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Dietary corn fractions reduce atherogenesis in low-density lipoprotein receptor knockout mice



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ABSTRACT

Accumulating evidence has suggested that intake of whole grains is a protective factor against pathogenesis of coronary artery disease. The exact mechanisms, however, are still not clearly understood. In this study, we hypothesized that adequate intake of corn fractions (aleurone, endosperm and germ) can modify lipid profiles in relation to atherosclerotic lesion development in low-density lipoprotein receptor knockout (LDLr-KO) mice. The purpose of the present study was to investigate the potential cardiovascular benefits of corn fractions in LDLr-KO mice through a number of biomarkers including lipid profile, and morphologic and morphometrical analysis of atherosclerotic lesions in aortic root. Four groups of male LDLr-KO mice were fed with the experimental diets supplemented with (3 treated) or without (control) 5% (wt/wt) of each of corn fractions for 10 weeks. All diets were supplemented with 0.06% (wt/wt) cholesterol. Compared with mice in the control group, atherosclerotic lesions in the aortic roots were significantly reduced ($P = .003$) in the mice that were fed diet supplemented with aleurone and germ fractions. This effect was associated with significant reductions in plasma total ($P = .02$) and LDL ($P = .03$) cholesterol levels, and an increase in fecal cholesterol excretion ($P = .04$). Furthermore, abdominal fat mass was significantly reduced by consumption of aleurone ($P = .03$). In summary, the consumption of aleurone and germ may help attenuate atherosclerosis by reducing plasma total and LDL cholesterol levels.

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Abbreviations: CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; H&E, hematoxylin and eosin; LDL-C, low-density lipoprotein cholesterol; LDLr-KO, low-density lipoprotein receptor knockout mice; TC, total cholesterol; TG, triglyceride; VLDL, very low density lipoprotein cholesterol.

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1. Introduction

Atherosclerotic cardiovascular disease (CVD) is a major source of morbidity and mortality worldwide [1,2]. Atherosclerosis is a complex process in which the lumen of a blood vessel becomes narrowed by cellular and extracellular substances to the point of obstruction. Atherosclerotic lesions tend to form at the branch points of arterial blood vessels and progress through 3 stages: the first stage being the fatty streak lesions, which is characterized by the presence of lipid-filled macrophage (foam cells) in the subendothelial space; the second stage is the fibrous plaque, which consists of a central lipid-rich area, mainly derived from necrotic foam cells, covered by a fibrous cap containing smooth muscle cells and collagen; and in the final stage, these lesions get complicated through thrombus formation with deposition of fibrin and platelet [2]. Elevated plasma low-density lipoprotein (LDL) cholesterol levels and LDL oxidation are among the well-established risk factors for atherosclerotic CVD. Dietary modification has been suggested to play a crucial role in reduction of CVD risk [3].

Several epidemiologic studies have consistently reported that intake of whole grains is inversely associated with CVD and other chronic disorders [4–8]. Whole grains including wheat, corn, rice, barley, rye, and oats are composed of different fractions, including endosperm at approximately 80% to 85%, bran at 12% to 18%, and germ by 2% to 12% of the grain's weight [9–13]. The bran is a composite multilayer consisting of outer pericarp, inner pericarp, testa, hyaline layer, and aleurone layer. The aleurone layer makes 5% to 8% of the kernel and 45% to 50% of the bran fraction. The aleurone layer is nutritionally rich [12]. The starchy endosperm fraction contains most of the protein and carbohydrates, whereas bran is rich in fiber, minerals, unsaturated fats, vitamins, and other phytochemicals [14,15]. The germ fraction is also rich in both macronutrient and micronutrients plus several phytochemicals such as carotenoids, flavonoids, phytosterols, and policosanols [11,12,16–19]. A few studies have further suggested that the bran, not the endosperm or germ of whole grains, is the major component for disease prevention [4,14,20]. The content of phenolic acid in bran is 10% to 20% times higher than that of endosperm [21,22]. This property has been suggested to be one of the disease protective mechanisms of bran [5,23].

Wheat and corn (*Zea mays* L.) are the most commonly consumed whole grain in North America. Corn has the highest total phenolic content and antioxidant activities among 4 main grains, namely, wheat, corn, rye, and rice [22,24]. Thus, these natural products may have the potential to reduce the risk of oxidative stress-induced diseases. Increased intakes of antioxidant rich foods are consistently shown to beneficially impact CVD risk [25]. Shane and Walker [26] showed that corn bran supplementation of low-fat controlled diet (20 g bran/d) lowers plasma total cholesterol (TC) in men with hypercholesterolemia.

The aim of the present study was to investigate the effects of supplementation with corn fractions (aleurone, endosperm, and germ) on the reduction of atherosclerotic lesion development, particularly through alteration of lipid metabolism in LDL

receptor knockout (LDLr-KO) mice. We specifically hypothesized that supplementation with corn fractions would regulate lipid-related cardiovascular risk factors in male LDLr-KO mice. The LDLr-KO mouse model closely resembles the disease in humans and has been frequently used by us and others to test the impact of dietary agents on atherosclerosis [27,28].

2. Methods and materials

2.1. Ethics statement

The experiment was approved by the University of Manitoba Animal Care Committee under project number 13-053/1 (AC10879) and followed the University of Manitoba *Guide for the Care and Use of Laboratory Animals*.

2.2. Corn fractions preparation

Dasca-flint corn was selected for this investigation because it had been previously characterized in terms of its phytochemical profiles [11,22]. Corn (flint) was hand dissected to separate the aleurone, germ, and endosperm according to the procedure described by Stewart et al [29] with slight modifications [11]. The grains were brushed at the ends and germs were removed by a sharp scalpel. The degermed grains were soaked in 0.1% (vol/vol) sodium hypochlorite (NaClO) for 15–20 minutes to sterilize the surfaces and rinsed using sterile deionized water. The seeds were then placed in 10-cm petri dishes lined with 2 ashless filters, moistened with 10 mL of sterile deionized water. The petri dishes were wrapped in aluminum foil and kept at 20°C for 48 hours. The aleurone and endosperm were separated using a scalpel and stored at –20°C. Samples were freeze dried thereafter and ground using a multiuse blade grinder, model PCC 770 (Loblaws Inc, Winnipeg, Manitoba, Canada), to pass through a 0.5-mm sieve. The ground samples were stored at –20°C before use.

2.3. Animals

Thirty-two male LDLr-KO mice (4-week-old) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). The mice were housed in groups of 2 or 3 in conventional mouse cages in a room with controlled temperature (24°C ± 1°C) and a 12:12-hour light-dark cycle.

2.4. Treatments and diets

All the diets were prepared in our laboratory using Mouse Diet 9F (LabDiet, St Louis, MO, USA). The diets were stored in dark bags and closed containers in a cold room (0°C–4°C) during the entire course of the study.

The mice were fed with one of the following diets for 10 weeks: (a) Mouse Diet 9F supplemented with 0.06% (wt/wt) cholesterol and used as the control diet; the control diet was supplemented with (b) 5% (wt/wt) aleurone or (c) 5% (wt/wt) endosperm or (d) 5% (wt/wt) germ. The experimental diet composition is summarized in Table 1.

Table 1 – Ingredient composition of the diets fed to mice

Ingredients	g/kg diet			
	Control	Aleurone	Endosperm	Germ
Protein	205	194.8	194.8	194.8
L-Cystine	3	2.85	2.85	2.85
Sucrose	100	95	95	95
Starch	380.5	361.5	361.5	361.5
Dyetrose	132	125.4	125.4	125.4
Fat	90	85.5	85.5	85.5
Cellulose	27	25.7	25.7	25.7
Mineral mix (AIN-93-MX)	48	45.6	45.6	45.6
Vitamin mix (AIN-93-VX)	10	9.5	9.5	9.5
Choline bitartrate	2.5	2.4	2.4	2.4
Cholesterol	0.6	0.6	0.6	0.6
Aleurone	0	50	0	0
Endosperm	0	0	50	0
Germ	0	0	0	50

After consuming standard mouse chow for 1 week, blood samples were collected through jugular vein under light anesthesia after 4-hour fasting and baseline plasma TC was measured. The animals were divided into 4 groups ($n = 8$ /group; control, aleurone, endosperm, germ) with similar mean plasma TC concentrations and body weight as previously described [30,31]. The experiment period was 10 weeks. The experimental diets were provided to the animals on a weekly schedule. The leftover food was discarded. The food was made as needed and stored in dark bags and closed containers in a cold room (at 4°C) until used. During the experiment, we collected blood samples at 3-week intervals from the jugular vein in lightly anesthetized animals. Body weight and food intake were recorded every week. Fecal samples were collected during the last week of the study. Collected fecal materials stored at -80°C . During the 10-week dietary treatment, the animals had free access to food and water.

2.5. Tissue preparation

After 10 weeks of dietary treatments, the mice were fasted for 4 hours and then euthanized for tissue collection. Mice were killed using carbon dioxide. Their blood was collected through

cardiac puncture in heparinized tubes, per our standard procedures [30,31]. The whole blood samples were then centrifuged at 3000 rpm for 10 minutes at 4°C to yield the plasma fraction, which was used for subsequent analyses. Various internal tissues including heart, aorta, spleen, abdominal fat, liver, and kidneys were also collected, weighed, and stored at -80°C until analysis. Parts of the tissues were fixed in 10% buffered formalin and sectioned for histologic examinations.

2.6. Lipid profiles

2.6.1. Plasma lipids

All mice were fasted for approximately 4 hours before blood collection. Blood samples were drawn through jugular vein of lightly anesthetized animals during the study (at baseline and at weeks 3, 6, and 9) and through cardiac puncture at the end of the study. Plasma was used for determination of TC and triglyceride (TG) levels using our standard enzymatic procedure [32]. Plasma high-density lipoprotein cholesterol (HDL-C) and LDL cholesterol (LDL-C) levels were determined by standard enzymatic kit (LDL and HDL Ultra Sekisui Diagnostics; LLC, Framingham, MA, USA) according to the manufacturers' instructions. Plasma very low density lipoprotein cholesterol (VLDL-C) levels were calculated according to the Friedewald formula: $\text{VLDL-C} = (\text{TG}/5)$ [33].

2.6.2. Liver and fecal lipids

Fecal and liver tissue lipids extractions were performed according to Folch et al [34] with slight modification [28]. The weight and percentage of lipids to total fecal weight were calculated. About 0.5 g of fecal samples was weighed and soaked overnight in 5 mL of 0.025% calcium chloride. The mixture was then vortexed for 30 seconds to homogenize the softened fecal samples. For extraction of liver lipids, approximately 0.15 g of liver tissue was homogenized in 5 mL of calcium chloride for 3 minutes using tissue-tearor homogenizer (BioSpec 985370; BioSpec Product, Inc, Bartlesville, OK, USA). Both fecal and liver tissue homogenates were extracted in 25 mL of chloroform and methanol (2:1, vol/vol) and then homogenized. The suspension was vortexed at room temperature for 1 minute and left for 1 hour to separate into 3 layers. The top 2 layers were discarded. The bottom layer containing lipids was collected and dried at 55°C on a heating block using

Table 2 – Body weights, tissue weights, and food intake of mice fed the different diets

		Control	Aleurone	Endosperm	Germ
Body weight (g)	Initial	20.01 $\pm$ 1.51 ^{NS}	19.83 $\pm$ 1.15	19.88 $\pm$ 1.88	19.79 $\pm$ 0.85
	Final	28.00 $\pm$ 1.31 ^{NS}	25.43 $\pm$ 0.89	27.15 $\pm$ 1.01	26.46 $\pm$ 0.58
	Liver	1.43 $\pm$ 0.07 ^{NS}	1.31 $\pm$ 0.05	1.46 $\pm$ 0.06	1.34 $\pm$ 0.05
	Kidney	0.36 $\pm$ 0.018 ^{NS}	0.35 $\pm$ 0.016	0.363 $\pm$ 0.012	0.37 $\pm$ 0.010
	Spleen	0.078 $\pm$ 0.004 ^{NS}	0.072 $\pm$ 0.004	0.080 $\pm$ 0.005	0.077 $\pm$ 0.002
Tissue weight (g)	Heart	0.20 $\pm$ 0.016 ^{NS}	0.17 $\pm$ 0.008	0.18 $\pm$ 0.007	0.16 $\pm$ 0.021
	Abdominal Fat	0.59 $\pm$ 0.085 ^a	0.36 $\pm$ 0.021 ^b	0.39 $\pm$ 0.049 ^{ab}	0.45 $\pm$ 0.031 ^{ab}
Food Intake (g/d)		2.91 $\pm$ 0.11 ^{NS}	2.79 $\pm$ 0.15	2.79 $\pm$ 0.13	2.79 $\pm$ 0.16

Values are means $\pm$ SE ($n = 8$). The different letters (a, b) within a row indicate significant differences at $P < .05$ determined by Tukey-Kramer multiple range test ($a > b$). Abbreviation: NS, not significant.

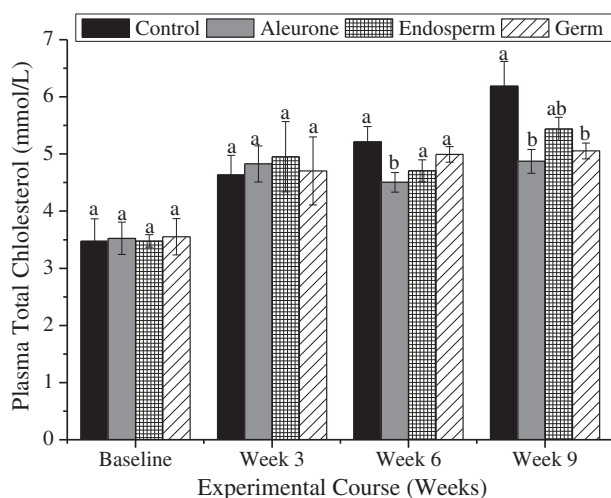


Fig. 1 – Total plasma cholesterol levels of mice fed the different diets. Values are means $\pm$ SE ($n = 8$). The different letters (a, b) indicate significant differences ($P < .05$), determined by Tukey-Kramer multiple range test ($a > b$).

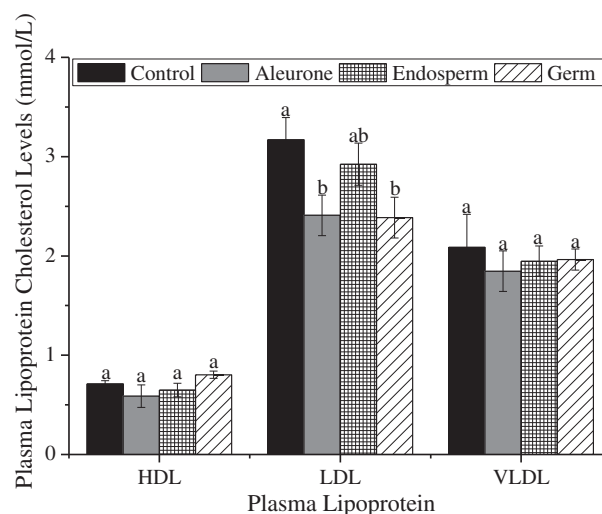


Fig. 2 – Plasma lipoprotein cholesterol levels of mice fed the different diets. Values are means $\pm$ SE ($n = 8$). The different letters (a, b) indicate significant differences ($P < .05$) determined by Tukey-Kramer multiple range test ($a > b$).

an evaporating unit and nitrogen gas. For TC and TG, the samples were reconstituted with ethanol. Total cholesterol and TG levels in the fecal and liver samples were analyzed as described in Section 2.6.1. Gas chromatography (GC) was used for determination of fecal sterol derivatives.

2.6.3. Derivatization of fecal sterols

Samples were reconstituted in 1 mL of chloroform/methanol (2:1, vol/vol). Then 100 μ L of the reconstituted sample was dried at 55°C on a heating block using an evaporating unit and nitrogen gas, followed by the addition of 200 μ L of 1,1,1,3,3,3-hexamethyldisilane + trimethylchlorosilane + pyridine, chlorotrimethylsilane, hexamethyldisilazane, Syton HTP, trimethylchlorosilane, and trimethylsilyl chloride (HMDS + TMCS + Pyridine, 3:1:9 [Syton HTP]). The mixture was dried and then reconstituted in 300 μ L of hexane, vortexed, and left to sit for 15 minutes before centrifuging at 3000 rpm for 1 minute at 4°C. The supernatant was transferred to the newly labeled GC vials. Fecal sterol GC-mass spectrometry analysis was performed according to a method by Wu et al [35].

2.7. Heart and liver histology

Specimens of the base part of hearts containing aortic roots were collected at sacrifice and fixed in 10% buffered formalin. Sections from aortic roots were cut and stained with hematoxylin and eosin (H&E) [36]. Six, 10- μ m sections representing the whole aortic root length were used for morphologic and morphometrical analysis of atherosclerotic lesions. Light microscopy at $\times 100$ magnification and Image ProPlus digitizing system (Media Cybernetics, Inc, Rockville, MD 20850 USA), respectively, were used. Details of these procedures can be found elsewhere [36]. Similarly, H&E-stained liver sections were examined using light microscopes.

2.8. Statistical analyses

Eight mice were used in each group to have sufficient biological samples including plasma for various biochemical analyses. $n = 7$ is the minimum requirement for statistical analysis to detect significant differences among the groups at $P < .05$ and a power of 80%. Data were analyzed using 1-way analysis of variance with GLIMMIX Procedure of SAS, release 9.3 (SAS 2014; SAS Institute, Cary, NC, USA). Sample means were compared using Tukey-Kramer multiple comparison procedure, and data were considered significantly different at $P < .05$. All results are presented as means $\pm$ SE.

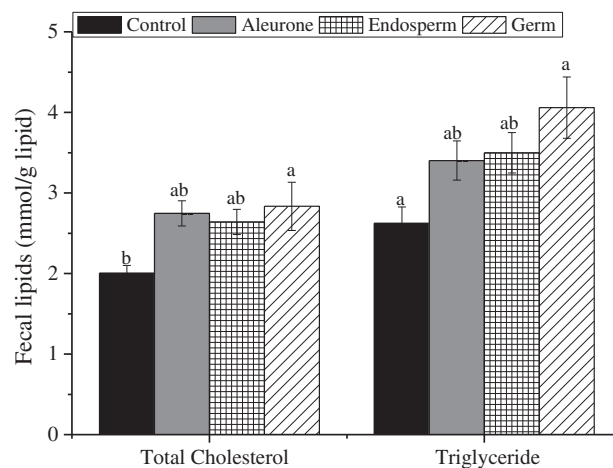


Fig. 3 – Fecal total cholesterol and triglyceride levels of mice fed the different diets. Values are means $\pm$ SE ($n = 8$). The different letters (a, b) indicate significant differences ($P < .05$) determined by Tukey-Kramer multiple range test ($a > b$).

Table 3 – Fecal sterol excretion of mice fed the different diets

	mg of Sterol per g feces				
	Control	Aleurone	Endosperm	Germ	P
5 α -Cholestane	4.22 $\pm$ 0.73 ^{NS}	4.27 $\pm$ 0.23	3.68 $\pm$ 1.26	4.32 $\pm$ 0.38	.93
Cholesterol	42.2 $\pm$ 1.64 ^{NS}	38.9 $\pm$ 3.00	39.1 $\pm$ 5.58	45.0 $\pm$ 3.81	.66
Desmosterol	21.0 $\pm$ 14.1 ^{NS}	36.1 $\pm$ 2.07	42.8 $\pm$ 1.05	35.4 $\pm$ 5.11	.25
Campesterol	38.6 $\pm$ 11.4 ^a	13.0 $\pm$ 1.62 ^b	12.7 $\pm$ 3.31 ^b	17.0 $\pm$ 1.16 ^b	.01
Stigmasterol	4.56 $\pm$ 2.01 ^{NS}	2.14 $\pm$ 0.31	2.64 $\pm$ 0.66	3.88 $\pm$ 0.27	.12
β -Sitosterol	28.6 $\pm$ 3.38 ^{NS}	26.6 $\pm$ 4.51	30.3 $\pm$ 5.15	36.1 $\pm$ 2.01	.40

Values are means $\pm$ SE (n = 8). The different letters (a, b) within a row indicate significant differences (P < .05) using Tukey-Kramer multiple range test (a > b). Abbreviation: NS, not significant.

3. Results

3.1. Body weight, tissue weight, and food intake

Changes in body and tissue weight and the level of food intake are presented in Table 2. A steady, body weight gain was observed in all groups over the study period (data not shown). At the end of 10 weeks, the body weight including heart, kidney, liver, and spleen weights were comparable among all the groups. However, consumption of aleurone significantly reduced abdominal fat mass (P = .03) as compared with the control group. The mean food intake was not statistically significant among the experimental groups (P = .354).

3.2. Plasma lipid levels

The plasma TC levels are shown in Fig. 1. The TC levels were significantly higher in the control group than in the aleurone- and germ-supplemented groups from week 6 to the end of the study, but the levels were not significantly different between the endosperm group and the control group. However, the levels of TG remained similar among all treatment groups during the study course (data not shown).

The plasma lipoprotein cholesterol levels are shown in Fig. 2. The HDL-C, LDL-C, and VLDL-C levels were determined at the end point. The mice fed with aleurone (P = .041) or germ (P = .031) fraction exhibited lower LDL-C when compared with the control and endosperm-treated diet groups. However, the levels of VLDL-C and HDL-C were comparable among all the experimental groups. Comparisons of the LDL-C/HDL-C ratios among groups were as follows: control, 4.53 $\pm$ 0.36; aleurone, 4.12 $\pm$ 0.68; endosperm, 4.82 $\pm$ 0.57; and germ, 2.97 $\pm$ 0.22. Mice consuming germ fraction-supplemented diet had the lowest LDL-C/HDL-C ratio when compared with the aleurone, endosperm, and control groups.

3.3. Fecal lipid levels and sterols

The fecal TC and TG levels are shown in Fig. 3. In this study, we report that consumption of germ fraction was associated with increased fecal cholesterol excretion when compared with those in the control group (P = .04). In addition, germ-treated animals had significantly increased fecal TG levels (P = .015). All the treated groups showed a significant reduction in fecal campesterol when compared with the control

(P = .01; Table 3), whereas there were no significant differences with other sterols (Table 3).

3.4. Liver TC and TG levels

The liver TC and TG levels are shown in Fig. 4. Triglyceride levels of the mice fed on the diet supplemented with aleurone, endosperm, and germ fractions were significantly increased compared with that in the control diet group (P = .0001). The TC levels of germ fraction-administered group were significantly increased compared with that in the control group (P = .013).

3.5. Liver and heart histology

Histologic analysis and photomicrographs of aortic roots of the heart from each group are shown in Fig. 5. Representative photomicrographs of aortic roots showing various degrees of atherosclerotic lesions are shown in Fig. 5A. Advanced atherosclerotic lesions were present in the aortic root of the control mice. The atherosclerotic lesions size was decreased in the heart aortic root of mice whose diet was supplemented with aleurone (P = .002) and germ (P = .049) fractions (Fig. 5B). The atherosclerotic lesion area was significantly different among the experimental groups (P = .003; Fig. 5B). Histologic

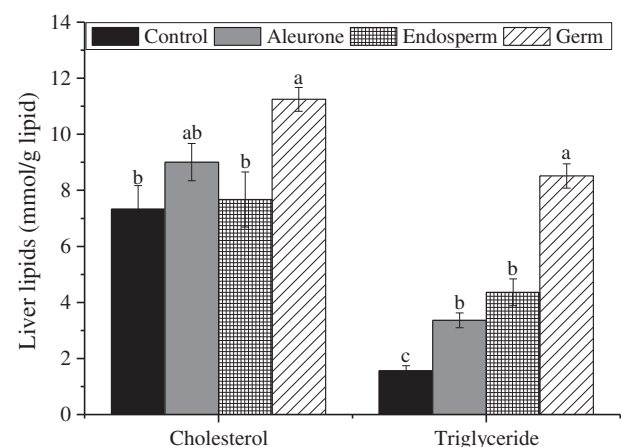


Fig. 4 – Liver total cholesterol and triglyceride levels of mice fed the different diets. Values are means $\pm$ SE (n = 8). The different letters (a, b, c) indicate significant differences (P < .05) determined by Tukey-Kramer multiple range test (a > b > c).

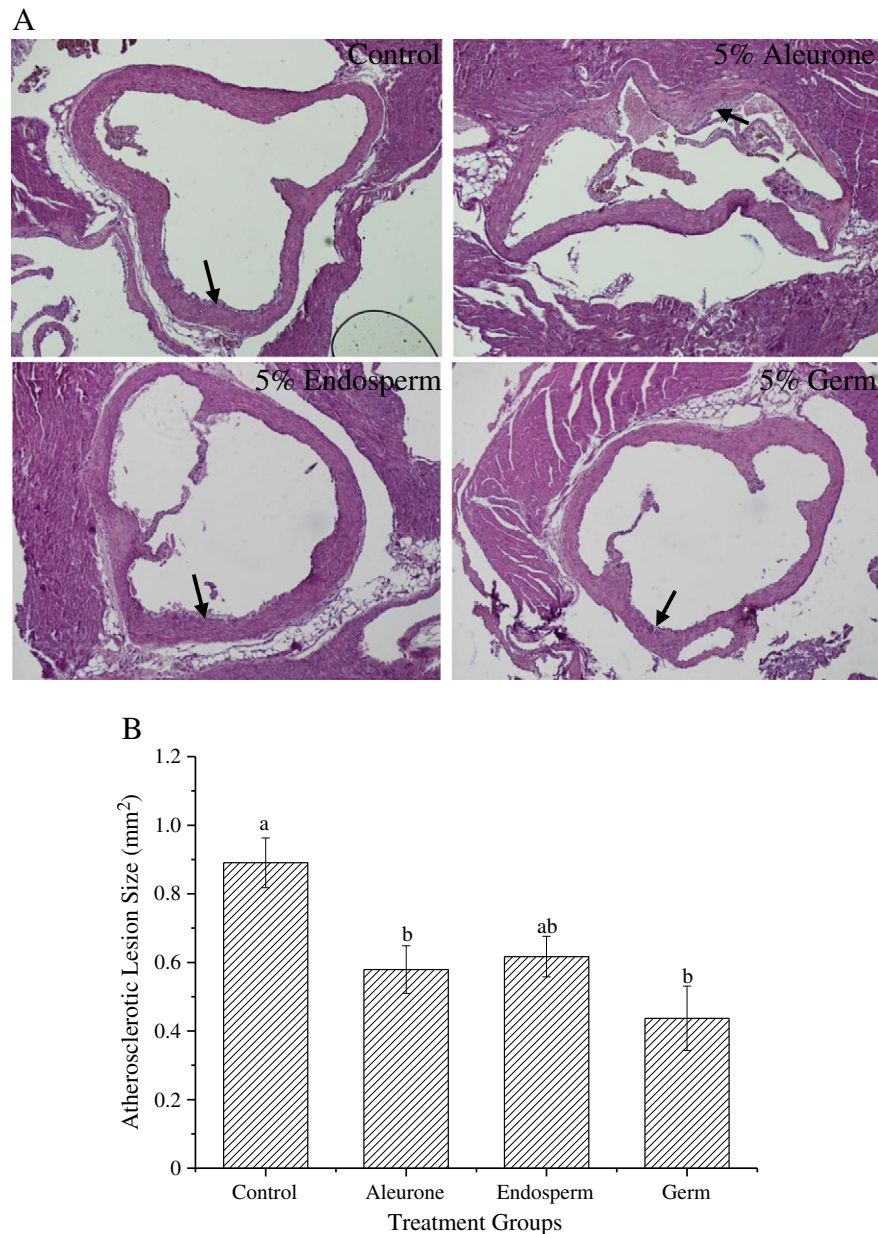


Fig. 5 – A, Photomicrographs of transverse sections at the level of the aortic valve cusps showing atherosclerotic lesion of mice fed the different diets. B, Atherosclerotic lesion area in aortic sinuses measured in H&E-stained sections from aorta of mice fed the different diets. Sections were taken from 6 different levels of the aortic root length. Values are means $\pm$ SE (n = 6). The different letters (a, b) indicate significant differences ($P < .05$) determined by Tukey-Kramer multiple range test ($a > b$).

analysis of the livers in each group is shown in Fig. 6. Representative photomicrographs of the liver droplets are presented in Fig. 6A. The liver samples showed a small degree of nonspecific vacuolation; this feature may represent hepatic fatty changes. The extent of changes was reduced in the mice fed on a diet supplemented with corn fractions (Fig. 6A and B) as compared with the control group.

4. Discussion

Atherosclerosis has been recognized as a chronic inflammatory disease. The use of natural antioxidants and plant

compounds that affect atheroprotection has been implicated to be of great clinical relevance [37].

This study was designed to examine the effects of corn fraction (aleurone, endosperm, and germ) supplementation on the dietary cholesterol-induced atherosclerotic lesion in LDLr-KO mice. Data from the histologic analysis presented in this article support our previous reports by showing that 0.06% (wt/wt) cholesterol induces atherosclerosis in LDLr-KO mice. Our data also demonstrated the effects of aleurone and germ supplementation in cholesterol-fed mice. Both aleurone and germ supplementation reduced atherosclerotic lesion development, and this was associated with reduced plasma TC and LDL-C and increased fecal cholesterol excretion.

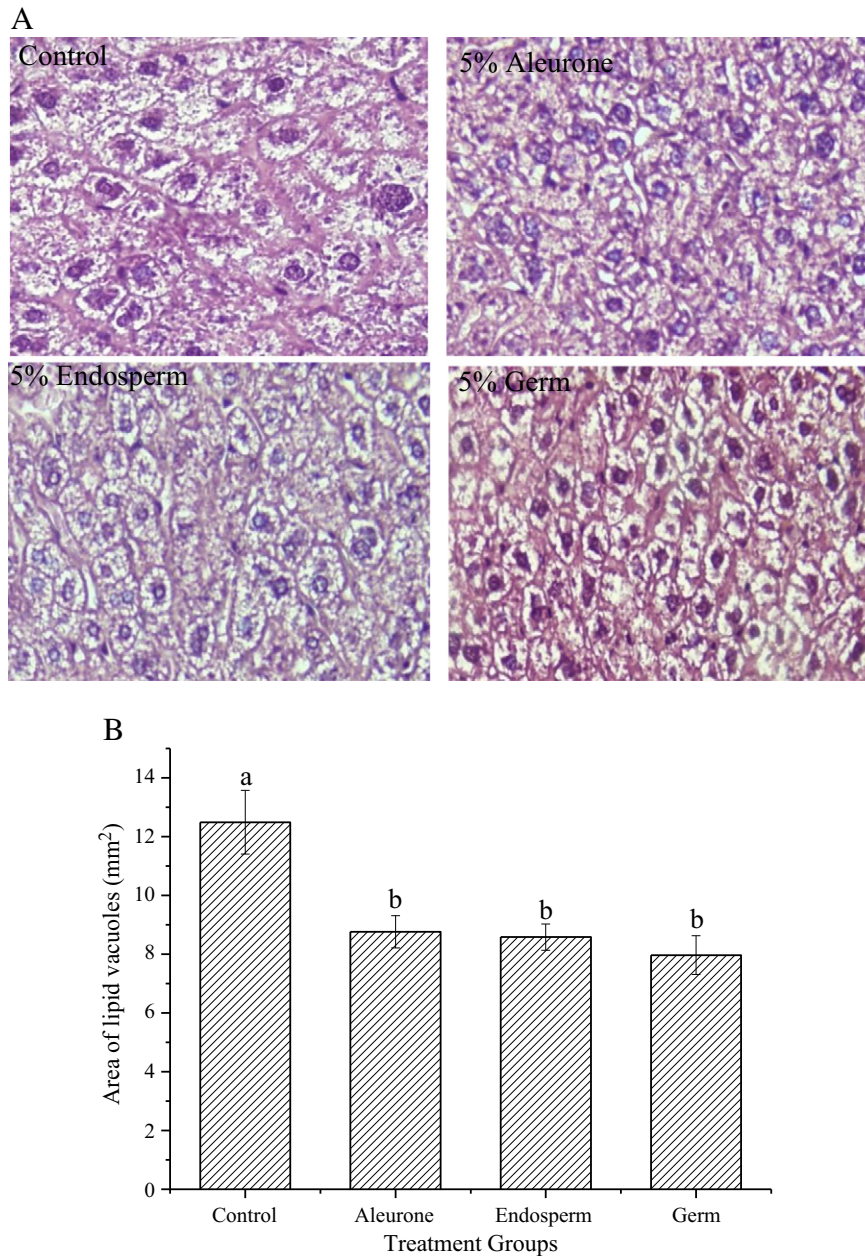


Fig. 6 – A, Histologic images of liver tissues from mice fed the different diets. B, Fatty changes measured in H&E-stained sections from each liver section. Sections were taken from six different levels representative of the liver length. Values are means $\pm$ SE (n = 8). The different letters (a, b) indicate significant differences ($P < .05$) determined by Tukey-Kramer multiple range test ($a > b$).

Cholesterol has been widely used by our group and others to further induce atherosclerosis in experimental rodent models. These LDLr-KO mice on a cholesterol-rich diet had significant body weight increases, although supplementation with corn fractions did not affect the final body weight. This is in agreement with our previous studies showing no significance in food intake and body weight changes in apolipoprotein E-knockout (apoE-KO) mice fed wheat or corn bran [38] and LDLr-KO mice fed wild rice [28], and Chinese brown rice (unpublished data). However, mice that were given diet supplemented with aleurone fraction had significantly lower abdominal fat, whereas the food intake was comparable with

that in other groups. This suggests that aleurone fraction could influence the changes in fat mass of animals with atherosclerosis despite the lack of a direct change in body weight. Zhang et al [39] reported that replacing white rice and processed wheat starch with wild rice as a chief carbohydrate source in high-fat cholesterol rats lowered body weight and abdominal fat mass, despite similar dietary energy intake among the experimental groups.

Elevated plasma concentrations of LDL are a potent risk factor for atherosclerosis [40]. The LDLr-KO mice have elevated LDL-C levels, and the high cholesterol diet produced a further increase in these values, which was significantly

inhibited by supplementation with aleurone and germ fractions to the diets. The LDLr-KO mice on a cholesterol-rich diet had significant plasma TC and LDL-C increases; however, supplementation with aleurone and germ fractions significantly lowered plasma TC and LDL-C. A few studies have suggested that the bran but not the endosperm or germ of whole grain is the major component for disease prevention [4,8,20]. The content of phytochemicals including phenolic acids in the bran is 10 to 20 times higher than that in endosperm [21,22]. This property has been suggested to be one of the disease-protective mechanisms of bran [5,23]. Conflicting results on association between grain bran and lipid profiles have been published in randomized clinical trials. Shane and Walker [26] showed that corn bran supplementation of low-fat controlled diet lowers plasma TC, TG, and VLDL-C in men with hypercholesterolemia; however, such effects were not found with wheat bran. Anderson et al [41] 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. Mongeau [42] found that supplementation of diets with 14% wt/wt hard red wheat bran could lower plasma TC in rats. Zhao et al [38] reported that 18-week consumption of corn bran and wheat bran did not significantly alter lipoprotein profiles or reduce the atherosclerotic lesion development in apoE-KO mice.

Whole grains containing bran provide good amounts of dietary fiber and many micronutrients including potassium, magnesium, iron, zinc, thiamin, niacin, folate, vitamin E, lignin, and phenolic antioxidants [43]. Most of these nutrients have been shown to reduce risk factors for coronary artery disease such as hypercholesterolemia, hypertension, diabetes, insulin resistance, hyperhomocysteinaemia, and lipid oxidation [15]. However, the contents of these potential functional nutrients in bran vary largely with the type and strain of the grain.

Our data demonstrated that supplementation with germ fraction significantly increased fecal TC and TG excretion. In addition, supplementation with germ fraction significantly reduced campesterol in feces. The major route of sterol elimination from the body is by excretion of unabsorbed cholesterol and bile acids as fecal, neutral, and acidic sterol [44]. Moreover, increased bile acid secretion was found to be inversely correlated with elevated cholesterol levels and the prevalence of coronary artery disease [45]. Germ supplementation significantly increased hepatic cholesterol levels, whereas hepatic TG levels significantly increased with supplementation of all corn fractions. When the input rate of fatty acid is greater than the output rate, steatosis develops. Various interactions between fatty acid oxidation, hepatic fatty acid uptake, fatty acid export within VLDL-TG particles, and de novo lipogenesis affect the TG levels in hepatocytes [46].

Accumulations of fatty substances and cholesterol are 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 [47]. Total cholesterol/HDL-C and LDL-C/HDL-C ratios are used to predict the risk of ischemic heart disease [48]. Results of prospective studies [49,50] have suggested that

a high LDL-C/HDL-C ratio combined with hypertriglyceridemia is a major risk factor for coronary artery disease. Corn is richer in phytochemicals compared with other main grains, namely, wheat, rye, oats, and rice [11,22,24]. Data from the heart histologic analysis presented in this article support our previous reports by showing a limited extent of lesion involvement in diet supplemented with germ and aleurone fractions. Previously, our laboratory showed no significant reduction in atherosclerotic lesions in apoE-KO mice fed corn bran or wheat bran over an 18-week study [35]. However, we previously reported significant reductions in atherosclerotic lesion development in LDLr-KO mice fed wild rice for 24 weeks [28]. Moreover, liver histologic analysis after diet supplementation with corn fractions demonstrated an overall normal appearance, with lesser apparent fatty change. Reduced atherosclerotic lesion area in aortic roots and fatty changes in the corn fraction-treated mice may be related to the inhibition of intestinal cholesterol absorption. The reduction of plasma cholesterol in corn fraction-treated animals most likely resulted in the absence of cholesterol clefts in the attenuated atherosclerotic lesions of the treated animals.

There are limitations of this study that need to be addressed. First, LDL oxidation could not be measured because the sample size was limited. Oxidation of LDL particles is widely recognized as the key step in the initiation of atherosclerosis development [51]. The LDL oxidation profile might allow for more appropriate interpretation of the effects of supplementation of corn fractions on atherosclerosis. The relatively short period of the experimental protocol because of the limited amounts of corn fractions could be considered as another limitation. Many studies demonstrate that mice with induced atherosclerosis show severe atherosclerotic damage histologically after 20 weeks [28,38]; however, atherosclerotic lesions already exist in the LDLr-KO mice and their size increases as the study progresses.

In summary, we conclude that supplementation with aleurone and germ fraction could reduce plasma TC and LDL-C levels, increase fecal lipid excretion, and reduce the size of atherosclerotic lesions, which is partially consistent with our original hypothesis. The findings of this study add to the current knowledge about the effects of bran fraction supplementation on CVDs, particularly with respect to lipid metabolism. Thus, these results provide clinically relevant insight into the use of germ and aleurone fractions as a supplement to prevent atherosclerosis risk factors.

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