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Lack of Evidence for Antiatherogenic Effects of Wheat Bran or Corn Bran in Apolipoprotein E-Knockout Mice

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Epidemiological studies have suggested that intake of whole grains is inversely associated with coronary artery disease. The mechanisms, however, are not completely clear. We tested the hypothesis that intake of wheat bran or corn bran would (1) increase the plasma concentration of phenolic antioxidants and (2) reduce atherosclerosis in apo E-knockout mice. Apo E-knockout (E-KO) mice were fed for 18 weeks with a 0.1% cholesterol-supplemented diet in the absence of grain brans or the presence of 1.7% yellow dent corn bran or 3.3% hard red spring wheat bran. The concentration of antioxidant ferulic acid in plasma and urine was measured by HPLC to monitor the bioavailability of grain phenolics. Plasma lipoprotein profiles were determined by a combination of HPLC and online enzymatic methods. Urinary 15-isoprostane F_{2t}, an in vivo LDL oxidation biomarker, and atherosclerotic lesions were analyzed by ELISA and histological methods, respectively. Dietary supplementation with corn or wheat bran resulted in a 4- and 24-fold increase, respectively, in urinary excretion of ferulic acid. The urinary recovery rate of ferulic acid from the two brans in apo E-KO mice was approximately 1.9−2.9%. Dietary corn bran but not wheat bran also significantly increased the concentration of total ferulic acid in plasma. Nevertheless, the supplementation with either bran product for 18 weeks did not significantly alter the urinary excretion of 15-isoprostane F_{2t}, change the lipoprotein profiles, nor reduce the atherosclerotic lesion development in this animal model. The results suggest that phenolic antioxidants from the two types of bran may not be sufficient to reduce atherosclerosis in this animal model.

KEYWORDS: Whole grain; wheat bran; corn bran; phenolic antioxidants; ferulic acid; atherosclerosis; lipoprotein profile

INTRODUCTION

Many prospective epidemiological studies consistently report that intake of whole grains is inversely associated with coronary artery disease and other chronic disorders (*1*−*4*). Whole grains including whole grain wheat, corn, rice, barley, rye, and oats are composed of approximately 80% endosperm, 15% bran, and 5% germ by weight (*5*). The endosperm contains most of the protein and carbohydrates, while bran is rich in fiber, minerals, unsaturated fats, vitamins, phenolic antioxidants, and other phytochemicals (*5*,*6*). A few studies have further suggested that the bran but not the endosperm or germ of whole grains is the major component for disease prevention (*1*, *3*, *7*). The content of phenolic antioxidants in bran is 10−20 times higher than that in endosperm (*8*). This property has been suggested to be one of the disease protective mechanisms of bran (*4*,*9*). Ferulic acid (FA) is the principal phenolic antioxidant accounting for 60−70% of total phenolic compounds in bran of wheat, corn, and rye (*8*, *10*, *11*). Antiatherosclerotic effects of ferulic acid in hyperlipidemic rabbits have been reported (*12*). Furthermore, in vitro studies have reported other bioactive effects of FA including inhibition of LDL oxidation, platelet aggregation, and endothelial and vascular smooth muscle cell proliferation (*13*−*15*). Such effects may lead to the prevention of atherosclerosis.

Wheat and corn are the most common sources of whole grain in North America. Wheat and corn have the second highest and the highest total phenolic contents and antioxidant activities, respectively, among four main grains, namely wheat, corn, rye, and rice (*10*). Thus, these natural products may have the potential to reduce the risk of oxidative stress-induced diseases. For example, antioxidant and anti-inflammatory effects of black rice pigment fraction may be responsible for its antiatherogenic effects observed in apo E-knockout (Apo E-KO) mice (*16*).

In the present study, we tested the hypothesis that intake of wheat bran or corn bran would (1) increase the plasma concentrations of phenolic antioxidants and (2) reduce atherosclerosis in apo E-KO mice. The apo E-KO mouse model of atherosclerosis is one of the best animal models that closely resembles the disease in humans and has been frequently used by us and others to test the impact of dietary agents on atherosclerosis (*17*−*19*). Thus, we believed that this animal model will be very suitable for testing our current hypothesis.

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MATERIALS AND METHODS

Chemicals and Materials. Ferulic acid (FA), salicylic acid, β-glucuronidase (EC3.2.1.3 1) type H-2 (with both β-glucuronidase and sulfatase activities), β-glucuronidase type B-1 (with β-glucuronidase activity only) were purchased from Sigma Chemical. Total cholesterol (TC) and triglyceride (TG) assay kits were from Diagnostic Chemicals Ltd. Other chemicals were of analytical or high-performance liquid chromatography (HPLC) grade from Fisher Scientific. Corn bran was Canadian Harvest stabilized yellow dent corn bran, and wheat bran was red hard spring wheat bran, from SunOpta Ingredients Group. Their compositions are shown in **Table 1**. The contents of total phenolics and total FA, respectively, were extracted from grain bran after alkali-hydrolysis as previously described (*20*). The contents of total phenolics in wheat bran and corn bran were 7.0 and 53.2 g/kg, respectively, which were measured by a microplate spectrophotometer and expressed as gram of FA equivalents. The contents of total FA were 4.0 and 30.3 g/kg, respectively, which were measured by HPLC as previously described (*20*).

Mice and Diets. Twenty-four 4-week-old male apo E-KO mice were purchased from Jackson Laboratory. After a 7 day adaptation period, the mice were divided into 3 groups of eight on the basis of body weight and plasma cholesterol levels as previously described (*17*). Thus, all 3 groups of animals had comparable mean body weight and plasma total cholesterol levels at the outset of the experiment. The mice were fed with one of the following diets for 18 weeks (**Table 1**): (a) AIN-93G-based mouse chow supplemented with 0.1% (w/w) cholesterol and used as the control diet; the control diet supplemented with (b) 1.7% (w/w) corn bran or (c) 3.3% (w/w) wheat bran. The AIN 93G base (950 g) contained 397.5 g of cornstarch, 200 g of casein, 132 g of dextrinized cornstarch, 100 g of sucrose, 70 g of soybean oil, 35 g of mineral mix, 10 g of vitamin mix, 3 g of L-cystine, and 2.5 g of choline bitartrate. The doses of corn bran (~5 mg/kcal dietary intake) and wheat bran (~10 mg/kcal dietary intake) correspond to the human consumption of bran per calorie intake in the top quintile as estimated through epidemiological studies (≥9.6 g/day) and based on a 2000 kcal diet; such doses have been shown to be beneficial against coronary heart disease and diabetes (*1*, *7*). The content of dietary fiber for all of the experimental diets was adjusted with the addition of cellulose. During the experiment, we collected approximately 160 μL blood samples at week 0 and at 4 week intervals from the jugular vein in lightly anesthetized animals. A 24 h urine collection was completed in week 17. Body weight of the mice was recorded every month. At the end of the experiment, the mice were sacrificed using CO_2. Blood, heart, aorta, spleen, cecum, liver, and kidneys were collected at sacrifice. The study was approved by the Animal Care Committee at the University of Manitoba.

Table 1. Composition of Yellow Dent Corn Bran and Red Hard Spring Wheat Bran[a]

	units/100 g	
ingredients	corn bran	wheat bran
energy (kJ)	0.27	0.8
total fat (g)	1.25	3.83
total carbohydrate (g)	89.1	65.4
dietary fiber (g)	80	45.6
protein (g)	4.1	17.5
cholesterol (mg)	0	0
sodium (mg)	2.8	8.2
magnesium (mg)	35.4	607
potassium (mg)	323	1,220.0
phosphorus (mg)	68.8	1,290.0
calcium (mg)	12.6	89.7
iron (mg)	1.1	14.2
folic acid (μg)	nd	142
niacin (mg)	nd	28.1
vitamin E (IU)	nd	2.3
vitamin A (IU)	0	50
water (g)	4.78	6.85
ash (g)	0.81	6.36

[a] Data is from SunOpta Ingredients Group. nd, not detected.

Analysis of FA in Diets, Plasma, and Urine by High-Performance Liquid Chromatography (HPLC). FA is the main phenolic antioxidant in grain bran (*8*, *10*) and thus serves as a biomarker of grain phenolics intake (*21*). We, therefore, measured the concentration of FA in plasma and urine to monitor the bioavailability of phenolic antioxidants in apo E-KO mice. Total FA in the diets and free FA, FA glucuronides, and total FA in the plasma and urine were determined with HPLC following the preparation of samples by combined enzymatic hydrolysis as previously described (*20*, *22*, *23*). Briefly, plasma or urine was divided into three equal portions. The first portion was treated with β-glucuronidase type H-2 solution containing both β-glucuronidase and sulfatase to determine the amount of all forms of FA (total FA); the second portion was treated with β-glucuronidase type B-1 containing only β-glucuronidase to determine FA-glucuronides; the third portion was used directly for HPLC analysis without any enzyme treatment to determine free FA. By this method, the amount of free FA subtracted from that quantified in the first portion was referred to as the amount of FA-glucuronides. Salicylic acid was used as an internal standard. Identification of the compounds was confirmed by comparing retention times and absorption spectra with those of standard FA. Quantification was accomplished using calibration of the FA standard. The detection limit was 0.04 μmol/L.

Analysis of Lipoprotein Profile by HPLC. HPLC with gel permeation column is a valid method for classifying and quantifying lipoproteins on the basis of particle size and TC contents of the particles (*24*). Plasma lipoprotein profile was determined according to the methods previously described (*24*, *25*). Briefly, plasma lipoproteins were first separated through a gel permeation column on the basis of their particle size. Cholesterol contents of the fractions were then assayed using a postcolumn reactor containing appropriate buffer and enzymes as provided by commercial TC kits (Diagnostic Chemicals Ltd.).

A superose 6 10/300 GL gel column (GE Healthcare) was used for the analysis of lipoprotein profile. The column was calibrated using a gel filtration HMW calibration kit (GE Healthcare) and standard particles with a series of different diameters, by which the conversion of elution time to particle size was built. The fractions of lipoprotein subclasses were then defined by their retention time according to Okazaki's method (*24*, *25*). The retention time of VLDL, LDL, and HDL were further verified using human plasma lipoprotein standards (Sigma Chemical).

Assay for Total Plasma Lipids. TC and TG were measured at baseline, during, and at the end of the study using standard enzymatic methods as previously described (*17*, *18*).

Assay for Urinary 15-Isoprostane F_{2t}. Isoprostanes are chemically stable prostaglandin isomers that are produced by free radical-mediated peroxidation of lipoproteins (*26*). 15-Isoprostane F_{2t} (also known as 8-iso-$PGF_{2\alpha}$) is a representative isoprostane that has become an important LDL oxidation marker in clinical and in vivo studies (*27*). To determine whether the intake of grain bran was able to prevent LDL oxidation, we measured the levels of urinary 15-isoprostane F_{2t} using a commercial enzyme-linked immunoassay (ELISA) per the manufacturers' instructions (EA85, Oxford Biomedical Research). The urine samples were treated with glucuronidase to measure all forms of 15-isoprostane F_{2t}. All values were normalized with the urinary creatinine levels determined by Creatinine Assay Kits (CR01, Oxford Biomedical Research).

Histology and Morphometric Evaluations of Atherosclerotic Lesions. Sections at aortic roots were cut and stained with hematoxylin and eosin to estimate the atherosclerotic lesion size and the lesion to lumen ratio using computer assisted image analysis (Olympus CX41 light microscope, DP70 color video camera, and Image Pro-Plus Software) as previously described (*17*, *18*).

Statistical Analysis. Data are shown as means ± SEM. Significant differences among the groups were determined using one-way ANOVA, followed by Tukey's test as previously described (*20*, *22*).

RESULTS

Effects of the Supplementation with Grain Bran on the Growth of Apo E-KO Mice. Supplementation of wheat or corn bran did not change the weight gain rate of apo E-KO mice. The weights of the wheat and corn bran groups at the end of the study (18 weeks) were 29.6 ± 2.7 and 28.4 ± 0.8 g, respectively, which did not

significantly differ from that of the control group (29.1 ± 1.5 g). Similarly, the weights of internal organs including the liver, kidney, spleen, and heart were comparable among the mice.

Effects of Supplementation with Grain Bran on the Concentration of Antioxidant FA in the Plasma and Urine of Apo E-KO Mice. FA levels in both experimental diets and biofluids were measured as a biomarker of the bioavailability of dietary phenolic antioxidants. Supplementation with wheat bran or corn bran increased the contents of FA in the diets by 14 and 51 times, respectively, as compared to that in the control diet (Table **3**). We assumed that each mouse might have consumed up to 4.5 g of food per day. This gives us an estimate of 0.58 and 2.25 mg of FA consumption per day in the wheat bran or corn bran groups, respectively. The plasma concentration of total FA in the corn bran group was significantly higher ($p < 0.05$) than that in either the control or wheat bran groups (**Table 3**). The urinary excretion of total FA in the wheat bran and corn bran diets increased by 4 and 24 times, respectively, as compared with that in the control group (**Table 3**). The diets also slightly affected the composition of total ferulic acid in urine; the percentage of FA-glucuronides (FA-G) to the total FA was 8.3 ± 0.8% for the wheat bran group, which significantly differed from that in the control or corn bran group (**Table 3**).

Effects of Supplementation with Grain Bran on Plasma Total Lipids and Lipoprotein Profile. Neither corn bran nor wheat bran significantly altered plasma TG or TC levels (**Figure 1A** and **B**). Similarly, the profiles of plasma lipoproteins did not differ among the three groups of animals at the end of the experiment (**Figure 1C**). The three lipoprotein classes VLDL, LDL, and HDL contribute to the plasma TC by 64%, 34%, and 2%, respectively (inset table in **Figure 1C**).

Effect of Supplementation with Grain Bran on Urinary Excretion of 15-Isoprostane F_{2t}. To test whether the phenolic antioxidants in corn and/or wheat bran could reduce the oxidation of lipids, we compared the urinary excretion of 15-isoprostane F_{2t}, a well-studied biomarker of lipid oxidation. Supplementation of wheat bran or corn bran did not significantly alter the urinary excretion of 15-isoprostane F_{2t} (**Figure 2**).

Effect of Supplementation with Grain Bran on Atherosclerotic Lesion Development. At the end of the experiment, all mice had mature atherosclerotic lesions in their aortic roots (**Figure 3**). Mean lesion area and lesion to lumen ratio were similar among all groups of mice (**Table 4**).

Table 2. Composition of Diets

	units (kg)		
group	control	corn bran	wheat bran
energy (kJ)	15756	15748	15783
cholesterol (g)	1	1	1
AIN93G base [a] (g)	949	949	949
total dietary fiber (g)	50	50	50
cellulose (g)	50	36.4	35
fiber from grain bran (g)	0	13.6	15
(supplemented grain bran)	0	17	33

[a] The AIN93G base was prepared in the absence of cellulose according to the formulation of AIN93G. The AIN93G base (950 g) contained 397.5 g of cornstarch, 200 g of casein, 132 g of dextrinized cornstarch, 100 g of sucrose, 70 g of soybean oil, 35 g of mineral mix, 10 g of vitamin mix, 3 g of L-cystine, and 2.5 g of choline bitartrate.

DISCUSSION

We examined whether the intake of wheat bran or corn bran could prevent atherosclerosis in apo E-KO mice. Dietary supplementation with 3.3% wheat bran or 1.7% corn bran resulted in a 4- and 24-fold increase, respectively, in urinary FA excretion, a biomarker of phenolic antioxidants. The intake of corn bran also significantly increased the concentration of the total FA in plasma (**Table 3**). However, such significant increases in plasma FA levels were not observed in the wheat bran group. The possible reasons for this observation could be (a) that the content of FA in the wheat bran diet was 3.6 times lower than that in the corn bran diet and/or (b) that the absorbed FA was quickly eliminated from circulation (*22*) since the plasma samples were taken after 12 h of fasting. Nevertheless, the supplementation with either wheat or corn bran for 18 weeks did not significantly lower urinary excretion of 15-isoprostane F_{2t}, a biomarker of lipid oxidation, nor did either diet prevent atherosclerotic lesion development in this animal model.

LDL oxidation is thought to be a major cause of injury to the endothelium and underlying smooth muscle cells, recruiting macrophages and resulting in an early stage of atherosclerosis, namely, foam cell formation (*28*, *29*). Consequently, antioxidants that inhibit LDL oxidation are expected to have potential antiatherogenic effects (*30*). Grain bran is rich in phenolic antioxidants, and therefore, the phenolic antioxidants are thought to produce health benefits through their antioxidant properties (*4*, *6*). However, wheat or corn bran was not able to reduce the LDL-oxidation biomarker, urinary 15-isoprostane F_{2t}, in the present study (**Figure 2**). One reason for this observation could be a high degree of oxidative stress in the apo E-KO mouse model. Another reason could be low systemic concentrations of active phytochemicals such as FA in this case. Probably because of the low absorbability of bran FA, the concentrations of total FA in the 12-h fasting plasma of apo E-KO mice were very low in wheat and corn bran groups, 0.02 and 0.07 μmol/L, respectively (**Table 3**). Therefore, higher circulating concentrations of FA may be needed to prevent LDL oxidation and consequent atherogenesis in apo E-KO mice. The results of this in vivo study may suggest that the grains with higher contents and/or higher bioavailability of phenolic antioxidants may be necessary to observe benefits of whole grains/grain bran. The bioavailability of phenolic antioxidants varies largely with the type of grain bran. For example, the urinary recovery rate of FA from rye bran in humans is 28% (*21*), which is much higher than

Table 3. Total Ferulic Acid (FA) in Experimental Diets and in Plasma and Urine of Apo E-KO Mice Fed for 18 Weeks with a 0.1% Cholesterol-Supplemented AIN-93G Diet in the Absence of Grain Bran (Control) or the Presence of Either 1.7% Yellow Dent Corn Bran (Corn Bran) or 3.3% Hard Red Spring Wheat Bran (Wheat Bran)[a]

	control	wheat bran	corn bran
total FA in diet (g/kg)[b]	0.009 ± 0.001 a	0.140 ± 0.006 b	0.510 ± 0.003 c
total FA in plasma (μM)	0.01 ± 0.01 a	0.02 ± 0.01 a	0.07 ± 0.01 b
total FA in urine (μmol/mg creatinine)	2.2 ± 0.3 a	8.9 ± 0.6 b	53.5 ± 5.4 c
% of free FA to total FA in urine	0.3 ± 0.3 a	2.0 ± 0.9 a	1.6 ± 1.0 a
% of FA-G to total FA in urine	12.7 ± 0.7 a	8.3 ± 0.8 b	10.9 ± 0.3 a

[a] Data are means ± SE. Values in a row without a common letter differ from each other ($p < 0.05$). $n = 4$ per group for diet; $n = 7$ for plasma and urine of control and corn bran groups; $n = 6$ for wheat bran group. Plasma samples were prepared from 12-h fasting blood samples; 24-h urine samples were collected using a metabolic cage in week 17 of the study. [b] Total FA, all forms of FA, containing free FA and its conjugates; FA-G, FA-glucuronides.

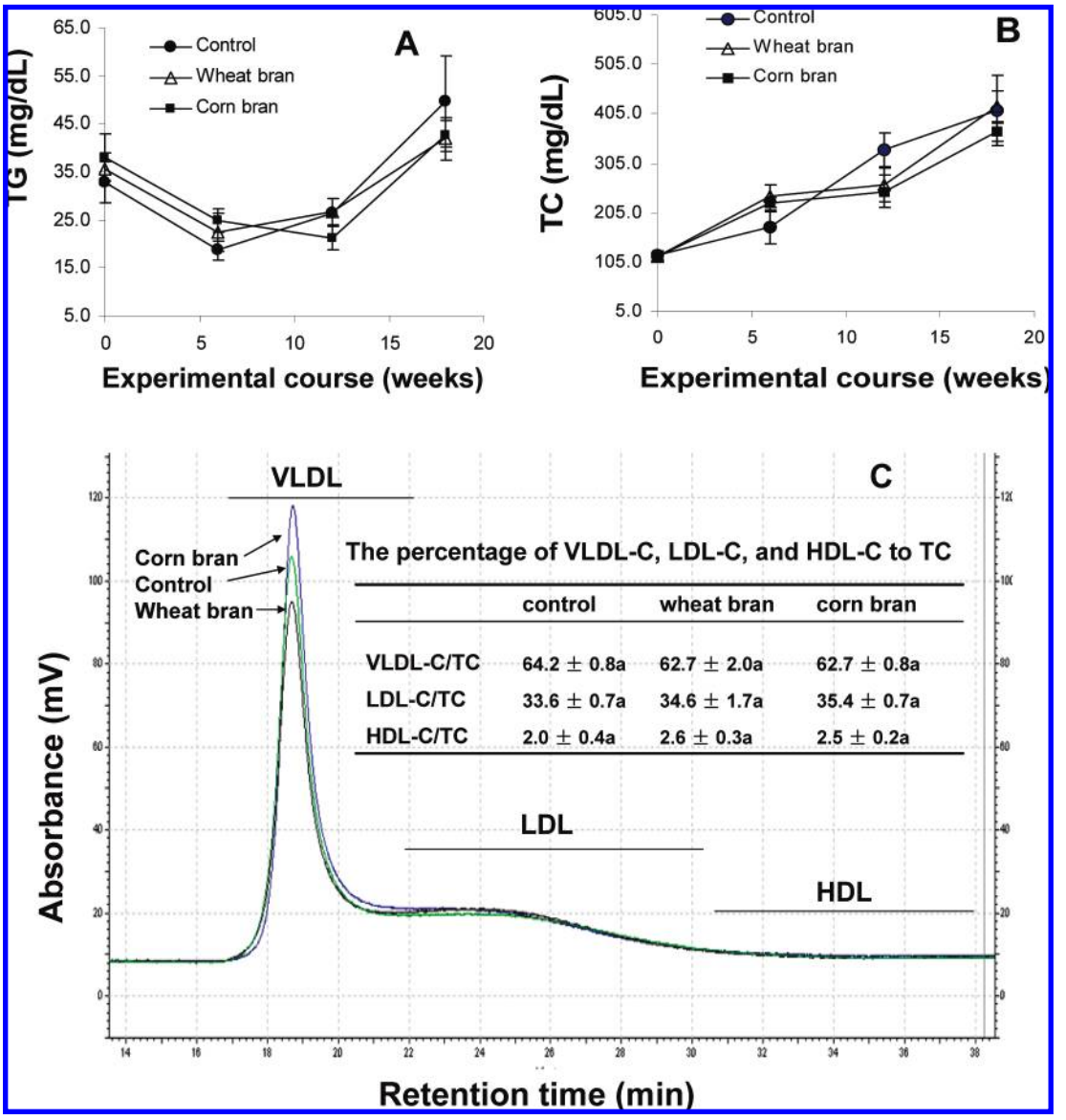

Figure 1. Concentrations of plasma triglyceride (TG, panel **A**) and total cholesterol (TC, panel **B**) during the course of the study were not significantly changed by either of the bran treatments. The lipoprotein profile (panel **C**) and the percentage of TC in each class of lipoprotein are shown in panel **C**. The data are means ± SE, $n = 6-8$. Values in a row without a common letter differ from each other ($p < 0.05$).

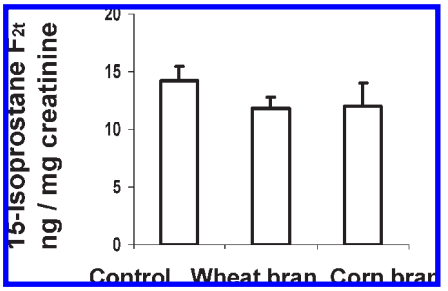

Figure 2. 15-Isoprostane F_{2t} urinary excretion in apo E-KO mice fed with a 0.1% cholesterol-supplemented AIN-93G diet in the absence of grain bran (control) or the presence of 1.7% yellow dent corn bran (corn bran) or 3.3% hard red spring wheat bran (wheat bran). The data were normalized by urinary creatinine levels and shown as means ± SE; $n = 7$ for control and corn bran groups, and $n = 6$ for wheat bran group. There are no significant differences among the groups tested by ANOVA ($p = 0.51$).

that from wheat bran in humans (3.1% (*31*)) or that from refined corn bran in rats (0.5% (*32*)).

Whole grains/grain bran provide important amounts of fiber and many micrometerutrients including potassium, magnesium, iron, zinc, thiamin, niacin, folate, vitamin E, lignin, and phenolic antioxidants (*33*). Most of these nutrients have been shown to reduce CAD risk through preventing hypercholesterolemia, hypertension, diabetes, insulin resistance, hyperhomocysteinaemia, and lipid oxidation (*6*). However, the contents of these potential functional nutrients in bran vary largely with the type and strain of grain. For example, the content of total folate in wheat bran and rice bran is 15−20 times higher than that in corn bran (*34*), whereas the content of total phenolics in corn bran is 2−3 times higher than that in wheat bran and rice bran (*10*). Oat bran contains more soluble fiber, whereas wheat bran contains more minerals and vitamins (*34*). Also, the outer layer of black rice contains 6 times higher flavonoids and 2 times higher vitamin B-1 and niacin than those in white rice (*16*).

The various causes of atherosclerosis combined with the multiple effects of grain bran on atherosclerosis contribute to the complexity and difficulty in identifying the efficacious factors in bran. Nevertheless, current research is focused on specifying the

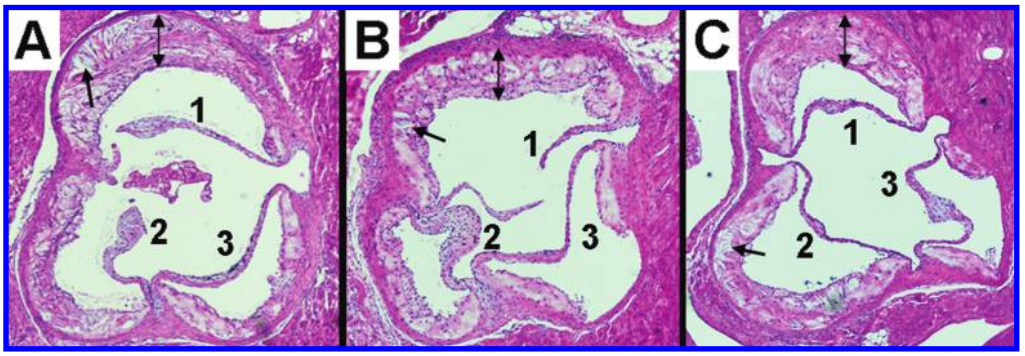

Figure 3. Representative photomicrographs taken from sections at the level of the aortic valve cusps of Apo E-KO mice. The mice were fed for 18 weeks with 0.1% cholesterol-supplemented AIN-93G diet without grain bran (**A**) or with 1.7% yellow dent corn bran (**B**) or 3.3% hard red spring wheat bran (**C**). Advanced atherosclerotic plaques containing cholesterol crystals (monoarrows) are present in aortic roots. The intimal thickening (double arrows) and the three leaflets (numbers) are shown on the sections (H&E ×40).

Table 4. Atheroslerotic Lesion Size in the Aortic Roots of Apo E-KO Mice Fed for 18 Weeks with a 0.1% Cholesterol-Supplemented AIN-93G Diet in the Absence of Grain Bran (Control) or the Presence of Either 1.7% Yellow Dent Corn Bran (Corn Bran) or 3.3% Hard Red Spring Wheat Bran (Wheat Bran)

groups	lesion area mm^2	lesion/lumen ratio
control (n = 7)	0.43 ± 0.12	0.32 ± 0.05
wheat bran (n = 6)	0.38 ± 0.10	0.32 ± 0.05
corn bran (n = 7)	0.40 ± 0.13	0.33 ± 0.06

functional types of grain bran (*6*, *16*, *32*, *35*−*38*). Shane et al. showed that corn bran supplementation of a low-fat controlled diet (20 g bran/d) lowers plasma TC, TG, and VLDL-C in men with hypercholesterolemia; however, such effects were not found for wheat bran (*36*). Harder et al. reported that the intake of 58 g of rye bran per day does not change the susceptibility of human LDL to oxidation ex vivo (*32*). Anderson et al. showed that oat bran but not wheat bran (6 g in 100 g diet) significantly lowered liver cholesterol in rats; oat bran contains more soluble fiber, whereas wheat bran contains significant amounts of insoluble fiber (*37*). Mongeau found that supplementation of diets with 14% w/w hard red wheat bran but not soft white wheat bran could lower the plasma TC in rats (*38*). To mimic the consumption of grain bran by humans, we used 1.7 g of corn bran and 3.3 g of wheat bran per 100 g diets, respectively, in our study. It is possible that higher doses of wheat or corn bran provide higher amounts of soluble fiber and thereby reduce plasma TC concentrations (*37*). Regardless, a lack of cholesterol-lowering effects of either of the bran types in this study may be the main reason for a lack of antiatherogenic effects. Unlike in our study, Ling et al. reported that the outer layer fraction of black but not white rice (5 g in a 100 g diet) had antiatherogenic effects in apo E-KO mice (*16*). The outer layer fraction of black and white rice varies significantly from each other in their content of phenolic compounds. The black rice bran contains 6.4 g of phenolic compounds per 100 g, which contributed 320 mg of phenolic compounds in 100 g of prepared black rice bran diet (*35*); this is 6 times higher than that in white rice bran. These researchers suggested that these phenolic compounds (mainly as anthocyanins and flavonoids) in black rice beneficially modify the plasma lipid profile, enhance antioxidative capacity, and lower CD4+T lymphocyte expression in the artery, leading to reduced atherosclerosis (*16*, *35*). The wheat bran and corn bran tested in this study contained 0.7 and 5.3 g of phenolic compounds per 100 g, respectively, which contributed 23 mg and 90 mg of phenolic compounds in 100 g of prepared wheat and corn bran diets, respectively. These phenolic compounds are mainly phenolic acids such as FA, which are different from those found in black rice. The fact that neither of the bran types reduced atherosclerosis could be due to insufficient dose and/or less efficacious phenolic compounds in wheat or corn bran as compared to those in black rice bran.

In addition, it is also possible that the animal model used was not appropriate for testing antiatherogenic properties of grain bran. In this regard, we have also observed that unlike humans, apo E-KO mice do not respond to the lipid lowering effects of fenofibrate or niacin (*39*, *40*). Therefore, one may suggest that this animal model of severe atherosclerosis may not be suitable for testing compounds with modest antiatherosclerotic activities as also suggested by other researchers (*39*). Another insight from this study is that the benefits of whole grains may be attributable to the synergistic effects of multiple functional components that may not be present in the two types of bran tested in this study. The type and genetics of grains and their products, such as bran, may also play a crucial role in their biological effects.

In conclusion, this study showed that the intake of hard red spring wheat bran and yellow dent corn bran increases the phenolic antioxidant levels in urine and that the intake of corn bran also increases the phenolic antioxidant levels in plasma. However, this does not reduce atherosclerosis in apo E-KO mice. This could be because of (1) a lack of an antiatherogenic effect of the specific wheat bran and corn bran tested in this study; (2) low bioavailability of phenolic antioxidants from bran that would preclude beneficial responses in apo E-KO mice; and (3) a deviation in the biology of atherosclerosis in apo E-KO mice from that in humans. Further studies should continue to test the antiatherogenic effect of other strains of wheat or corn bran and of other types of grain bran in other animal models. Investigations of the bioavailability of phenolic antioxidants from grain bran in humans and in experimental animals are also important to understand the mechanisms of action of whole grains and grain bran in preventing atherosclerotic cardiovascular diseases.

ABBREVIATIONS USED

Apo E-KO, apo E-knockout; TC, total cholesterol; TG, triglycerides; HPLC, high-performance liquid chromatography; FA, ferulic acid; total FA, all forms of FA, containing free FA and its conjugates.

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