase 1 ($P \leq 0.01$).

Tissue Copper

Copper in the femur was too low for the determination by flame atomic absorption spectroscopy. Liver copper increased from phase 1 to 2 (Table IV); the change in the males was minimal. Copper levels in the liver and kidney were about 40–80% higher in the females than in the males, but sex differences in muscle and heart were much smaller. The effect of age was appreciable only in the kidneys of the females, which increased almost 40% from phase 1 to 2. Based on liver copper, the availability of copper in the stock diet appeared to be low, since copper content was the highest (16 μg per gram of diet), but copper concentration in the livers (14.7 μg per gram of dry weight) was not higher than were the levels for the rats in the other diet groups (15.2–16.4 μg per gram of dry weight) that received 7–11 μg per gram of diet. Although copper content of the diets varied widely, the effect on tissue levels in different groups was relatively small. Ballam et al (1984) also observed higher levels of liver copper in female versus male Wistar rats, but Burch and Hahn (1982) found no difference associated with sex in Fischer-344 rats.

Total acid detergent fiber levels of 5.8–10.6% from rice bran, corn, and soybean meal, and phytate content of 0.8–1.3% did not show a significant effect on the liver copper levels in Wistar males or females fed for three weeks (Ballam et al 1984), or in Sprague-Dawley males fed a 3% wheat bran diet for five weeks when the diet provided adequate copper (6 μg per gram) (Rockway et al 1987). Our results are in agreement with these observations. The effect of age on the liver and kidney copper levels of Fischer-344 female rats (Burch and Hahn 1982) were similar to our findings, the increases in males from two to six months reported by the same authors were not seen in our data. This may be due to a strain difference.

Dietary fiber from wheat or corn bran had no adverse effect on the copper balance in men consuming these sources of fiber for 30 days (Sanstead et al 1978, Morris et al 1988). In a long-

term study using monkeys, a diet containing about 10% psyllium husk increased the apparent copper absorption, but no effect on liver or kidney levels (Paulini et al 1988) was noted. These results illustrate the difficulty of depending only on the apparent absorption data for determining the effect of a dietary factor on trace element metabolism.

Pure phytate added to a rat diet at 1% reduced whole-body retention of copper (Davies and Nightingale 1975), but comparable phytate content in two of the diets in the present study (H-WHT-14 and S-WHT-14) had no adverse effects on tissue copper levels. This was probably due to the natural phytase in the cereal brans that hydrolysed the phytate (Sandberg and Andersson 1988).

Liver Manganese

In the female rats, liver manganese content increased about 10% from 8–29 weeks (8.2 ± 0.9 versus 9.2 ± 1.2 μg per gram of dry weight). In phase 2, the level was about 15% higher in females than males (9.2 ± 1.2 versus 7.9 ± 1.1 μg per gram of dry weight). The effect of diet on this parameter was minimal. Apparent manganese absorption, however, generally decreased from phase 1 to 2 (−1% versus −11%), although the difference was significant only for diets H-WHT-4 and H-WHT-14. Burch and Hahn (1982) found no effect of sex or age up to 12 months on liver manganese levels in rats. This differs from our observation, but it must be emphasized that the differences we observed were small. No adverse effect of any bran on the manganese retention was evident in the liver, although in chicks, wheat bran but not rice bran reduced key tissue levels (Halpin and Baker 1986).

Although synthetic phytate (1% of diet) was reported to reduce whole-body retention of manganese in young rats (Davies and Nightingale 1975), the diets in the present work did not affect liver levels in spite of a comparable phytate content of two high wheat bran diets. This may be the result of the natural phytase in cereal brans hydrolyzing the phytate (Sandberg and Andersson 1988).

In summary, tissue levels of iron, zinc, copper, and manganese were better indicators of the effects of the diets on mineral retention than on apparent absorption. Dietary fiber (up to 14%) from cereal brans, together with a maximum of 1% phytate, produced practically no adverse effects on tissue levels of male or female rats. The main effects were associated with age and sex. The trace element levels in liver, muscle, kidney, heart, and femur generally increased from week 8 to 29 and were higher in females than in males.

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