Low-Cholesterol and High-Fat Diets Reduce Atherosclerotic Lesion Development in ApoE-Knockout Mice

Lucía Calleja, Miguel A. París, Antoni Paul, Elisabet Vilella, Jorge Joven, Antonio Jiménez, Gabriel Beltrán, Marino Uceda, Nobuyo Maeda, Jesús Osada

Abstract—We have investigated the effect of most common oils used in human nutrition on the development of atherosclerosis in apoE-knockout mice. Seven groups of animals, separated according to sex, were fed for 10 weeks either chow diet or the chow diet 10% (wt/wt) enriched with different oils (palm, coconut, 2 types of olive oil, and 2 types of sunflower oil) without addition of cholesterol. At the end of this period, plasma lipid parameters were measured and vascular lesions scored. None of the diets induced changes in plasma cholesterol concentrations, whereas plasma triglycerides were uniformly reduced in all diet groups. Some diets caused significant reductions in the size of atherosclerotic lesions in males and others in females; males responded most to sunflower oils and females to palm oil and one olive oil (II). The lesion reduction in males consuming sunflower oils was associated with the decrease of triglycerides in triglyceride-rich lipoproteins, whereas the decrease in females consuming olive oil II or palm oil was accompanied by an increase in plasma apoA-I. The increase in plasma apoA-I in the latter condition, is mainly due to overexpression of hepatic message elicited by a mechanism independent of apoE ligand. The data suggest that the different diets modulate lesion development in a gender specific manner and by different mechanisms and that the development of atherosclerosis, due to genetic deficiencies, may be modulated by nutritional maneuvers that may be implemented in human nutrition. (Arterioscler Thromb Vasc Biol. 1999;19:2368-2375.)

Key Words: cholesterol ■ apolipoprotein ■ atherosclerosis ■ dietary fat

ApoE is a 34-kDa glycoprotein that circulates in plasma as a component of several lipoproteins such as chylomicron remnants, IDL, VLDL, β-migrating VLDL, and HDL. It is involved in the uptake and degradation of chylomicron and VLDL remnants by the LDL receptor and the LDL receptor related protein. Besides its role in lipoprotein metabolism, other biological functions have been attributed, such as macrophage differentiation and mobilization and utilization of lipids in the central nervous system. Genetic deficiency of apoE in humans has shown accumulation of plasma remnant lipoproteins and development of atherosclerosis. ApoE-deficient mice develop severe hypercholesterolemia and atherosclerosis on a regular low-fat/low-cholesterol diet. The progression and histopathology of lesions in this animal model show similar features to those observed in humans and other species, including fatty streaks, necrotic cores, and fibrous caps.

Dietary fat is one of the most important environmental factors associated with the incidence of cardiovascular diseases. Diets high in cholesterol and saturated fat have shown to promote the development of atherosclerosis. Conversely, dietary polyunsaturated fats have shown to reduce the development of atherosclerosis in several species.

Studies dealing with the effects of monounsaturated fatty acids on lesion development are scarce. This is surprising because traditional Mediterranean diets are followed in a geographical area with a low incidence of coronary heart disease, and they combine a relatively low cholesterol content and the use of olive oil as the main source of fat. Therefore, the hypothesis that a combination of high dietary oil consumption with reduced cholesterol is protective for atherosclerosis, needs to be tested in more detail.

Due to the above described properties, apoE-knockout mice are excellent animal models for evaluating the influence of pharmacological and nutritional agents on atherosclerosis development. The absence of the apoE, specific ligand to remove remnants, adds an additional value to this model to test the influence of different nutrients on the regulation of some apolipoproteins. In this study, we report the effect of the most common oils used in human nutrition, provided in diets of low cholesterol content, on the development of atherosclerotic lesions and the regulation of apoA-I gene expression in the apoE-deficient mice.

Methods

Animals

Homozygous apoE-knockout mice, hybrids of C57BL/6J and 129 Ola strains, were progeny of those described by Piedrahita et al. and
breds in the Unidad Mixta de Investigación, Zaragoza, Spain. Seventy-seven males and sixty-three females, aged 2 months, were randomly allocated into 7 groups for each sex and housed in sterile filter-top cages. Animals had ad libitum access to food and water. Normal mouse chow was supplied by B & K Universal Ltd. Body weights were recorded throughout the experiment. None of the animals were lost during the study.

Diets

Regular mouse diet was supplemented with fat of different origins to achieve isocaloric diets, differing only in the source of fat. No extra cholesterol was provided. Experimental diets were fed for 10 weeks, achieve isocaloric diets, differing only in the source of fat. No extra cholesterol was provided. Experimental diets were fed for 10 weeks, during the study. None of the animals were lost during the study.

Diet Analysis

Diet analysis. For a more detailed analysis of the plasma lipoprotein profiles, 200 μL of pooled plasma samples from 11 males and 9 females from each dietary group, were subjected to fast protein liquid chromatography gel filtration using a Superose 6B column (Pharmacia LKB Biotechnology), according to conditions described by Vilella et al.18 Fractions (0.5 mL) were collected, and their total cholesterol and triglyceride content were measured as described above.

RNA Preparation and Analysis

At the moment of killing, livers and small intestines were obtained and quickly frozen in liquid nitrogen. RNA was isolated using Trigent reagent MRC following the manufacturer’s instructions. A pool of 5 μg of each group was denatured by 2.2 mol/L formaldehyde and 50% formamide at 68°C for 15 minutes, run on a 1% agarose gel with formaldehyde, transferred to a nylon membrane (Hybond-N, Amersham), and hybridized to probes following current protocols.19 The mouse Apoa1 probe was a 180bp Ecorl/NheI fragment corresponding to the fourth exon of the gene. A mouse G3PDH probe obtained from Ambion was used to normalize the amount of RNA loaded on the gel. Both probes were labeled using α-32P-dCTP and Rediprime (Amersham). Filters were exposed to Biomax film (Kodak, Amersham) and analyzed using Molecular Analysis (BioRad).

TABLE 1. Composition of the Experimental Diets

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>Coconut</th>
<th>Olive Oil I</th>
<th>Olive Oil II</th>
<th>Palm Oil</th>
<th>Sunflower Oil I</th>
<th>Sunflower Oil II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energetic content (kJ/g)</td>
<td>13</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
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<tr>
<td>Carbohydrate</td>
<td>57</td>
<td>51</td>
<td>51</td>
<td>51</td>
<td>51</td>
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<td>51</td>
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<tr>
<td>Protein</td>
<td>16</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
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<td>14</td>
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<tr>
<td>Fat</td>
<td>3</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Vitamin E (UI %)</td>
<td>13</td>
<td>12</td>
<td>16</td>
<td>17</td>
<td>15</td>
<td>22</td>
<td>20</td>
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<tr>
<td>Cholesterol (mg %)</td>
<td>31</td>
<td>29</td>
<td>28</td>
<td>29</td>
<td>29</td>
<td>28</td>
<td>28</td>
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<tr>
<td>Fatty acids, &lt;C12</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Myristic (14:0)</td>
<td>1.5</td>
<td>13.9</td>
<td>0.3</td>
<td>0.5</td>
<td>1.0</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Palmitic (16:0)</td>
<td>21.6</td>
<td>12.8</td>
<td>15.0</td>
<td>11</td>
<td>35.3</td>
<td>9.5</td>
<td>6.9</td>
</tr>
<tr>
<td>Margaric (17:0)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>Trace</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>7.0</td>
<td>11.6</td>
<td>2.9</td>
<td>4.5</td>
<td>5.0</td>
<td>5.1</td>
<td>4.5</td>
</tr>
<tr>
<td>Arachidic (20:0)</td>
<td>2.5</td>
<td>0.5</td>
<td>0.9</td>
<td>0.2</td>
<td>0.4</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Behenic (22:0)</td>
<td>0.9</td>
<td>0.2</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
<td>0.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Lignoceric (24:0)</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>Trace</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Palmitoleic (16:1)</td>
<td>2.2</td>
<td>0.4</td>
<td>1.4</td>
<td>0.9</td>
<td>0.5</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Margaroleic (17:1)</td>
<td>0.4</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Oleic (18:1)</td>
<td>32.2</td>
<td>8.4</td>
<td>62.3</td>
<td>69.5</td>
<td>42.6</td>
<td>26.6</td>
<td>70.5</td>
</tr>
<tr>
<td>Gadoleic (20:1)</td>
<td>0.4</td>
<td>0.2</td>
<td>0.4</td>
<td>0.2</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Linoleic (18:2n-6)</td>
<td>30.1</td>
<td>8.7</td>
<td>15.7</td>
<td>11.4</td>
<td>14.1</td>
<td>56.0</td>
<td>15.2</td>
</tr>
<tr>
<td>Linolenic (18:3n-3)</td>
<td>0.3</td>
<td>0.1</td>
<td>0.3</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Saturated</td>
<td>33.8</td>
<td>88.2</td>
<td>19.9</td>
<td>17.5</td>
<td>42.3</td>
<td>16.4</td>
<td>12.9</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>35.2</td>
<td>9.1</td>
<td>65.3</td>
<td>70.9</td>
<td>43.6</td>
<td>27.5</td>
<td>71.4</td>
</tr>
<tr>
<td>P/S ratio</td>
<td>0.9</td>
<td>0.1</td>
<td>0.8</td>
<td>0.6</td>
<td>0.3</td>
<td>3.4</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Dietary components are expressed as g% (w/w). Other components of chow diet are crude fiber (4.5%) and minerals (6.8%). A total dry matter of 87.5%. P/S ratio indicates polyunsaturated/saturated fatty acid ratio.
Evaluation of Atherosclerotic Lesions

The heart and the arterial tree were perfused with phosphate-buffered formalin (4%, pH 7.4, Panrec) under physiological pressure. Hearts and aortas were dissected, cleaned, and stored in neutral formaldehyde. The base of the hearts and the aortic roots were taken for analysis and transferred to liquid OCT (Bayer Diagnostic), where they remained for 24 hours to eliminate bubbles. Hearts were placed in new OCT on a cryostat chuck (Microm HM505E). Serial cryosections of the proximal aorta and the aortic sinus were made and stained with Sudan IV B (Sigma Chemical Co) and counterstained with hematoxylin and eosin (Sigma Chemical Co). Average lesion sizes of 4 sections, spanning the region from the very proximal aorta to the point in the aortic sinus that contains 3 complete valve leaflets, were used for morphometric evaluations based on the method of Paigen et al. Images were captured using a Leica microscope equipped with a video camera connected to a computer. Quantification of atherosclerotic lesions was performed using WinQ500C software (Leica).

Statistical Analysis

Data were analyzed using Instat for Macintosh software (GraphPad). Most of the parameters in this study did not follow normal distribution according to Shapiro-Wilk test. Therefore, analysis of statistically significant differences was carried out using Wilcoxon test for paired data and Mann-Whitney U test for unpaired data. Differences were considered nonsignificant when P > 0.05. Association between variables was assessed by Spearman’s rank-order correlation coefficient (r).

Results

Dietary Characteristics

Table 1 summarizes the results of chemical analysis of the diets. As shown in this table, chow and experimental diets were almost isocaloric. Distinctive general features among them were lower carbohydrate, higher percentage of fat, and a slight decrease in cholesterol in the experimental diets. The vitamin E content was highest in sunflower oils, at an intermediate level in olive and palm oils, and at the lowest level in chow and coconut oil diets. Chow diet presented a monounsaturated fatty acid content of 35.2% and almost equal amounts of polyunsaturated and saturated fatty acids (P/S ratio 0.9). Coconut oil was a diet with a high content of short chain saturated fatty acids, whereas palm oil contained saturated fatty acids of long chain and monounsaturated fatty acids. Olive oil I and II diets were high in monounsaturated fatty acids, differing in their percentages of saturated, mono-unsaturated, and polyunsaturated fatty acids. Sunflower oil diets differed in their content of monounsaturated (oleic acid) and polyunsaturated fatty acids (linoleic acid).

Effects of Different Diets on Body Weight and Plasma Lipoprotein Concentrations

During the 10 weeks of experimental period, both male and female animals fed chow gained an average 6 g body weight (Tables 2 and 3). In contrast, all fat-fed female groups gained significantly less weight than animals consuming regular mouse chow (Table 2). This effect was less evident in males, and only those groups consuming diets supplemented with coconut oil, olive oil II, and sunflower oil I gained significantly less weight than their littermates fed chow, olive oil I, palm oil, or sunflower oil II diets (Table 3). No significant variations in the amount of food consumption was found, with the exception of the coconut oil diet (consumed less than other diets), although the difference was not statistically significant (data not shown).

Plasma cholesterol, triglycerides, and apoA-I were determined at the beginning and at the end of the experimental period in both female and male animals, as summarized in Tables 2 and 3, respectively. In females, (Table 2) plasma cholesterol levels did not change significantly, with the exception of a significant decrease in animals fed sunflower oil I. At the beginning, males in all groups had similar cholesterol levels and, on average, 100 mg/dL higher than females, but no significant change was observed with the dietary intervention (Table 3). In contrast, all the high-fat diets induced significant decreases in plasma levels of triglycerides in both sexes compared with chow diet (Tables 2 and 3). Plasma apoA-I in the male of all groups (Table 3) showed uniform baseline levels, whereas there was a high variability among the females in different groups (Table 2). Diets supplemented with olive oil II or palm oil significantly induced their plasma apoA-I concentrations in both sexes (Tables 2 and 3), and sunflower oil I diet reduced apoA-I levels only in females (Table 2). The effect of sunflower oil was not present when it was enriched in oleic acid (sunflower oil II) (Table 2).

The distribution of cholesterol and triglycerides among the different plasma lipoproteins was analyzed by Superose 6B
Lesion (Figure 3). Taking into consideration the responses to high oleic acids presented statistically significant decreases of compared with palm oil diet, both sunflower oils with low or this decrease did not reach statistical significance. When to the supplement of fat compared with chow diet. However, males (Figure 3) showed a trend of decreased lesion sizes due oleic acid (sunflower oil II) did not have any additional effect P0.08). Enrichment of sunflower oil with significance (.

...infiltration into the intima (data not shown). There was a variability in both groups (Figures 2 and 3). In either sex, reduced them in females. An opposite trend was found in males. The triglycerides were mainly carried in TRL. Olive protein fractions in males. The distribution of triglycerides containing coconut, palm, or olive oil II decreased cholesterol in the diet is low. To address this issue and to study possible mechanisms by which dietary fat regulates cholesterol in the diet has an effect on lipid metabolism and for cardiovascular diseases, it is not well understood whether whether mRNA induction was involved in some changes in plasma apoA-I, we determined apoA-I message levels in liver and intestine by triplicate Northern blot analyses. Data in Figure 4 are expressed as arbitrary units and refer to the level of glyceraldehyde 3-phosphate dehydrogenase. When mice were fed chow diets, no differences were found between sexes in hepatic or intestinal expression, and intestinal levels were slightly lower than hepatic levels. In females, sunflower oil I diet induced a significant decrease of intestinal expres- oil I diet induced a significant decrease of intestinal expres- sion of apoA-I compared with chow (Figure 4A), whereas no significant changes by the effect of other diets were observed. In the liver, no significant change in the expression of apoA-I mRNA (Figure 4B) was seen in males. Administration of high-fat diets induced significant increases (200%) in the hepatic expression of this message in females consuming saturated and polyunsaturated diets. All these data suggest a differential response to fat in different organs in a sex-dependent manner.

**Discussion**

Although dietary fat is considered to be one of the risk factors for cardiovascular diseases, it is not well understood whether a high fat content in diet has an effect on lipid metabolism and on atherosclerotic lesion development when the amount of cholesterol in the diet is low. To address this issue and to study possible mechanisms by which dietary fat regulates such parameters, we have conducted a dietary trial in which apoE-knockout mice were given 7 different diets for 10 weeks. We found that dietary fat supplement without addition of cholesterol does not increase lesion sizes either in males or females. On the contrary, palm and olive II oils significantly reduced lesion size but only in female mice. Furthermore, different types of fat have different effects, and even a particular oil from different cultivars has different effects, as

### TABLE 3. Effects of Experimental Diets on Male ApoE-Knockout Mice

<table>
<thead>
<tr>
<th></th>
<th>Chow (n=11)</th>
<th>Coconut Oil (n=11)</th>
<th>Olive Oil I (n=11)</th>
<th>Olive Oil II (n=11)</th>
<th>Palm Oil (n=11)</th>
<th>Sunflower Oil I (n=11)</th>
<th>Sunflower Oil II (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight change, g</td>
<td>+6±2</td>
<td>+2±2†</td>
<td>+5±4</td>
<td>+3±2†</td>
<td>+6±2</td>
<td>+4±2‡</td>
<td>+5±2‡</td>
</tr>
<tr>
<td>Plasma cholesterol (mg/dL⁻¹)</td>
<td>Before diet</td>
<td>637±194</td>
<td>519±187</td>
<td>541±160</td>
<td>553±186</td>
<td>505±121</td>
<td>687±141</td>
</tr>
<tr>
<td></td>
<td>10-week diet</td>
<td>627±97</td>
<td>575±100</td>
<td>590±141</td>
<td>660±99</td>
<td>558±102</td>
<td>586±66</td>
</tr>
<tr>
<td>Plasma triglycerides (mg/dL⁻¹)</td>
<td>Before diet</td>
<td>232±35</td>
<td>328±81</td>
<td>417±121</td>
<td>218±47</td>
<td>240±79</td>
<td>247±69</td>
</tr>
<tr>
<td></td>
<td>10-week diet</td>
<td>237±55</td>
<td>124±26†</td>
<td>175±62†</td>
<td>145±40*</td>
<td>176±21</td>
<td>166±46*</td>
</tr>
<tr>
<td>Plasma apoA-I (mg/dL⁻¹)</td>
<td>Before diet</td>
<td>42±22</td>
<td>20±10</td>
<td>39±22</td>
<td>27±10</td>
<td>31±10</td>
<td>45±20</td>
</tr>
<tr>
<td></td>
<td>10-week diet</td>
<td>41±15</td>
<td>33±18</td>
<td>39±22</td>
<td>58±18†</td>
<td>49±17*</td>
<td>44±19</td>
</tr>
</tbody>
</table>

Results are expressed as means±SD. Mice were fed chow or experimental diets for 10 weeks and fasted overnight before blood collection. Statistical analysis to evaluate dietary response in each group was done using paired Wilcoxon test. *P=0.01, †P=0.002 vs initial value. Statistical analysis to evaluate body weight change was done using Mann-Whitney U test. †P=0.002, ‡P=0.03 vs chow diet.
in the case of olive oil I and II. Reduction in lesion size was independent of plasma cholesterol levels because no change in the latter was observed in any of the studied conditions, and no correlation between total plasma cholesterol levels and lesion size was found (data not shown).

Overexpression of apoA-I has been shown to reduce atherosclerosis in apoE-deficient mice. We found that the females consuming olive oil II and palm oil (the groups with significant decreases in lesions) had a significant elevation of plasma concentration of apoA-I. The decreased lesion in these groups may therefore be explained by the increase in apoA-I. However, changes in total apoA-I levels do not appear to be responsible for the sizes of lesions in other diets because a constant reduction in lesion occurred in all fat-fed groups, but no such parallel increase in plasma apoA-I was observed. Furthermore, although increase in apoA-I was present in males fed olive oil II and palm oil, it did not have the same relevance as in females regarding lesion development.

The implication of hypertriglyceridemia in atherosclerosis is a matter for open discussion because it is not easy to delineate its role without considering other related parameters. For example, in apoE-null mice made openly chylomicronemic by crossing them with mice overexpressing apoC-III, no further increase in lesion was observed. This suggests that in a dramatic lesion forming condition, as in the case of apoE knockout, additional increase in plasma triglyceride levels is not a further risk factor. The opposite situation appeared when mice lacking apoA-I were crossed with apoB-transgenic mice, in which an increase in triglycerides together with an increase in cholesterol was related to accelerated lesion development. In apoB overexpressing apoE-null mice, a negative correlation between triglyceride and lesion development was seen, although this effect was overlapped with the increase in cholesterol. A clear correlation between triglyceride levels and atherosclerotic lesion, independent of cholesterol, was present in apoE mice treated with estrogens and fed chow diet. In fact, their correlation coefficient values are similar to those obtained by us ($r_s=0.24$, $P<0.05$ for females and $r_s=0.19$, $P<0.05$ for males), suggesting that a decrease in triglyceride may be a

Figure 1. Plasma lipoprotein distribution in apoE-knockout mice after different experimental diets. Pooled plasma samples were fractionated by Superose 6B FPLC column chromatography as described in Methods and fractions analyzed for cholesterol (dotted line) or triglyceride (solid line) content. Results are shown as $\mu g$ of lipid per fraction. Triglyceride-rich lipoproteins (TRL) are found in fractions 1 to 8, remnants in fractions 9 to 13, LDL in fractions 14 to 23, and HDL in fractions 25 to 37.
direct cause of a key for the reduction of vascular lesion formation, but this contribution would not be higher than 20%. Clearly, a decrease in the amount of triglycerides in TRL, when their cholesterol is high (Figure 1), may explain the decrease in lesion observed in males consuming sunflower oils (Figure 3). The influence of this particular dietary condition and the complex genetics, as emerging from the referred animal models, might explain the controversial results obtained for triglyceride as risk factor in human studies.26–27

Plasma triglycerides at the beginning of the present study were higher than those initially described for this mouse model.5–6 Although our colony was established from 1 pair of apoE−/− mice, some of the animals that were randomly allocated into the olive oil I group were openly hypertriglyceridemic. Different authors have found both more and less intense hypertriglyceridemia in different progenies of this animal.22,28

A remarkable and surprising finding in our study is the decrease in lesions in females fed palm oil. This oil was found to increase lesion in C57BL/6J females by Nishina et al.10 The main differences between their studies and ours are that we did not add cholesterol or cholate to the diet and no plasma cholesterol change was observed by the palm oil used. Another important aspect to be considered is that we did not add cholesterol or cholate to the diet and no plasma cholesterol change was observed by the palm oil used. Additionally, the percentage of fat used (10%) in our study was lower than that used by Nishina et al10 (15%). Furthermore, we used genetically manipulated apoE−/− animals, whereas Nishina et al10 used C57BL/6J inbred mice. Lesion development in these 2 models may be influenced differently by other aspects apart from plasma lipids, such as monocyte adhesion and endothelium function,30 which can be modulated by different diets.

The decrease in body weight induced by these diets was sex-specific and more pronounced in females than in males. This sex difference was not attributable to differences in the amount of food consumed. Interestingly, we observed significant correlations (r=0.35, P<0.0026 for males and r=0.32, P<0.01 for females) between body weight and plasma triglyceride concentrations at the end of diet experiment. A fat-enriched diet with low cholesterol content therefore not only fails to induce obesity in this animal model but helps to reduce body weight. These data suggest a presence of important physiological aspects that are regulated in a gender-specific way and modulated by the dietary amount of fat.

The cellular response of hepatic apoA-I mRNA elicited by the different diets points out that remnants are taken by the liver through an apoE-independent mechanism, as suggested
by Chang et al.\textsuperscript{31–32} and Quarfordt et al.\textsuperscript{33} and poses an exciting question regarding the ligand that facilitates the uptake in these conditions. Induction of the hepatic transgene cholesterol ester transfer proteins expression in apoE-null mice in response to dietary cholesterol has been reported by Masucci-Magoulas et al.\textsuperscript{34} Their results and ours clearly show that transcription machinery in animals lacking apoE ligand is sensitive to dietary manipulations. Compared with previous results in rats that showed that a minimum threshold of 40% dietary fat was necessary to obtain significant responses of apoA-I expression,\textsuperscript{35} apoE$^{-/-}$ mice responded more acutely with lesser percentage of fat (10%, wt/wt). Our observation of the tissue expressions of apoA-I also corroborate dietary expression studies carried out by Sorci-Thomas et al.,\textsuperscript{36} who showed that hepatic expression of apoA-I is more adaptable to diet than its intestinal expression. A low-cholesterol high-fat diet increases apoA-I expression in female liver, and this effect is independent of the type of fat. This particular behavior may be specific to mice, and the response to different diets may differ among species. Indeed, a hypertriglyceridemic response to high-fat diets, with or without cholesterol,\textsuperscript{10,23} has only been shown in mice. Our findings with the apoE-knockout mice may, therefore, not be directly applicable to humans. Nevertheless, it is surprising that diets rich in polyunsaturated fatty acid from sunflower oil did not decrease hepatic expression (Figure 4). An enrichment of this type of oil with oleic acid may be crucial to maintain levels of apoA-I in plasma in both males and females. This effect was not observed between olive oil I and II, perhaps because the difference of oleic acid content between 2 olive oil diets is <10%, whereas the difference in 2 sunflower oil diets is 43%. It is also possible that other components of oils, such as sytosteroles and polyphenols, may also have influence. The sources of polyunsaturated (corn oil, sunflower oil) or monounsaturated fatty acids (olive oil, canola oil) are not often considered to be important. However, our data, analyzing apoA-I concentration as well as lesion size, clearly show that even olive oils from different cultivars are not all the same in their biological properties. They also suggest that the previous assumption of similar effects for different oils based on their fatty acid contents may be too simplistic.

In conclusion, our results in apoE-knockout mice show that a dietary intervention is a useful tool to palliate the consequences of severe genetic backgrounds. The approach, although possibly not as effective as potential gene therapies,\textsuperscript{37} is more readily adaptable in populations for controlling incidence of atherosclerosis. Our data also show that some fat diets with low cholesterol content could reduce atherosclerotic lesions and help maintain body weight in a gender-specific fashion.

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References


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