High-Fat, High-Cholesterol Diet Increases the Incidence of Gastritis in LDL Receptor–Negative Mice

Aino Laurila,* Sheri P. Cole,* Shiva Merat, Marygorret Obonyo, Wulf Palinski, Joshua Fierer, Joseph L. Witztum

Abstract—Transgenic and knockout mice are widely used as models for atherogenesis studies. While developing a Helicobacter infection model in LDL receptor–negative (LDLR−/−) mice, we noticed that mice fed a high-fat, high-cholesterol diet often contracted gastritis independent of infection. To further investigate this finding, we studied 27 male and 18 female LDLR−/− mice fed high-fat, 1% or 1.25% cholesterol diets for 3 to 4 months. The extent of atherosclerosis was morphometrically analyzed in the whole aorta, and the degree of gastric inflammation was scored histologically in hematoxylin-eosin–stained stomach sections. The autoantibody titers to epitopes of oxidized LDL were also measured. Mice fed high-fat, high-cholesterol diets had a significantly higher incidence of gastritis than mice fed normal chow, 62% versus 5%, respectively (P<0.0001). This effect was specific for LDLR−/− mice, because no difference in gastritis was found in wild-type mice fed either diet. Animals with gastritis showed slightly more atherosclerosis than animals without gastritis: 16.3±6.4% versus 12.8±3.4% in males and 9.4±3.5% versus 6.5±3.3% in females. Cholesterol-fed mice also had significantly higher IgG autoantibody titers against modified LDL than normal chow–fed animals, but no difference was seen between the gastritis and nongastritis groups. We conclude that the standard high-fat, high-cholesterol diet commonly used in many murine models to induce atherosclerosis increased the incidence of gastritis significantly in LDLR−/− mice. (Arterioscler Thromb Vasc Biol. 2001;21:991-996.)

Key Words: inflammation ▪ atherosclerosis ▪ gastritis

Mice are rapidly becoming a preferred model for studies of atherosclerosis. Most wild-type mice are generally resistant to hypercholesterolemia and atherogenesis, even when placed on a high-fat, high-cholesterol diet. C57BL/6 mice, however, have an increased susceptibility to atherosclerosis,1 and a large number of transgenic and gene-knockout models based on this genetic background develop substantial hypercholesterolemia and atherosclerosis.2 In these models, the development of transitional and advanced atherosclerotic lesions in the aortic origin and throughout the aorta is generally dependent on prolonged feeding of high-fat, high-cholesterol diets. For example, LDL receptor–negative (LDLR−/−) mice fed normal murine chow develop plasma cholesterol levels of approximately 250 mg/dL and limited atherosclerosis, whereas high-cholesterol diets induce cholesterol levels >1200 mg/dL and extensive atherosclerosis throughout the aortic tree.3,4 In contrast, apolipoprotein (apo) E–deficient (apoE−/−) mice spontaneously develop substantial hypercholesterolemia and atherosclerosis even on a normal chow diet.5,6 Nevertheless, many studies in apoE−/− mice use “Western” diets with increased cholesterol content to further raise plasma cholesterol levels and accelerate lesion formation.3,7-9

Mice fed high-cholesterol diets have been used extensively to study atherogenic mechanisms of apoproteins and lipoproteins, lipoprotein and scavenger receptors, the role of the immune system, and the effects of diabetes.2 A number of observations have been made, however, that may complicate the interpretation of results from these studies. One such observation is the fact that high-fat diets induce not only hypercholesterolemia but also insulin resistance in LDLR−/− mice.10 In the present article, we report another potentially confounding effect associated with high-fat diets.

Helicobacter pylori infection (and subsequently gastritis) was recently associated with cardiovascular diseases,11,12 although the link is controversial. During a preliminary study attempting to develop a murine model in which the influence of Helicobacter infection on atherogenesis could be investigated, we noticed that LDLR−/− mice fed high-fat, high-cholesterol diets more frequently showed signs of gastritis than mice fed standard chow, independently of Helicobacter infection. Because atherosclerosis has many characteristics of an inflammatory disease, it might be anticipated that any chronic inflammation might lead to systemic effects that could accelerate the atherogenic process. Therefore, we decided to test the hypothesis that gastritis would accelerate...
the development of atherosclerosis and could in part explain some of the variability in the lesion formation between individual animals in many previous reports.

Method

Animals and Diets

Study 1

In each of 3 separate experiments, we used fifth-generation LDLR−/− mice (C57BL/6J×129Sv) from a breeding colony established from animals originally obtained from Jackson Laboratories (Bar Harbor, Me). In a preliminary experiment, sixteen 3-month-old female LDLR−/− mice were fed a high-fat, 1.25% cholesterol diet (TD 92121, Harlan Teklad) for 8 weeks. Eight of the mice were infected with Helicobacter felis, a strain known to infect and induce gastritis in mice.13 An additional 8 noninfected female mice of the same age were fed normal chow (Rodent Diet 8604, Harlan Teklad). The composition of the diets is presented in Table 1. Although the infection was unsuccessful, as based on urease breath test, histology, and bacterial culture, we noticed that the incidence of gastric inflammation was high in the noninfected cholesterol-fed animals compared with normal chow–fed animals.

Study 2

To further investigate this unanticipated finding, we examined a group of male LDLR−/− mice that were available from another ongoing study. In that study, 27 animals had been fed a high-fat, 1% cholesterol diet (TD 95286, Harlan Teklad) for 4.5 months. Twenty controls had been fed normal chow.

Study 3

In addition, we carried out a third prospective study using twenty-six 8- to 9-month-old female LDLR−/− mice. Of these, 18 were fed the high-fat, 1.25% cholesterol diet and 8 the normal chow for 3 months. In all 3 studies, mice were weighed and 100-μL blood samples were obtained by retro-orbital bleeding at 4- to 6-week intervals. Total plasma cholesterol and triglyceride levels were determined by an enzyme assay kit (Boehringer Mannheim Diagnostics). In study 1, the animals were euthanized at age 4 to 5 months, in study 2 at age 7 to 8 months, and in study 3 at age 11 to 12 months.

Study 4

To determine whether cholesterol-induced gastritis was specific for LDLR−/− mice, wild-type C57BL/6 mice were also fed high-fat, high-cholesterol diets in a control experiment. Ten female mice were fed the high-fat, 1.25% cholesterol diet (TD92121) and 10 mice were fed normal chow for 3.5 months.

For all of these studies, mice were housed in separate groups, ≤4 to a cage, on a 12-hour light cycle and with access to food and water ad libitum. Mice were killed and the stomachs processed as described below.

Morphometric Determination of Atherosclerosis

The morphometric analysis of atherosclerosis was performed as previously described.5 In brief, anesthetized mice were killed by exsanguination from the inferior vena cava. The systemic circulation was perfused with PBS containing EDTA through a needle in the left ventricle until the effluent was free of blood and then fixed by perfusion with 4% paraformaldehyde–5% sucrose solution for 5 minutes. The aorta was cleaned, opened, pinned on a black wax surface, and stained with Sudan IV as described earlier.9 The relative aortic surface area covered by atherosclerotic lesions was measured by computer-assisted image analysis as described.4 Atherosclerosis was determined in 10 cholesterol-fed mice from study 2 and in all mice from the prospective study 3.

Evaluation of Gastric Inflammation

The stomach was removed, split longitudinally, and fixed for 24 hours with paraformaldehyde. Fixed tissue was embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The presence of inflammation was analyzed on whole longitudinal sections of the stomach.13 Three separate characteristics were evaluated, and each was graded on a scale of 0 to 3. The characteristics were (1) the number of inflammatory cells, (2) the depth of the inflammation in the mucosa, and (3) the extent of inflammation in different parts of the stomach, ie, antrum, fundus, and nonglandular area. The scores were added to obtain a single “inflammation score” for each animal. A score of 5 shows a clinically significant degree of inflammation and corresponds to a histological diagnosis of gastritis. For statistical analysis, the animals were therefore divided according to the inflammation score into a “gastritis” (score ≥5) and a “nongastritis” (score <5) group. In addition, absolute inflammation scores for each animal were also used for some analyses.

Measurement of Antibodies Against Oxidation-Specific Epitopes

The binding of circulating IgG, IgA, and IgM autoantibodies to malondialdehyde-modified LDL (MDA-LDL), a model epitope of oxidized LDL, was measured as previously described, with a plasma dilution of 1:250.14 IgG and IgM antibodies were measured from the end-point samples of studies 2 and 3. IgA antibodies were measured from 15 cholesterol-fed mice, from 7 control mice in study 2, and from all animals in study 3.

Statistical Analysis

ANOVA was used to compare the means of variables between the high-cholesterol diet and normal chow groups. Mann-Whitney U test was used to analyze the data in the 2 gastritis groups (gastritis, score ≥5 and nongastritis, score <5) in studies 2 and 3. Bivariate correlations were examined by Pearson correlation coefficient. Multiple regression analysis was used to evaluate the influence of sex, plasma cholesterol levels, and the degree of inflammation on the development of atherosclerosis.

Results

Effects of Diets on Gastritis

The gastric mucosa of each animal was examined histologically and assigned an inflammatory score, as described in Methods. The young control animals fed normal chow in study 1 showed no sign of inflammation in the gastric mucosa. Figure 1A provides an example of the normal gastric mucosa with essentially no inflammatory cells and normal submucosa and muscle wall. The inflammatory score would be 0 for this animal. The gastric sections of older control animals in studies 2 and 3 frequently displayed some lymphocytes and plasma cells in the mucosa, especially at the border of the fundus and nonglandular area, and were scored from 1 to 4. A comparable slight increase in cell number with increasing age is often found in human samples.15 In cholesterol-fed animals, a variable but generally much higher

### Table 1. Composition of High-Fat, High-Cholesterol Diets and Normal Chow

<table>
<thead>
<tr>
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<th>High-Fat, 1% Cholesterol</th>
<th>High-Fat, 1.25% Cholesterol</th>
<th>Normal Chow</th>
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<tbody>
<tr>
<td>Protein</td>
<td>245.0</td>
<td>195.0</td>
<td>150.0</td>
</tr>
<tr>
<td>Fat</td>
<td>44.0</td>
<td>341.5</td>
<td>150.0</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>10.0</td>
<td>341.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Fiber</td>
<td>41.5</td>
<td>210.0</td>
<td>39.0</td>
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Values are grams per kilogram.
A number of inflammatory cells was found throughout the gastric mucosa. Figure 1B shows an example of gastric inflammation with abundant accumulation of inflammatory cells throughout the mucosa and extending into the submucosa. The micrograph represents a stomach section that was categorized as 7. Cell infiltrates consisted mainly of lymphocytes, with some plasma cells and neutrophils. In some animals, even more severe gastritis with frank ulcerations was found, as shown in Figure 1C, scored as 9.

The degree of gastric inflammation in individual animals expressed as the inflammation score is shown in Figure 2. In each study, the incidence of gastritis (score ≥5) was significantly higher in animals fed a high-cholesterol diet than in animals given normal chow. In study 1, 37% (3/8) of the cholesterol-fed group had an inflammation score ≥5, compared with 0% (0/8) of the control animals (P<0.05). In study 2, 63% (17/27) of cholesterol-fed animals had gastritis, versus 10% (2/20) of the control animals (P<0.0001), and in study 3, 72% (13/18) of the cholesterol-fed animals had gastritis, versus 0% (0/8) of control animals (P<0.001). The mean inflammation scores are shown in Table 2. For all 3 studies, the average inflammation score of all cholesterol-fed animals was 5.3±2.4 and that of all control animals 2.2±1.4 (P<0.0001).

In contrast, in study 4, wild-type C57BL/6 mice fed a high-cholesterol diet had the same low gastritis scores as animals fed normal chow. Ten mice were fed a high-fat, high-cholesterol diet, and 10 control mice were fed normal chow for 3.5 months. Neither group had gastritis, with a

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**Figure 1.** H-E staining of the longitudinal stomach sections of LDLR<sup>−/−</sup> mice. A, Shows normal gastric wall of a young normal chow-fed control animal. Glands are closely packed and submucosa is clear (arrows). B, Shows a moderate gastritis in a high-fat, high-cholesterol diet-fed animal. Abundant inflammatory cells infiltrate the mucosa, widening the space between the glands (arrowheads) and extending to the submucosa (arrows). C, Shows severe gastritis with an ulceration (arrowheads) in a cholesterol-fed animal. Inflammatory cells and fibrosis extend to the submucosa (arrows). Bar=25 μm.

**Figure 2.** Distribution of gastric inflammation scores of high-fat, high-cholesterol diet-fed LDLR<sup>−/−</sup> mice and normal chow-fed control animals in the 3 studies. Bars represent the mean value in each group.
mean score of 2.4±1.5 for the control mice and 3.2±1.2 for the cholesterol-fed mice. The highest score was 5.0, which represented 1 mouse from the normal chow group. The difference between the 2 groups was not significant (P>0.1).

In all studies, the cholesterol-fed mice gained significantly more weight than the control mice (Table 2). The weight gain of the cholesterol-fed LDLR−/− mice tended to be slightly higher in animals with gastritis than in animals without gastritis, but the difference was not statistically significant (Table 3).

**Effects of Gastritis on Plasma Cholesterol Levels and Extent of Atherosclerosis**

As expected, the total plasma cholesterol levels in the LDLR−/− mice were 6 to 8 times higher in cholesterol-fed mice than in control animals (Table 2). When cholesterol-fed mice with or without gastritis were compared, the mean plasma lipid levels tended to be slightly higher in animals without gastritis than in animals with gastritis (Table 3). However, no correlation between the inflammation score and cholesterol levels was found.

Female mice of study 3 appeared to develop less atherosclerosis throughout the aorta than male mice in study 2, as shown in Table 2. In cholesterol-fed animals, the mean lesion area was 8.6% in females and 14.9% in males. This is in agreement with previous reports from our laboratory.3 In the old control female animals, the lesion area varied from 2.5% to 6.3% (mean 4.5%). In studies 2 and 3, cholesterol-fed animals with gastritis had somewhat more atherosclerosis than animals without gastritis, but the differences were not statistically significant (Table 3).

To explicitly test the hypothesis that the inflammation associated with gastritis would accelerate atherosclerosis, we examined the correlation between the degree of lesion formation and the gastric inflammation score. As shown in Figure 3, the extent of atherosclerosis did show a weak correlation with the inflammation score, consistent with the hypothesis tested (r=0.32; P=0.047, 1-tailed analysis). In stepwise multiple regression analysis, however, only sex and plasma cholesterol levels were found to be significantly correlated with the degree of atherosclerosis.

Because it is conceivable that inflammatory processes associated with gastritis lead to increased lipid oxidation and because this may modulate the titer of circulating antibodies to oxidation-specific epitopes, we also measured the binding of antibodies to MDA-LDL, an oxidation-specific epitope found during the oxidation of LDL in the dietary groups (Table 4). In study 2, IgG and IgM antibodies to MDA-LDL were significantly higher in cholesterol-fed mice than in controls. Study 3 yielded similar results, although the difference in IgM binding did not reach significance. An analogous increase in IgG and IgM antibody during increasing atherogenesis has been reported previously in mice16 and in many studies in humans with coronary artery disease.17,18 IgA antibody binding was significantly increased in the cholesterol-fed animals of study 3, but not those of study 2. When data were compared between animals with or without gastritis...
tis, however, no significant differences were found (data not shown).

Discussion

The present study provides the first evidence that a standard high-fat, high-cholesterol diet used for atherosclerosis studies in LDLR<sup>−/−</sup> mice is associated with a high incidence of gastritis in these animals. Thirty-five percent of the cholesterol-fed LDLR<sup>−/−</sup> mice developed moderate to severe gastritis within ≈3 months. Even those LDLR<sup>−/−</sup> mice that did not develop overt gastritis showed more gastric inflammation when placed on a high-fat, high-cholesterol diet (Figure 2). In contrast, feeding the same high-fat, high-cholesterol diet to wild-type C57BL/6 mice did not increase the incidence of gastritis, suggesting that hypercholesterolemia and/or the absence of LDLR plays a role in the cholesterol-induced gastritis.

LDLR<sup>−/−</sup> mice develop severe hypercholesterolemia when fed such high-cholesterol diets. In this setting, we have previously demonstrated an enhanced formation of autoantibodies to epitopes of oxidized LDL, consistent with the enhanced atherogenesis. These observations have been confirmed here and strongly suggest the enhanced presence of oxidized LDL (and/or the associated oxidized lipids) in LDLR<sup>−/−</sup> mice that are fed high-cholesterol diets. Oxidized LDL has been reported to increase the expression of nuclear factor-κB (NF-κB), a transcriptional regulator that stimulates production of a number of proinflammatory cytokines. Indeed, a number of cellular signaling molecules are upregulated in experimental hypercholesterolemia, including NF-κB. Upregulation of this DNA binding protein is also a hallmark of gastritis, resulting in the induction of proinflammatory cytokines and the recruitment of neutrophils, lymphocytes, and macrophages to the stomach mucosa. Thus, it is possible that NF-κB, or another common signaling factor, is upregulated in both the coronary vascular endothelium and gastric mucosa as a response to high cholesterol levels. Because mucosal damage in H pylori–induced gastritis is predominantly due to overstimulation of host inflammatory cells, it is likely that the relatively high levels of oxidized LDL or oxidized lipids present in the LDLR<sup>−/−</sup> mice serve as a similar chronic stimulus.

Alternatively, other components of the high-fat, high-cholesterol diet may contribute to the gastritis. As shown in Table 1, the high-fat, high-cholesterol diet differed in many respects from the control chow. Nevertheless, the high fat and/or high cholesterol content appears to be primarily responsible for the resulting gastritis. Indeed, Bagchi et al recently reported that a high-fat diet induces gastric mucosal damage in rats. They showed that the superoxide anion production in the gastric mucosa was increased 5.7-fold and lipid peroxidation 2.6-fold by the high-fat diet. In dogs, gastric pathology similar to that found in this study was attributed to irritant or toxic effects of chemicals or food supplements. The standard high-fat, high-cholesterol diet used in the present experiments, however, did not contain drugs or artificial nutrients. It was also free of cholic acid, used in many murine models in which only moderate hypercholesterolemia is achieved by dietary means. A high-fat diet may induce mucosal damage by direct chemical irritation. Moreover, it has been shown

![Figure 3. Correlation of the degree of atherosclerosis and the gastric inflammation score in high-fat, high-cholesterol–fed LDLR<sup>−/−</sup> mice (r=0.32; P<0.05).](http://atvb.ahajournals.org/)

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Values are mean±SD, expressed as relative light units/msec, as described. *One animal excluded because of out-of-bounds values. †IgA antibodies measured from 15 cholesterol-fed and 7 normal chow–fed mice. ‡P<0.05, §P<0.01, ||P<0.0001, high-fat, high-cholesterol diet vs normal chow.
that high cholesterol intake increases cholic acid synthesis and bile acid pools. 25 Conceivably, the effect might be due to bile reflux, a known cause of gastritis, 15 rather than a direct effect of lipids on the gastric mucosa.

In conclusion, the present study demonstrates that a standard high-fat, high-cholesterol diet is associated with a marked increase in gastritis in LDLR−/− mice and suggests that this may constitute a complicating factor in many studies using this model.

Acknowledgments

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References


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