Dietary flaxseed inhibits atherosclerosis in the LDL receptor-deficient mouse in part through antiproliferative and anti-inflammatory actions

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Dietary flaxseed inhibits atherosclerosis in the LDL receptor-deficient mouse (LDLrKO), and to identify the cellular mechanisms for these effects. LDLrKO mice were administered a regular diet (RG), a 10% flaxseed-supplemented diet (FX), or an atherogenic diet containing 2% cholesterol alone (CH) or supplemented with 10% flaxseed (CF), 5% flaxseed (CF5), 1% flaxseed (CF1), or 5% coconut oil (CS) for 24 wk. LDLrKO mice fed a cholesterol-supplemented diet exhibited a rise in plasma cholesterol without a change in triglycerides and an increase in atherosclerotic plaque formation. The CS mice exhibited elevated levels of plasma cholesterol, triglycerides, and saturated fatty acids and an increase in plaque development. Supplementation of the cholesterol-enriched diet with 10% (wt/wt) ground flaxseed lowered plasma cholesterol and saturated fatty acids, increased plasma ALA, and inhibited plaque formation in the aorta and aortic sinus compared with mice fed a diet supplemented with only dietary cholesterol. The expression of proliferating cell nuclear antigen (PCNA) and the inflammatory markers IL-6, mac-3, and VCAM-1 was increased in aortic tissue from CH and CS mice. This expression was significantly reduced or normalized when flaxseed was included in the diet. Our results demonstrate that dietary flaxseed can inhibit atherosclerosis in the LDLrKO mouse through a reduction of circulating cholesterol levels and, at a cellular level, via antiproliferative and anti-inflammatory actions.

linseed; nutrition; α-linolenic acid; cardiovascular disease; inflammation

It is becoming increasingly evident that atherosclerotic heart disease is largely attributable to factors that can be altered or prevented by lifestyle modification. Yusuf et al. (54) recently reported that >90% of heart disease can be influenced through behavior modifications such as nutritional interventions, cessation of smoking, and regular exercise. Nutritional recommendations have recently promoted the increased need to consume omega-3 fatty acids to provide cardioprotection against ischemic heart disease and significantly reduce the incidence of myocardial infarcts and stroke (19, 24, 33). The most common way to consume omega-3 fatty acids has been in the form of marine oils like fish.

Recently, flaxseed has been identified as a significant alternative source of omega-3 (n-3) fatty acids (19). Flaxseed is one of the richest sources of α-linolenic acid (ALA). ALA has been identified in several epidemiological trials as having significant beneficial effects versus heart disease (11, 12, 14, 21, 26). However, the data have been indirect, and the mechanism of action for this cardioprotection is unclear. Dietary flaxseed is also a rich source of soluble and insoluble fibers and the lignan secoisolariciresinol diglucoside (SDG). Inclusion of flaxseed or one of its derived components in the diet in animal studies has shown that flaxseed can inhibit arrhythmogenesis during ischemia-reperfusion (1), inhibit atherogenesis (39–43), and protect against vascular dysfunction during hypercholesterolemic conditions (15). Although these studies strongly support the argument that dietary flaxseed is an important antiatherogenic agent, all of these studies have used the cholesterol-fed rabbit as the model of human atherosclerotic disease. This animal model is not an ideal representation of the human atherosclerotic process. Genetically manipulated mouse models of atherosclerotic disease such as the LDL receptor-deficient (LDLrKO) mouse more closely mimic the human condition (48, 52). The LDLrKO mouse is also considered a superior model for use in dietary intervention trials compared with other mouse models, because the LDLrKO mouse only develops diet-induced atherosclerotic lesions, as opposed to the spontaneous atherosclerotic development observed in, for example, the ApoE receptor-deficient mouse (51). It is important to evaluate the effects of flaxseed in an animal model that mimics the human condition as closely as possible before costly human trials are undertaken. The purpose of this study, therefore, was to assess the antiatherogenic effects of three concentrations of dietary flaxseed in LDLrKO mice and to determine the mechanism of action of flaxseed at a cellular level.

MATERIALS AND METHODS

Animals and dietary interventions. The protocol for this study was approved by an independent Animal Use Committee at the University of Manitoba. One hundred five female C57BL/6J LDLrKO mice (Jackson Laboratory, Bar Harbor, ME), 5–7 wk old, were randomly assigned, following a 1-wk acclimatization period, to 7 dietary treatment groups of 15 animals. The seven diets included a regular RMH 3000 rodent chow (TestDiet, Richmond, IN) diet (RG), a 10% ground...
flaxseed (Promega Flax; Polar Foods, Fisher Branch, MB, Canada)-supplemented chow diet (FX), or an atherogenic chow diet supplemented with 2% cholesterol alone (CH) or supplemented with 10% ground flaxseed (CF), 5% ground flaxseed (CF5), 1% ground flaxseed (CF1), or 5% coconut oil (CS). The Promega flaxseed contained 71% ALA. Mice were given 4 g of one of the seven diets daily. Water was provided ad libitum. The mice were housed in plastic cages (maximum 5 animals per cage) in a room with controlled temperature, humidity, and a 12-h light cycle. Guidelines for the ethical care and treatment of animals from the Canadian Council on Animal Care were strictly followed (34).

The nutritional composition of the experimental diets was analyzed by Norwest Laboratories (Lethbridge, AB, Canada) for proximate analysis of crude protein, carbohydrate, fat, fiber, ash, and digestible energy. Lipids were extracted by chloroform-methanol from a 1-g sample of ground diet by using the Folch method (18). Fatty acids were esterified into their corresponding methyl esters with the use of an acetylchloride-methanol-benzene solution as described previously (15, 27). Subsequent analysis by gas chromatography with flame ionization detection yielded the amounts of fatty acid methyl esters quantitatively. The fatty acid content of the samples was identified by comparison with authentic standards (NuChek Prep, Elysian, MN).

**Blood and tissue collection.** Following 24 wk of dietary intervention, plasma was collected and stored at −80°C until analyzed for fatty acid, triglyceride, and cholesterol content. Total fatty acids were extracted from the plasma samples and derivatized as described above to assess the circulating fatty acid profiles of the animals. Plasma triglyceride and cholesterol levels were quantified using commercial enzymatic kits (Thermo Electron).

**Assessment of atherosclerotic lesion formation.** The aorta was cleaned of adventitial tissue and washed in cold PBS solution before the tissue was evaluated for atherosclerotic lesions by en face and cross-sectional analysis. For en face analysis, the aorta from the ascending arch to the iliac bifurcation was cleaned of peripheral tissue, opened longitudinally, pinned flat, and digitally photographed, and the luminal images were analyzed using Silicon Graphics Imaging (SGI) software. The lesion area index was calculated as the ratio of areas with lesions versus total luminal surface area × 100 (mean ± SE). The aortic tissues were subsequently flash frozen and stored at −80°C until used for Western blots.

The heart samples were embedded in tissue freezing medium (O.C.T. compound), frozen at −20°C, and sectioned using a cryostat and were thaw-mounted onto positive glass slides. The entire aortic sinus was recognized by the disappearance of the three aortic valve cusps as previously described (7, 36). Every sixth section was stained with Oil red O and hematoxylin, digitally photographed under ×40 magnification, and evaluated for atherosclerotic lesions with a morphometric imaging system. The measurements were expressed in arbitrary units (pixels). The lesion area index was calculated as the percentage of aortic lumen area covered by atherosclerotic lesions (mean ± SE).

**RESULTS**

**Experimental diets.** The nutritional composition of the various diets used in this study was evaluated. As shown in Table 1, the nutritional content of the experimental diets reported was calculated based on the SDG content of the flaxseed multiplied by the amount of flaxseed used in the study.

**Western blot analysis.** The expression levels of proliferating cell nuclear antigen (PCNA), the macrophage marker M3/84 (mac-3), interleukin-6 (IL-6), vascular cell adhesion molecule-1 (VCAM-1), and peroxisome proliferative-activated receptor-γ (PPARγ) were measured using Western immunoblotting techniques. Frozen aortas were homogenized using a mortar and pestle and liquid nitrogen. The homogenates were lysed with RIPA buffer (50 mM Tris, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 1 mM EDTA, and 1 mM EGTA, pH 7.5, with 1 mM PMSF, 1 mM benzamidine, and a protease inhibitor cocktail) and centrifuged at 14,000 g for 15 min at 4°C to remove cellular debris. Aliquots of lysates were analyzed by SDS-PAGE electrophoresis, and proteins were transferred onto nitrocellulose membranes using either a wet or semidyed transfer protocol. The membranes were then blocked and probed with the following primary antibodies: anti-PCNA (1:2,000 dilution; 13-3900, Zymed Laboratories), anti-mac-3 M3/84 (1:100 dilution; sc-19991, Santa Cruz Biotechnology), anti-IL-6 (1:500 dilution; MAB406, R&D Systems), anti-VCAM-1 (1:500 dilution; sc-1504, Santa Cruz Biotechnology), and PPARγ (1:500 dilution; sc-7196, Santa Cruz Biotechnology). The membranes were incubated with an appropriate horseradish peroxidase-conjugated secondary antibody, and the signal was developed using West Pico chemiluminescence substrate (Pierce) and quantified by densitometric analysis using Quantity One software on a Bio-Rad GS-670 imaging densitometer. Equal protein loading and transfer were verified using Coomassie blue and Ponceau staining. Protein levels were normalized against total actin (Sigma) expression and are represented as percentages of the control (RG) group (arbitrary unit).

**Immunohistochemistry.** Aortic arch cross sections were immunostained with antibodies against mac-3 M3/84 (1:50 dilution), IL-6 (1:50 dilution), and PCNA (1:50 dilution). After washing, sections were incubated with anti-rat ( Sigma) and anti-mouse (Chemicon) horseradish peroxidase-conjugated secondary antibodies at 1:200 dilutions. Immunocomplexes containing mac-3, IL-6, and PCNA antibodies were detected using diaminobenzidine tetrahydrochloride dihydrate substrate (DAB, Sigma). A brown to black precipitate was indicative of the presence of mac-3, IL-6, or PCNA. Negative controls were performed in the absence of both primary and secondary antibodies as well as the DAB substrate. Adventitial tissue surrounding the exterior of aortic sections was also detected by DAB staining. Sections were mounted in Permount and digitally photographed using a Nikon microscope under ×20 magnification.

**Statistical analysis.** Data are means ± SE. Statistical comparisons were made using one-way analysis of variance, followed by Fisher’s least significant difference test for multiple parametric comparisons using SigmaStat software. Differences between means were considered significant when P < 0.05.

**RESULTS**

Experimental diets. The nutritional composition of the various diets used in this study was evaluated. As shown in Table 1, the nutritional content of the experimental diets reported was calculated based on the SDG content of the flaxseed multiplied by the amount of flaxseed used in the study.

**Table 1. Nutritional composition of experimental diets**

<table>
<thead>
<tr>
<th>Diets</th>
<th>Group</th>
<th>Ash, %</th>
<th>Protein, %</th>
<th>Fiber, %</th>
<th>Fat, %</th>
<th>CHO, %</th>
<th>ME, kcal/g</th>
<th>SDG, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular</td>
<td>RG</td>
<td>7.5</td>
<td>25.4</td>
<td>4.5</td>
<td>7.1</td>
<td>55.8</td>
<td>3.61</td>
<td>1.37</td>
</tr>
<tr>
<td>10% Flaxseed</td>
<td>FX</td>
<td>6.8</td>
<td>25.0</td>
<td>4.3</td>
<td>10.4</td>
<td>53.5</td>
<td>3.81</td>
<td>0.69</td>
</tr>
<tr>
<td>2% Cholesterol</td>
<td>CH</td>
<td>7.2</td>
<td>24.0</td>
<td>4.4</td>
<td>8.6</td>
<td>55.8</td>
<td>3.70</td>
<td>0.69</td>
</tr>
<tr>
<td>2% Cholesterol + 10% flaxseed</td>
<td>CF</td>
<td>6.5</td>
<td>25.8</td>
<td>4.2</td>
<td>12.5</td>
<td>51.0</td>
<td>3.93</td>
<td>1.37</td>
</tr>
<tr>
<td>2% Cholesterol + 5% flaxseed</td>
<td>CF5</td>
<td>6.9</td>
<td>24.1</td>
<td>3.9</td>
<td>10.5</td>
<td>54.6</td>
<td>3.82</td>
<td>0.69</td>
</tr>
<tr>
<td>2% Cholesterol + 1% flaxseed</td>
<td>CF1</td>
<td>6.3</td>
<td>24.8</td>
<td>4.1</td>
<td>9.2</td>
<td>55.1</td>
<td>3.75</td>
<td>0.14</td>
</tr>
<tr>
<td>2% Cholesterol + 5% coconut oil</td>
<td>CS</td>
<td>6.5</td>
<td>23.2</td>
<td>3.8</td>
<td>13.6</td>
<td>52.9</td>
<td>3.98</td>
<td>0.14</td>
</tr>
</tbody>
</table>

CHO, carbohydrates; ME, metabolic energy; SDG, the lignan secoisolariciresinol diglucoside. The Promega flaxseed contained 13.73 mg/g SDG. The lignan content of the experimental diets reported was calculated based on the SDG content of the flaxseed multiplied by the amount of flaxseed used in the study.
the ash, protein, fiber, and carbohydrate content of all the diets was controlled and similar among the groups. The metabolic energy levels within the diets were also similar among the groups. The lipid content was slightly higher in the flax-fed diets. The coconut oil-supplemented group was created to provide an internal control for this enhanced lipid load in the diet. SDG is the principal lignan found in flaxseed. As expected, the SDG concentration was lower as the content of flaxseed in the chow decreased.

The inclusion of flaxseed in the mouse diet resulted in significantly higher levels of ALA (C18:3 n-3) and reduced levels of linolenic acid (LA; C18:2 n-6) in the chow compared with the control, regular mouse chow (Fig. 1). As expected, these changes were graded by the amount of flaxseed included in the diet. The cholesterol-supplemented diet contained no changes in either fatty acid, unless flaxseed was also included in the mouse chow. The coconut oil-supplemented diet had a significantly lower content of LA but no change in ALA content compared with the control chow.

Circulating lipid profiles. Differences in the plasma fatty acid profile of the animals following the 24-wk feeding period were observed (Fig. 2). The flaxseed-enriched diets induced significant increases in the plasma ALA and eicosapentaenoic acid (EPA; C20:5 n-3) levels and reduced the arachidonic acid (AA; C20:4 n-6) levels compared with control. Supplementation of the diet with cholesterol resulted in increased LA levels without changes in the other fatty acid species. The addition of flaxseed to the cholesterol-enriched diet partially mitigated the cholesterol-induced rise in the plasma LA content and elevated the plasma ALA levels beyond what was observed with flaxseed feeding alone. The extent of the change in these plasma fatty acids was dependent on the concentration of flaxseed in the chow. All of the dietary treatments had no impact on docosahexaenoic acid (DHA; C22:6 n-3) levels in the plasma. The addition of coconut oil in the diet had no effect on plasma fatty acids compared with the changes observed in the cholesterol-fed group. The extent of the change in these plasma fatty acids was dependent on the concentration of flaxseed in the chow.

As expected, the inclusion of cholesterol in the diet induced a significant increase in plasma cholesterol (Fig. 3A). Flaxseed on its own did not alter plasma cholesterol levels compared with control levels, but when cholesterol was included in the diet, flaxseed mitigated this hypercholesterolemic effect in a concentration-dependent manner. Coconut oil did not have a cholesterol-raising effect beyond that seen with cholesterol feeding alone. However, this dietary intervention was the only approach that induced a significant increase in plasma triglyc-
extend the rise in plasma SFA levels observed with cholesterol feeding. The addition of flaxseed to the atherogenic diets partially mitigated the effects of cholesterol feeding on plasma SFA levels in a concentration-dependent manner.

**Dietary flaxseed prevents atherosclerotic development.** The inclusion of cholesterol in the diet of the LDLrKO mice induced a significant atherogenic action compared with the control diets. Representative results are shown in Fig. 4. Flaxseed included in the diet with cholesterol demonstrated a protective effect (Fig. 4D). The results from many animals were pooled and are shown in Fig. 5. Mice fed a control diet or one supplemented with flaxseed did not exhibit appreciable atherosclerotic plaque formation. However, a cholesterol-enriched diet induced plaque coverage to ~20% of the luminal surface of the aortic vessel. Flaxseed inhibited this atherogenesis in a dose-dependent manner. Coconut oil added to the diet also induced atherosclerotic plaque formation to the same degree as the cholesterol-enriched diet.

Cross-sectional analysis of atherosclerotic development at the aortic sinus revealed lipid deposition with the aid of Oil red O staining. Representative images are shown in Fig. 6. Little lipid deposition was detected in control tissue (Fig. 6A) and in animals fed a flaxseed diet (Fig. 6B). However, cholesterol supplementation to the diet induced extensive lipid deposits (Fig. 6C) that were reduced by the inclusion of flaxseed in the diet (Fig. 6D). The extent of atherosclerotic development at the aortic sinus was quantified as a percentage of aortic cross-sectional luminal area occupied by Oil red O-stained lipid deposits \((n = 6–10)\): RG, 10.51 ± 1.85%; FX, 13.77 ± 1.78%; CH, 55.87 ± 1.43%; CF, 50.37 ± 2.05%; CF5, 48.15 ± 1.94%; CF1, 51.60 ± 1.29%; and CS, 51.36 ± 1.67%. Atherosclerotic lesions at the aortic sinus were extensive following cholesterol feeding. The addition of 5 and 10% flaxseed to an atherogenic diet partially inhibited the development of atherosclerotic lesions at the aortic sinus.

**Antiproliferative and anti-inflammatory actions of dietary flaxseed.** Cell proliferation is associated with atherosclerotic plaque development. PCNA can be used as an independent marker of cell proliferation within the vessel wall (13, 16, 35, 56). PCNA expression was increased in aortic tissue obtained from mice fed the cholesterol-supplemented diet compared with control (Fig. 7), as detected by Western blots. Flaxseed supplementation on its own did not alter PCNA expression, but when included with cholesterol, flaxseed was capable of inhibiting cellular proliferation in a dose-dependent manner. Coconut oil in the diet also significantly stimulated cell proliferation. Levels of PPARγ expression in aortic tissue of LDLrKO mice were not affected by cholesterol, saturated fat, or flaxseed supplementation (results not shown).

Because inflammation is now considered to be an important mechanistic process within atherosclerosis, markers of inflammation were also examined as a function of the dietary interventions. The macrophage marker mac-3 has been used as an indicator of inflammatory reactions associated with atherosclerosis (55, 58). As shown in Fig. 8, mac-3 expression was increased significantly in aortic tissue obtained from mice fed cholesterol- or coconut oil-enriched diets. Including flaxseed in the cholesterol-supplemented diet significantly inhibited mac-3 expression in a dose-dependent manner.

Inflammation in the aortic tissues was also examined using the proinflammatory cytokine IL-6. Western blot analysis re-
revealed that IL-6 expression was increased in aortic tissue obtained from mice fed the cholesterol-supplemented diet and the cholesterol- and coconut oil-supplemented diet compared with control (Fig. 9). Flaxseed supplementation on its own did not alter IL-6 expression, but when included with cholesterol, flaxseed in the two highest concentrations (5 and 10%) was capable of mitigating the effects of cholesterol and coconut oil on IL-6 expression.

The effects of dietary flaxseed on the inflammatory and atherogenic marker VCAM-1 are shown in Fig. 10. VCAM-1 expression was significantly increased in aortic tissue from mice consuming cholesterol- or coconut oil-enriched diets. The addition of flaxseed to an atherogenic diet prevented the cholesterol- and saturated fat-induced rise in aortic VCAM-1 expression in a dose-dependent manner.

The expression of markers of proliferation and inflammation was confirmed in cross sections at the aortic sinus. Representative images from the RG, FX, CH, and CF groups are shown in Fig. 11. Little antibody staining was detected in the RG group. Flaxseed on its own had no effect on mac-3, IL-6, and PCNA expression. Abundant immunoreactivity for mac-3 (Fig. 11A) was detected throughout atherosclerotic lesions from LDLrKO mice fed a cholesterol diet, whereas IL-6 (B) and PCNA (C) expression predominated in the innermost region of atheromas. IL-6 staining was also detected within the media layer of aortic cross sections from the cholesterol diet group. The addition of dietary flaxseed to an atherogenic diet reduced the expression of mac-3, IL-6, and PCNA in aortic atherosclerotic lesions in LDLrKO mice compared with cholesterol feeding alone.

**DISCUSSION**

The dietary interventions used in this study induced significant changes in the plasma fatty acid profile of the LDLrKO mice. Dietary flaxseed increased plasma ALA levels significantly, as would be expected due to the high ALA content of flaxseed. ALA was metabolized in the mice to the longer chain omega-3 fatty acid EPA, but not to DHA. This differs from the inability of rabbits to metabolize ALA derived from dietary flaxseed to the longer chain omega-3 fatty acids (1, 15). The inclusion of cholesterol in the diet with flaxseed resulted in a significant stimulation of ALA levels in the plasma of the LDLrKO mice. This is similar to the response observed previously and likely represents an enhanced absorption of the fatty acid in the gastrointestinal tract (1, 15). This stimulatory effect of dietary cholesterol on the entry of ALA into the plasma was not saturated, because there was a clear dose-dependent rise in ALA with increasing flaxseed concentrations (1–10%) despite the cholesterol level in the mouse chow remaining the same. It is also interesting to note that this stimulatory effect was relatively specific to the medium-chain PUFA species, since AA, EPA, and DHA were not increased in the plasma when cholesterol was present in the diet.

The results of the present study demonstrate the antiatherosclerotic effects of dietary flaxseed in the LDLrKO mouse. This antiatherogenic action of flaxseed has been shown previously in the cholesterol-fed rabbit model (15, 39–43). However, in view of the limitations of the cholesterol-fed rabbit model of atherosclerosis (3, 32), it was important to confirm these findings in a model that more closely represents atherosclerosis in humans. Our data in the LDLrKO mice now support the direct evaluation of the antiatherogenic potential of dietary flaxseed in human trials where the effects of flaxseed on cardiovascular disease are not clear. Flaxseed supplementation (20–50 g/day) has been demonstrated to modestly reduce circulating total and LDL cholesterol levels and to have no effect on HDL levels in healthy people (5, 6, 23, 28, 29). Our results in the LDLrKO mouse agree with this cholesterol-lowering effect observed in humans but are in conflict with the results obtained in the cholesterol-fed rabbit, where flaxseed...
did not lower circulating cholesterol levels (15). This would again argue for the importance of using the data obtained in the LDLrKO mice as a template for what may occur in the future during any dietary studies with flaxseed in humans. It is important to note, however, that lipoprotein distribution and metabolism may differ between rodents and humans, since circulating cholesterol in mice is predominantly carried by the HDL lipoprotein.

It is evident from the results of this study that the effects of flaxseed on circulating cholesterol and atherosclerosis are sen-

![Fig. 6. Dietary flaxseed reduces atherosclerosis in LDLrKO mice. Representative images of cross sections taken of the aortic sinuses obtained from LDLrKO mice fed a RG (A), FX (B), CH (C), or CF diet (D). The sections were stained with Oil red O for lipid deposition (red) and cross-stained with hematoxylin (blue).](image)

![Fig. 7. Dietary flaxseed prevents the cholesterol-induced rise in cellular proliferation in atherosclerotic aortic tissues. Expression of proliferating cell nuclear antigen (PCNA) in aortic tissue was detected by Western blotting as a function of the various dietary interventions. Values are means ± SE; n = 7–8. A representative image of PCNA expression and total actin as a loading control is displayed at top. *P < 0.05 vs. RG and FX groups.](image)

![Fig. 8. Dietary flaxseed prevents macrophage infiltration into atherosclerotic aortic lesions. Expression of mac-3 in aortic tissue was detected by Western blotting as a function of the various dietary interventions. Values are means ± SE; n = 7. *P < 0.05 vs. RG and FX groups. †P < 0.05 vs. CH and CS groups.](image)
sitive to the dosage of flaxseed employed. A 10-fold reduction in the concentration of flaxseed given to the LDLrKO mice (1–10%) did not significantly reduce its capacity to lower circulating cholesterol levels, but it did reduce the ability of flaxseed to inhibit atherosclerotic development. This was similar when the flaxseed dosage was reduced by 50%. It appears that cholesterol levels must be lowered below a certain threshold level to yield an antiatherogenic effect. A 10% flaxseed-supplemented diet is similar in energetic load to the 50 g/day dosage used in human trials (6, 23). This may suggest that flaxseed dosages near 50g/day may be required to inhibit atherosclerosis significantly in humans. Of course, this still requires direct evaluation in human trials, but it does provide a useful starting point.

The mechanism for the antiatherogenic action demonstrated by dietary flaxseed was investigated in the present study. The cholesterol-lowering effect of flaxseed is likely the main contributing factor to its antiatherogenic potential; however, since atherosclerosis was only inhibited in animals fed a higher flaxseed dose, another mechanism, likely cellular, may be responsible for this antiatherogenic action. Our data reveal for the first time a significant antiproliferative and anti-inflammatory action of flaxseed. Atherogenesis is thought to involve an inflammatory reaction (49) and accelerated cell proliferation in the region of the obstructive plaque (35, 46). IL-6 has been implicated in the pathogenesis of atherosclerosis, including smooth muscle cell and fibroblast proliferation, oxidation of LDL cholesterol, activation of monocytes and macrophages, and amplification of the inflammatory cascade in atherogenesis (2, 22, 50). VCAM-1 has been associated with key steps in atherogenesis, including monocyte recruitment and infiltration into the arterial wall and differentiation into macrophages. Flaxseed effectively inhibited the expression of inflammatory markers such as IL-6, mac-3, VCAM-1, and the proliferative marker PCNA. Our data demonstrate for the first time that dietary flaxseed reduces the infiltration of macrophages into the subendothelial space and reduces the inflammatory and proliferative state of atherosclerotic lesions. These effects are likely due to the omega-3 fatty acid content of flaxseed. Dietary supplementation with ALA from flaxseed oil has been demonstrated to reduce circulating levels of several atherogenic and inflammatory markers, including C-reactive protein, serum amyloid A, IL-6, and soluble VCAM-1 in dyslipidemic patients (44, 45). Omega-3 PUFAs (ALA, EPA, and DHA) have demonstrated several direct antiatherogenic properties, including anti-inflammatory (9, 44) and immunomodulatory effects (10, 30, 57), as well as the ability to inhibit leukocyte adhesion (8, 10), decrease the production of proinflammatory eicosanoids, and inhibit cellular migration and proliferation (31, 37, 38, 47). The antiatherogenic effects described in this study may also be associated with the low omega-6-to-omega-3 fatty acid ratio in the plasma of the flaxseed-fed groups. Previous reports have shown that a low omega-6-to-omega-3 fatty acid ratio is associated with low levels of circulating inflammatory markers (17), decreased production of proinflammatory eicosanoids (4, 25), and reduced atherosclerotic development (53).

In summary, dietary flaxseed can inhibit the atherogenic effects of a high-cholesterol diet in the LDLrKO mouse. The present investigation demonstrated that this effect was achieved through a capacity to lower circulating cholesterol levels and, at a cellular level, by inhibiting cell proliferation and inflammation. This lends further support to the hypothesis that nutritional interventions have the capacity to alter disease through an anti-inflammatory action (20). Because this antiatherogenic effect of flaxseed has now been shown in more than one animal model, and because the LDLrKO mouse is a close representation of the clinical condition of coronary heart disease in humans, our study argues strongly for the initiation of careful, randomized controlled trials of dietary flaxseed in a patient population with symptoms of atherosclerotic heart disease.

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