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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.

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.