

# Absorption of Chromium as Affected by Wheat Bran

KATHRYN S. KEIM,<sup>1</sup> CLAIRE L. HOLLOWAY,<sup>2</sup> and MAREN HEBSTED<sup>2</sup>

## ABSTRACT

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This study was designed to investigate the influence of dietary fiber and phytate, as found in wheat bran, on the absorption of chromium. Twenty male Sprague-Dawley rats were divided into two groups of 10. The control group was fed a semipurified casein-based diet. The experimental group was fed the same diet but with soft red winter wheat bran added to a level of 35% of the diet. To determine chromium absorption and uptake by selected tissues, rats were fasted for 24 hr, fed 5 g of the respective diet, 2 hr later intubated with 100  $\mu\text{Ci}$  of  $^{51}\text{Cr}$ , and sacrificed 24 hr later. The rats were

housed in metabolic cages after the  $^{51}\text{Cr}$  intubation. The incorporation of wheat bran into the diet did not significantly affect chromium absorption as measured by percent dose of  $^{51}\text{Cr}$  in the 24-hr urine. The percent dose in urine in the casein group was  $0.64 \pm 0.20\%$  (mean  $\pm$  SEM) and in the wheat bran group  $0.60 \pm 0.20\%$  (mean  $\pm$  SEM; ns). The  $^{51}\text{Cr}$  uptake of liver, spleen, jejunum, and blood was not statistically different between groups. These results indicate that dietary fiber and phytate as found in wheat bran do not impair intestinal absorption of chromium.

Recommendations to increase dietary fiber intake have resulted in the increased consumption of dietary fiber and fiber-enriched food (Harmuth-Hoene and Schelenz 1980). The dietary fiber intake in the United States is currently about 20 g per day (Reiser 1984).

The Dietary Guidelines for Americans (1985) advocate an increased consumption of fiber in the diet because of beneficial effects such as the reduced absorption of harmful substances associated with colon cancer, an increased water content of the stool with a decreased transit time and intracolonic pressure, and a lowering of blood cholesterol or fasting triglyceride levels (Reiser 1984, Oku et al 1982, Harmuth-Hoene and Schelenz 1980). Persons with diabetes mellitus, in particular, have been advised to increase their consumption of fiber as a means of reducing postprandial blood glucose increase (Crapo 1983, American Diabetes Association 1987).

Dietary fiber refers to all polysaccharides and lignin that are not digested by endogenous secretions of the digestive tract (Trowell et al 1976). The main source of dietary fiber is foods such as fruits, vegetables, and cereal products. The polysaccharide component of fiber consists of cellulose, hemicellulose, pectic substances—including pectin, arabinoxylans, galactan, and arabinogalactan—and gums. The plant gums include mucilages, arabic, alginate, locust bean gum, kappa carageenan, and carboxymethylcellulose. The nonpolysaccharide lignin is an amorphous, high molecular weight, aromatic polymer composed of phenylpropane residues (Selvendran 1984).

Phytic acid (Myo-inositol 1,2,3,5/4,6-hexakis dihydrogen phosphate) is the chief storage form of phosphorous in plant seeds, with legumes and cereal grains being major dietary sources (Oberleas 1983, Maga 1982).

Dietary fiber and phytate can interfere with the absorption of several trace minerals, the effect being dependent on the mineral and fiber in question (Mercurio and Behm 1981, Godara et al 1981, Reinhold et al 1981, Simpson et al 1981). Studies to determine the effect of fiber or phytate on mineral absorption and the components in dietary fiber that are responsible for this effect are often inconsistent and may lead to contradictory conclusions (Fairweather-Tait 1982, Solomons and Jacob 1981).

Researchers have reported conflicting results when studying the effect of phytate on chromium absorption (Chen et al 1973, Keim et al 1987a). Limited data are available in the literature on the effect of fiber on chromium absorption. The present report describes the effect of fiber and phytate as found in wheat bran on the absorption of chromium.

## MATERIALS AND METHODS

### Animals and Diets

Ten male Sprague-Dawley rats were randomly assigned to each of two treatment groups. The dietary treatments consisted of a casein-based American Institute of Nutrition (AIN) 76 diet without wheat bran (control) and an experimental diet, which was a casein-based AIN 76 to which 35% wheat bran by weight was added with an adjustment of the casein, cornstarch, sucrose, and corn oil levels (Table I) (NAS 1978). The wheat bran was ground in a Wiley mill to a particle size of 1 mm.

Male Sprague-Dawley rats (150 g) were fed the casein (control) diet for two days. After this adjustment period, rats were randomly assigned to either the control or wheat bran diet. These diets were fed for 10 days during which time the animals were trained to mealfeed. Rats had free access to food and water unless otherwise noted in the protocol. Rat weights were recorded every other day. Animals were caged in stainless steel metabolic cages with attached feed cups. A paired *t* test with pairing based on day of experimentation was used to determine differences between group means at  $P < 0.05$  (Steel and Torrie 1960).

### Method

Radioactive carrier-free chromium  $^{51}\text{Cr}$  ( $^{51}\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ ; New England Nuclear, Boston, MA) with a specific activity of 71  $\mu\text{Ci}/\mu\text{g}$  (2,626,711 MBq/g) was used to determine absorption of chromium. The dose per rat was 100  $\mu\text{Ci}/0.2\text{ ml}$  (3.7 MBq/0.2 ml) acidic saline and contained 5.6  $\mu\text{g}$  of Cr. No cold Cr was added to the dose.

The rats were fasted for 24 hr and then fed 5 g of the respective diet. Two hours after the diet was fed the rats were intubated via stomach tube with  $^{51}\text{Cr}$ . Rats were kept in metabolic cages and urine and feces were collected for the following 24 hr. Blood was collected from the tail vein of each rat into heparinized microcentrifuge blood collection tubes at 30, 60, and 120 min after intubation. Twenty-four hours after  $^{51}\text{Cr}$  intubation, the rats were sacrificed under light ether anesthesia via cardiac puncture. The liver, spleen, kidney, and a 10-cm midsection of the jejunum rinsed with saline were removed. The rat tissues were frozen, minced, and placed in glass tubes for digestion. All samples were weighed, and then 50  $\mu\text{l}$  of  $\text{H}_2\text{SO}_4$  and 300  $\mu\text{l}$  of  $\text{HNO}_3$  were added to each tube. The tubes were heated in a dry block (100°C) until tissue digestion was complete. All tubes were brought up to equal volume (4.5 ml) with distilled water before assaying for radioactivity using a Packard model 5120 Autogamma counter (3.1% efficiency). The disintegrations per minute (dpm) corrected for background were divided by the weight of each organ to determine dpm per gram.

### Analytical Methods

Fiber levels were determined in the wheat bran diet, casein diet, and wheat bran using a procedure of Goering and Van Soest (1970) to determine neutral detergent fiber (NDF: hemicellulose,

<sup>1</sup>Division of Foods and Nutrition, University of Illinois, Urbana 61801.

<sup>2</sup>School of Home Economics, Louisiana State University, Baton Rouge 70803.

cellulose, and lignin), acid detergent fiber (ADF: cellulose and lignin), and hemicellulose (NDF-ADF). Phytate levels were determined in the diets, wheat bran, and in a phytate control of known phytate content (Harland and Oberleas 1977, Ellis and Morris 1983). Fiber and phytate were expressed as percent of the product or diet.

The diets and wheat bran were analyzed for chromium. The sample (150–200 mg) was weighed into a 12 × 75 mm borosilicate glass tube. The samples were dry ashed by starting at 100°C, holding for 1 hr and increasing 50°C every hour until 300°C was reached. The temperature was then raised to 480°C and ashing continued for 20 hr. To each cooled tube was added 50 µl each of glass distilled water, 50% hydrogen peroxide (Fisher Scientific, Springfield, NJ), and concentrated nitric acid (J. T. Baker Instra Analyzed, Phillipsburg, NJ). Tubes were dried at 100°C and ashed at 480°C for an additional 12 hr. The drying-ashing was repeated until a white ash formed. All samples were diluted with 1N HCl (J. T. Baker) for atomic absorption spectroscopy analysis.

An atomic absorption spectrophotometer with Zeeman background correction, graphite furnace, and HGA-500 programmer (Perkin-Elmer 5000, Norwalk, CT) was used for Cr analyses. A wavelength of 357.9 nm and a 0.7 low slit were used. The furnace program was drying, 110°C, 10-sec ramp and 30-sec hold; charring 1,000°C, 15-sec ramp and 20-sec hold; atomization 2,650°C, 1-sec ramp and 5-sec hold; and clean out, 2,700°C, 1-sec ramp, 3-sec hold. The internal gas flow rate during charring was 50 ml/min.

## RESULTS AND DISCUSSION

The levels of NDF, ADF, hemicellulose, and phytate in the casein diet were negligible compared to the wheat bran diet and wheat bran (Table II). The chromium content of the diets in the present study was well above 100 ppb, all diets being greater than 1,600 ppb. Diets below 100 ppb are considered chromium deficient (Mertz and Roginski 1969). The wheat bran contained 909 ppb of Cr. The original intent of the diet formulation was to make the diets similar in caloric density. This was not achieved in that the caloric density of the casein control diet was 397 kcal/100 g of diet compared to 338 kcal/100 g of wheat bran diet. During the study, both groups of animals had similar weight gains and were able to

receive enough calories by increasing consumption of the respective diets. The diets were isonitrogenous and similar in quantity of fat.

The absorption of <sup>51</sup>Cr in rats, when measured as the percent of dose appearing in the urine, was not significantly affected by the incorporation of wheat bran into the diet (Table III). The percent absorption of 0.64 ± 0.20% (mean ± SEM) for the casein-fed rats, and 0.60 ± 0.23% (mean ± SEM) for the bran-fed rats based on urine Cr are within the ranges reported by others. Donaldson and Barreras (1966) reported a chromium absorption of 0.5%, with a range of 0.1–1.2% when the isotope was administered orally as <sup>51</sup>CrCl<sub>3</sub>. Other researchers reported the absorption of trivalent chromium as 0.5% (Visek et al 1953), and 2–3% (Mertz et al 1965). In humans, Anderson and Kozlovsky (1985) determined a mean chromium absorption of 0.93 ± 0.06% for females and 0.64 ± 0.05% for males. The Anderson and Kozlovsky (1985) results are higher than earlier reports by Anderson and associates (1983), who calculated chromium absorption to be about 0.4%. The variation in percent absorption in these studies probably results from species differences and differences in amount of chromium fed. In the present study, there was a wide variation in chromium absorption. Of the 20 rats used in our study, four rats, two from each group, appeared to be high absorbers of chromium, with values of 2.52 and 0.99% (bran diet) and 2.27 and 1.13% (casein diet). The range for the bran-fed animals was 0.22–2.52% and for the casein-fed rats 0.24–2.27%.

It was not possible to measure <sup>51</sup>Cr in the feces because very little feces was recovered during the study. Only three animals had feces present in the metabolic cages, which amounted to very little. The chance exists that all animals were practicing coprophagy.

Measuring the radioactivity of blood and tissue is another way to determine the effect of wheat bran on the absorption of Cr. The increase in blood radioactivity, from 30 min to 2 hr after <sup>51</sup>Cr intubation, and the subsequent decrease after 24 hr was not significantly different between groups (Table IV). Sayato and co-workers (1980) noted a similar trend in <sup>51</sup>Cr rat blood levels. Although the <sup>51</sup>Cr content of blood was slightly higher in the bran-fed rats at all times, the differences were not statistically significant. The greater <sup>51</sup>Cr content of the blood of the bran-fed rats may have biological significance. Although these results are an interesting finding, and could possibly suggest an effect of wheat bran on improving chromium absorption, this effect cannot be assumed.

The rapid appearance of <sup>51</sup>Cr in the blood observed in the present study has been noted by other researchers (Oberleas and Stoecker 1987, Keim et al 1987b). Radioactive chromium (<sup>51</sup>CrCl<sub>3</sub>) mixed in with 1 g of low-chromium AIN diet resulted in the

TABLE I  
Diet Composition

Ingredient (g/100 g diet)	Casein Diet	Wheat Bran Diet
Casein, high nitrogen	20.0	14.0
Cornstarch	18.0	21.0
DL-Methionine	0.3	0.3
Sucrose	50.0	20.0
Corn oil	7.0	5.0
AIN mineral mix 76	3.5	3.5
AIN vitamin mix 76A	1.0	1.0
Choline bitartrate	0.2	0.2
Wheat bran <sup>a</sup>	...	35.0

<sup>a</sup>Soft red winter wheat bran composite of Hart and Logan (1:1), donated by Patrick Finney, North Central Region, Soft Wheat Quality Lab., O.A.R.D.C., Wooster, OH.

TABLE II  
Analyzed Composition of Diets and Wheat Bran<sup>a</sup>

Component	Casein Diet	Wheat Bran Diet	Wheat Bran
NDF, <sup>b</sup> %	0.07	21.05	49.20
ADF, <sup>c</sup> %	0.02	4.65	10.77
Hemicellulose, %	0.05	16.40	38.43
Phytate, %	0.05	0.5	1.6
Chromium, ppb	1,880	1,626	909

<sup>a</sup>Dry weight basis.

<sup>b</sup>Neutral detergent fiber.

<sup>c</sup>Acid detergent fiber.

TABLE III  
Radioactive Chromium Detected in Rat Urine<sup>a</sup>

Radioactivity	Casein Diet	Wheat Bran Diet
Total radioactive chromium, dpm <sup>b</sup>	1,327,000 ± 412,000	1,247,000 ± 471,000
Percent dose	0.64 ± 0.20	0.60 ± 0.12

<sup>a</sup>Mean ± SEM, *n* = 10. Values are not significantly different between diets at *P* < 0.05.

<sup>b</sup>dpm = Disintegrations per minute.

TABLE IV  
Radioactive Chromium Content of Rat Blood Over Time<sup>a</sup>

Time (hr)	Casein Diet (dpm/ml) <sup>b</sup>	Wheat Bran Diet (dpm/ml) <sup>b</sup>
0.5	11,290 ± 5,710	20,744 ± 7,290
1.0	13,516 ± 6,903	24,774 ± 9,065
2.0	19,871 ± 10,613	28,618 ± 10,581
24.0	6,355 ± 3,194	9,548 ± 3,387

<sup>a</sup>Mean ± SEM, *n* = 10. Values are not significantly different between diets at *P* < 0.05.

<sup>b</sup>dpm = Disintegrations per minute.

majority of  $^{51}\text{Cr}$  being detected at the midpoint of the small intestine 4 hr after eating, when the diet was still in the stomach. When the isotope was intubated 2 hr after consuming 1 g of diet, greater than 60% of the  $^{51}\text{Cr}$  reached the ileum 4 hr after intubation (Oberleas and Stoecker 1987). The question must be raised as to the effectiveness of  $^{51}\text{CrCl}_3$  as an extrinsic tag for food Cr. It appears that the  $^{51}\text{CrCl}_3$  moves ahead of the food mass and is not bound up by such components as fiber and phytate. Whether Cr as found in a mineral mix or food behaves in the same manner remains to be determined. The ability to move so quickly down the gastrointestinal tract may be one reason why Cr is poorly absorbed.

A comparison of the  $^{51}\text{Cr}$  content of the liver, spleen, kidney, jejunum, and rinsings from the jejunum of rats fed either diet did not show any significant differences (Table V). Of all the tissues studied, the kidney had the greatest concentration of  $^{51}\text{Cr}$ , with values of 45,484 dpm/g (casein diet) and 62,290 dpm/g (wheat bran diet), followed by the jejunum, spleen, and liver. The high concentration of chromium in the kidney is probably because the element is being filtered through the kidney for excretion in the urine (Hopkins 1965, Doisy et al 1971).

Hopkins (1965) found the distribution of injected  $^{51}\text{CrCl}_3$  24 hr after administration to be highest in the spleen, followed by the kidney and liver. The difference in results between the Hopkins study and the present one could be accounted for by the differences in route of chromium administration (intubation vs. intravenous injection).

Phytate and dietary fiber (ADF) as found in wheat bran did not affect chromium absorption in rats when the wheat bran was fed at a level of 35% of the diet. These results are in agreement with some related studies and contrary to others. Chen and associates (1973) observed that sodium phytate inhibited the absorption of chromium chloride in the rat. When sodium phytate was added to a chromium-containing solution (0.2  $\mu\text{Ci}$   $^{51}\text{CrCl}_3$  in a  $1 \times 10^{-4} M$  phytate concentration) (phytate:Cr ratio of 5,194), and administered orally, radioactivity in the circulating blood was reduced significantly. The phytate:Cr ratio of the present study was 242, and phytate did not impair absorption of Cr. The lower phytate:Cr ratio and the possibility that sodium phytate and phytate as found in food may not have the same effect on chromium absorption are possible reasons for the different results of these studies.

Several indigestible polysaccharides, when fed at the 10% level of the diet, were shown to inhibit chromium absorption in the rat (Harmuth-Hoene and Schelenz 1980). Although these results are contrary to those observed in the present study, the differences in the polysaccharides used, as opposed to wheat bran, and also the difference in methodologies used in the studies, do not allow for a very direct comparison of results. The content of chromium and other minerals in diet, feces, and ashed carcasses was assessed by instrumental neutron activation analysis in the Harmuth-Hoene and Schelenz (1980) study. Net mineral and trace element absorption was calculated as the difference between total dietary intake and fecal excretion. The fact that chromium absorption was not assessed by urinary excretion, and that radioactive chromium was not used in the Harmuth-Hoene and Schelenz (1980) study to determine the chromium concentration in rat tissues, is a possible explanation for conflicting results of the two studies.

When the source of phytate was a soy protein concentrate, Cr status was not impaired in rats even when the rats were fed a low-chromium diet (Keim et al 1987a). The level of phytate in the present study was 0.5% compared to 0.35% in the Keim et al (1987a) study. It appears that phytate as found in food components does not have a detrimental effect on Cr absorption or nutritional status.

The mechanism for transporting chromium across the intestinal wall and the significant function of its binding with chelating agents in biological systems are not well understood (Chen et al 1973). In the rat, the segment of the small intestine most diffusible to chromium appears to be the midsection of the jejunum, followed by the ileum and duodenum (Chen et al 1973). More recent work by Dowling et al (1986, 1987) using a double perfusion technique

TABLE V  
Distribution of Radioactive Chromium in Rat Tissues<sup>a</sup>

Organ	Casein Diet	Wheat Bran Diet
Liver, dpm/g <sup>b</sup>	4,871 $\pm$ 2,226	7,161 $\pm$ 2,290
Spleen, dpm/g	6,452 $\pm$ 3,355	8,613 $\pm$ 3,065
Kidney, dpm/g	45,484 $\pm$ 22,355	62,290 $\pm$ 25,419
Jejunum, dpm/g	8,484 $\pm$ 1,548	8,677 $\pm$ 968
Rinsings from jejunum, total dpm	2,290 $\pm$ 581	1,452 $\pm$ 258

<sup>a</sup> Mean  $\pm$  SEM,  $n = 10$ . Values are not significantly different between diets at  $P < 0.05$ .

<sup>b</sup> dpm/g = Disintegrations per minute per gram.

concluded that simple diffusion was the method of Cr absorption. When amino acids were omitted from the intestinal perfusate, a suppression in Cr absorption was noted.

This has implications for the present study in that there were nutrient differences in the two diets fed. In attempting to make the diets similar in quantity of protein, fat, and kilocalories, differences were created in type of macronutrients available. It is difficult to determine if these differences in source of carbohydrate (sucrose vs. cornstarch and carbohydrate from the wheat bran) and source of protein (casein vs. wheat bran) had an effect on Cr absorption. Because the diets were adequate in all nutrients, differences in sources of macronutrients may or may not have affected the results.

It has also been suggested that chromium and zinc may be absorbed by a common pathway in the intestine (Hahn and Evans 1975). Hahn and Evans (1975) observed that in zinc-deficient rats chromium absorption and intestinal contents of  $^{65}\text{ZnCl}_2$  and  $^{51}\text{CrCl}_3$  were increased. Oral zinc administration prevented this increase. Also, chromium decreased zinc absorption and intestinal contents of zinc in zinc-deficient rats. The researchers concluded that the ligand that binds the two metals may be one of the sites of antagonism between chromium and zinc. The finding that zinc and chromium may be absorbed by a common pathway in the intestines suggests that these minerals may be affected similarly by the addition to the diet of a substance such as wheat bran.

The possibility that Cr and Zn may share a common absorptive pathway does not mean that wheat bran would affect these two minerals the same way. In fact, the data are conflicting in regard to the effect of wheat bran on Zn absorption. Bagheri and Gueguen (1981, 1982), using radioactive and nonradioactive methodology and diets adequate in Zn, determined that 10–15% wheat bran in the diets of rats had no effect on zinc absorption.

The species of the animal model can affect results. Thompson and Weber (1981) found no effect of wheat bran on Zn absorption in chicks, whereas Kriek et al (1982) reported lower Zn status in baboons fed a high wheat bran yeast bread. In both of these studies, the level of wheat bran was less than 10% of the diet.

In contrast, Caprez and Fairweather-Tait (1982) and Davies et al (1980) observed decreasing Zn absorption with wheat bran. Methodology varied in that Davies et al (1980) fed zinc-deficient diets and Caprez and Fairweather-Tait (1982) used an isotope-dilution technique. These researchers also concluded that phytate, not fiber, was the cause of decreased Zn absorption. As with Zn, there may be differences in results attributable to differences in species and methodology used in regard to Cr absorption.

## CONCLUSION

This investigation was designed to examine the effect of dietary fiber and phytate, as found in wheat bran, on chromium absorption. Twenty male Sprague-Dawley rats were fed either a casein diet or casein with 35% wheat bran added. The addition of 35% wheat bran to the diet did not significantly affect chromium absorption in rats. The blood  $^{51}\text{Cr}$  content after intubation and the  $^{51}\text{Cr}$  uptake in the liver, spleen, kidney, jejunum, and rinsings from the jejunum were also not significantly affected by the wheat bran. The results indicated that addition of 35% wheat bran to the diet of rats had no effect on the absorption of trivalent chromium when the Cr is presented as  $^{51}\text{CrCl}_3$ .

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