Two groups of 18 rabbits were fed isocaloric, cholesterol-enriched diets for 8 weeks. The diet for one group was supplemented with 5% corn oil. The concentration of cholesterol in plasma was determined weekly and the amount of cholesterol in the diet was adjusted individually so that each rabbit had a mean plasma cholesterol concentration of about 45 mM during the experimental period. The aortic cholesterol concentrations were 122 ± 29 and 193 ± 38 (mean ± SEM) µmol/g protein for the corn-oil group and the control group, respectively (p<0.05). In a similar experiment, each of 36 rabbits was given a mean plasma cholesterol level of about 20 mM over a period of 12 weeks. One-third of the rabbits received 10% to 15% corn oil, another third 10% to 15% olive oil, while the last third served as a control group. The aortic cholesterol concentrations were 98 ± 25, 57 ± 9, and 131 ± 32 µmol/g protein, respectively. The value for the olive-oil group was significantly (p<0.01) lower than the value for the control group. The triglyceride concentrations and the distributions of cholesterol between HDL, LDL, and VLDL in plasma showed no significant differences between the plant-oil groups and their control groups. This suggests that plant oils have a direct effect on the aortic cholesterol metabolism.

Methods

All animals were male white rabbits of the Danish Country strain from Statens Seruminstitut in Copenhagen. The rabbits were housed individually under controlled environmental conditions.

Experiment A

In Experiment A, 36 rabbits were assigned to two groups based on body weights and plasma triglyceride and cholesterol concentrations. The control group received a diet enriched with cholesterol (CH-USP, Sigma Chemical). This was mixed with ethyl ether and added to the food; subsequently the ether evaporated. The rabbits were fed standard rabbit pellets (Boserup, Faxe, DK 4640, Denmark) consisting of oats (18%), alfalfa (32%), barley (30%), sunflower seed extract (15%), molasses (2%), fish-meal (1%), and mineral and vitamin mixture. The pellets contained 14% protein, 15% cellulose, 7% minerals, and 3% fat. The fatty acid composition was 16:0, 18%; 18:0, 3%; (18:1, n-9), 15%; (18:2, n-6), 40%; (18:3, n-3), 14%; (22:6, n-3), 1%. The composition data were provided by the manufacturer. For the corn oil diet, the cholesterol was first added to corn oil (Oleum Maidis, BP 80, Mecobenzon, Copenhagen) and the mixture was poured over the rabbit.
pellets and mixed thoroughly. Cholesterol- and corn oil-cholesterol-enriched diets were prepared once a week.

Every 5 days after the start of the cholesterol feeding period, blood samples were drawn from ear veins, and the concentrations of plasma total cholesterol and plasma triglycerides were measured (Enzymatic kits, Boehringer Mannheim). On the basis of the concentration of total cholesterol in plasma, the amount of cholesterol in the food of each rabbit was adjusted individually to obtain the same mean concentration of plasma total cholesterol in all rabbits during the experimental period.

**Experiment B**

In Experiment B, we repeated the first experiment but changed the experimental design and included a third group of rabbits which received an olive oil-enriched diet (Oleum olivae, Ph. Dan. 48, Mecobenzon, Copenhagen). The 36 rabbits were divided into three groups based on body weights, cholesterol, triglyceride, and high density lipoprotein (HDL) cholesterol concentrations in plasma. For 5 days, these rabbits were fed a diet with added plant oils but with no added cholesterol. In this experiment, a 1% cholesterol-enriched stock of rabbit pellets was prepared by dissolving cholesterol in ethyl ether and allowing the ether to subsequently evaporate. This cholesterol-enriched food was mixed with stock diet (no cholesterol) to obtain the proper amount of dietary cholesterol for each rabbit in the three groups. The diet for the plant-oil groups was then prepared by adding 15 g of plant oil to 75 g of rabbit pellets. Later in the experiment, 10 g of plant oil to 80 g of rabbit pellets was used. The diet was prepared once a week and stored at room temperature. In Experiment B, blood samples were drawn every 5 days and a concentration of cholesterol between 20 and 25 mmol/l was maintained.

**Analytical Procedures**

Two aliquots from the plasma samples obtained at Day 35 and Day 55 in Experiment A and at Day 35 and Day 77 in Experiment B were adjusted to a density of 1.019 and 1.063 g/ml, respectively. These were centrifuged at 4°C at 1.58 x 10^6 g x in a 40.3 Beckman Rotor. The cholesterol content in the aliquots of whole plasma and in the various ultracentrifuged fractions were determined by an enzymatic method.

At the end of the experiment, the rabbits were anesthetized by intravenous injections of a 5% pentobarbital solution. The thoracic aorta was dissected free, and the adventitia was carefully removed under running saline. The aorta was opened longitudinally and the surface was rinsed with saline. The vessel was fixed with pins on a corkboard, and the tissue was divided into proximal and distal parts at the level of the first intercostal arteries. From each of these parts, the inner layer containing the intima and part of the media was stripped from the underlying outer media, and all four parts were weighed. Within 1 hour, the tissues were stored at −20°C until analysis.

The aortic tissue was minced, and the lipids were extracted with chloroform/methanol (1:1, vol/vol) for 24 hours. Lipids and proteins were separated and the total cholesterol in the four different tissue specimens was determined by the Liebermann-Burchard method. Free and esterified cholesterol were separated by thin-layer chromatography and were eluted from the silica gel; values were determined by the Liebermann-Burchard method after saponification. The amount of protein in the tissue specimens was determined by the methods of Lowry et al. after extraction of the lipids and digestion of the residue for 24 hours with 5M NaOH. The reference serum of animal origin (Seronorm, Nyegård, and Company, Oslo, Norway) was treated in a similar way and was used to calibrate the protein determinations.

**Statistical Methods**

The difference between mean values was analyzed by Wilcoxon's nonparametric test.

**Results**

**General Conditions**

All the rabbits in Experiment A ate their daily food and, except for one rabbit in the corn-oil group that developed vestibular dysfunction at the end of the experiment, there were no visible side effects on general condition or behavior and there was no mortality during the experimental period.

After the rabbits in Experiment B had eaten the diets for 14 days, some animals in both the corn-oil and the olive-oil groups did not eat their entire daily allowance. At Day 21, the amount of these oils in the diet were reduced from 15% to 10%. Thereafter, all but two rabbits in the olive-oil group and two in the corn-oil group ate well. These rabbits ate an average of 90% of their food allowances during the remaining 52 days.

The experimental groups and the corresponding control groups showed no significant differences in body weight gain during the experimental periods.

**Cholesterol In Diet and Plasma**

During the first week of cholesterol feeding, all the rabbits in each group were fed the same amount of cholesterol, and the coefficient of variation of plasma cholesterol concentration was about 50%. When the amounts of dietary cholesterol were then individualized, the coefficients of variation were reduced to 10% to 25%.

At Day 14 in Experiment A, the plasma cholesterol concentration in the corn-oil group was lower than that in the control group (Figure 1). By allowing the experimental group's plasma cholesterol concentration to increase more than the control group's between Day 26 and Day 39, we found that the corn-oil group had mean levels slightly higher of total cholesterol calculated over the entire 55 days of the experiment than did the control group (Table 1).

In one rabbit in the olive-oil group, the plasma cholesterol concentration did not increase in spite of a daily intake of about 0.9 g cholesterol, which was about five times greater than that given to other rabbits. The average plasma cholesterol concentration of this rabbit was 14.9 mM, a value so different from those of the group as a whole that the...
Atherogenesis and Plant Oils

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Figure 1. Data from Experiment A. Left panel. The concentration of total cholesterol in plasma (mean ± SEM) for two groups of rabbits receiving cholesterol-enriched diets with 5% corn oil added (•) or with no added corn oil (○). Right panel. The concentration of total cholesterol in the aorta of each rabbit receiving a cholesterol-enriched diet with (•) and without (○) corn oil.

Figure 2. Data from Experiment B. Left panel. The concentration of total cholesterol in plasma (mean ± SEM) for three groups of rabbits receiving a cholesterol-enriched diet with 10% to 15% added olive oil (•), with 10% to 15% added corn oil (•), or with no added plant oil (○). Right panel. The concentration of total cholesterol in the aorta of each rabbit receiving a cholesterol-enriched diet supplemented with olive oil (•), corn oil (•), or with no added plant oil (○).

Table 1. Cholesterol in Diet, Plasma, and Aorta of Rabbits Fed a Diet with or without Added Plant Oils

<table>
<thead>
<tr>
<th></th>
<th>Experiment A</th>
<th></th>
<th>Experiment B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Corn oil</td>
<td>Control</td>
<td>Corn oil</td>
<td>Olive oil</td>
</tr>
<tr>
<td>Number of animals</td>
<td>18</td>
<td>18</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Duration (days)</td>
<td>55</td>
<td>55</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td>Added oils (g/day)</td>
<td>5</td>
<td>0</td>
<td>15, 10</td>
<td>15, 10</td>
</tr>
<tr>
<td>Standard pellets (g/day)</td>
<td>90</td>
<td>100</td>
<td>85 to 90</td>
<td>85 to 90</td>
</tr>
<tr>
<td>Cholesterol in diet (g/rabbit)</td>
<td>43 ± 3</td>
<td>67 ± 5</td>
<td>30 ± 4</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean total cholesterol (mM)</td>
<td>47.3 ± 0.1†</td>
<td>44.5 ± 0.1</td>
<td>19.0 ± 0.1</td>
<td>19.1 ± 0.1</td>
</tr>
<tr>
<td>VLDL cholesterol (%)*</td>
<td>85 ± 2</td>
<td>86 ± 2</td>
<td>70 ± 2</td>
<td>73 ± 2</td>
</tr>
<tr>
<td>LDL cholesterol (%)*</td>
<td>14 ± 2</td>
<td>13 ± 2</td>
<td>27 ± 2</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>HDL cholesterol (%)*</td>
<td>1.8 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>3.4 ± 0.4</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>Mean triglyceride (mM)</td>
<td>3.3 ± 0.2</td>
<td>2.8 ± 0.3</td>
<td>1.1 ± 0.1</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>Aortic cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entire aorta (μmol/g wet weight)</td>
<td>12.4 ± 3.0†</td>
<td>19.9 ± 3.4</td>
<td>10.9 ± 2.9</td>
<td>8.3 ± 1.1†</td>
</tr>
<tr>
<td>Inner proximal layer (μmol/g protein)</td>
<td>248 ± 66†</td>
<td>392 ± 79</td>
<td>150 ± 35</td>
<td>94 ± 16§</td>
</tr>
<tr>
<td>Outer proximal layer (μmol/g protein)</td>
<td>57 ± 5</td>
<td>94 ± 15</td>
<td>46 ± 5</td>
<td>36 ± 2‡</td>
</tr>
<tr>
<td>Inner distal layer (μmol/g protein)</td>
<td>69 ± 22</td>
<td>87 ± 25</td>
<td>78 ± 30</td>
<td>30 ± 6†</td>
</tr>
<tr>
<td>Outer distal layer (μmol/g protein)</td>
<td>126 ± 3</td>
<td>42 ± 3</td>
<td>29 ± 3</td>
<td>27 ± 2‡</td>
</tr>
</tbody>
</table>

Values in parentheses are aortic concentrations of esterified cholesterol. To convert cholesterol values to mg/dl, multiply by 38.7. Values are means ± SEM.

*Mean values of the concentrations of the cholesterol in the three fractions were based on determinations on Days 35 and 55 in Experiment A and on Days 35 and 77 in Experiment B. The values are expressed as percentages of the mean level of total cholesterol.

†p = 0.05, †p = 0.025, §p = 0.01, ‡p = 0.005.
rabbit was excluded from the experiment. Two rabbits in the corn-oil group of Experiment B showed the same low plasma cholesterol response to dietary cholesterol in the first 3 to 4 weeks of the experimental period. However, in the last 9 to 10 weeks of the experiment, these rabbits' plasma cholesterol concentration increased and the average level when calculated over the entire 13-week period was acceptable.

In Experiment A, the control group required significantly more dietary cholesterol than did the corn-oil group to keep the plasma cholesterol concentration at the same level. Similarly in Experiment B, the control group and also the corn-oil group received significantly more dietary cholesterol than did the olive-oil group (Table 1).

In the two experiments, the addition of dietary plant oils did not significantly change the concentrations of triglyceride in plasma or the distribution of cholesterol between the plasma lipoproteins from that observed in the control groups (Table 1). The cholesterol concentrations in plasma in Experiment A were about twice as high as those in Experiment B. The same ratio was present for the triglyceride concentrations in plasma in the two experiments.

**Cholesterol in Aorta**

In spite of a slightly, but significantly, higher plasma cholesterol concentration in the corn-oil group compared with the control group in Experiment A, the concentration of cholesterol in the thoracic aorta was significantly lower in the corn-oil group than in the control group (Figure 1A). This was mainly due to the difference in cholesterol concentrations in the inner layer of the proximal thoracic aorta between the two groups. The same tendency was observed for the cholesterol concentrations in the three other regions of the thoracic aorta (Table 1).

In Experiment B, the cholesterol concentrations in the aortic tissue specimens were generally lower than the values obtained in Experiment A. In all four aortic regions, the olive-oil group showed a significant reduction compared to the control group. This was also the case when only the aortic concentrations of esterified cholesterol were considered. The percentage of esterified cholesterol in aorta was significantly lower in the olive-oil group than in the control group. A comparison of the percentage of esterified cholesterol with the total cholesterol content in the tissues of all rabbits in Experiment B showed that the difference between the olive-oil group and the control group was associated with the difference in total aortic cholesterol content between these groups. The values for the corn-oil group were between the values for the olive-oil group and those for the control group (Figure 2). None of the cholesterol concentration values in the aortic tissues in the corn-oil group was significantly different from the values in the control group at the p<0.05 level, but most of them were significant at the p<0.1 level (Table 1). The significance levels shown in this table also held true when the tissue...
cholesterol was expressed per gram wet weight or per cm² luminal surface.

In Figure 3, the aortic cholesterol concentration in each rabbit in Experiment A is related to the amounts of cholesterol fed to that rabbit during the entire experiment. Figure 4 shows the values obtained in Experiment B. In neither experiment did we observe a significant correlation between the amounts of dietary cholesterol added in ether and aortic cholesterol concentrations.

Discussion

In this study, we investigated whether the addition of olive oil or corn oil to the diet of cholesterol-fed rabbits influenced the accumulation of cholesterol in the aortic wall by a mechanism not mediated by a change of total cholesterol concentration in plasma. Any effect the plant oils may have had on the level of total cholesterol in plasma was counteracted by adjustments in the amount of dietary cholesterol.

Dietary Cholesterol and Atherogenesis

When the mean values for dietary cholesterol and for aortic cholesterol concentration for each of the five groups in Experiments A and B are compared, the group with the highest intake of dietary cholesterol has the highest aortic cholesterol concentration, and the group with the lowest intake of dietary cholesterol has the lowest aortic cholesterol concentration (Table 1). A relation between the amounts of dietary cholesterol and atherogenesis not mediated through changes in plasma cholesterol level can be envisioned if the dietary cholesterol contains small amounts of cholesterol oxidation products, which are harmful for the arterial wall. Cholesterol auto-oxidation products given orally to rabbits are absorbed, and ultrastructural changes in the luminal endothelial surface of the aortic wall of normcholesterolemic rabbits after intravenous administration of some of the auto-oxidation products have been described.

The lack of a significant correlation between the amounts of dietary cholesterol and the aortic cholesterol concentrations among the 36 rabbits in Experiment A (Figure 3) or among the 35 rabbits in Experiment B (Figure 4) speaks against an important role of dietary cholesterol auto-oxidation products in the accumulation of aortic cholesterol. This is in accordance with the observation that, in rabbits, atherogenesis that is produced by endogenous hypercholesterolemia after consumption of a cholesterol-free, low-fat, semisynthetic diet is quantitatively similar to that produced by exogenous cholesterol in fat. Likewise, several other studies do not support the hypothesis that exogenous cholesterol through cholesterol auto-oxidation products have a major effect on aortic cholesterol accumulation in hypercholesterolemic rabbits or chickens.

Arterial lesions in rabbits fed cholesterol-free diets that elicit an endogenous hypercholesterolemia without auto-oxidation products can be modified by dietary fats. Therefore, in the present study, the difference in aortic cholesterol concentrations between the plant-oil group and the control group can probably not be ascribed to a difference in the amounts of dietary cholesterol.

Dietary Plant Oils and Atherogenesis

It has repeatedly been shown that addition of polyunsaturated fats to the diets of several animal species results in retardation of atherogenesis compared with the addition of dietary saturated fats. This property has usually been explained by the plasma cholesterol-lowering effect of the polyunsaturated oils compared with the more saturated oils. To investigate if the addition of unsaturated fats had an antatherogenic effect that exceeded the hypocholesterolemic effect, Kritchevsky and coworkers have suggested a so-called relative atherogenic effect by dividing the degree of aortic lesion by the plasma cholesterol concentration. Based on this calculation, feeding the rabbits with cholesterol in corn oil results in a less atherogenic hypercholesterolemia than feeding the cholesterol with other oils (such as coconut oil, lard, peanut oil, or butter). In pigs and monkeys also, dietary corn oil seems to have an antatherogenic effect that exceeds its hypocholesterolemic effect.

The theory of the relative atherogenic effect assumes a proportionality between the degree of atheromata and the plasma cholesterol concentration. Such a relation is only present over a narrow range of plasma cholesterol concentrations. However, when in the present Experiment A, we corrected for the corn-oil-induced differences in plasma cholesterol concentrations not by dividing, but by maintaining the plasma cholesterol concentration at the same level in the corn-oil and in the control groups, we still observed that corn oil had an antatherogenic effect (Figure 1). This effect cannot be explained by an altered distribution of cholesterol between VLDL, LDL, and HDL.

It is possible, however, that changes in lipoprotein structure and composition not detected by ultracentrifugation at d = 1.006 and 1.063 may be associated with the change in aortic cholesterol.

The same significant effect of corn oil on aortic cholesterol was not observed in Experiment B in which the rabbits had a lower plasma cholesterol level than in Experiment A and in which cholesterol in an evaporable solvent was added to the pellets of all three groups. In that experiment, only olive oil reduced atherogenesis significantly without a significant effect on the plasma lipoprotein concentration (Table 1).
The antiatherogenic effect of olive oil, which exceeds the effect of corn oil on atherogenesis in rabbits, has, to the best of our knowledge, not previously been noticed. In a study of heated versus unheated corn oil and heated versus unheated olive oil on atherogenesis in rabbits, the relative atherogenic effect for the unheated olive oil was lower than for the unheated corn oil. The atherogenic effects of coconut oil, peanut oil, corn oil, and combined safflower, cottonseed, and olive oil were compared in rabbits fed 2% cholesterol-enriched diet. Corn oil and the combined oil with 55% olive oil had a similar, but lower, atherogenic index than did coconut oil and peanut oil. In another study with rabbits fed a cholesterol-free, semipurified diet, olive oil was found less atherogenic than peanut oil. The results of these few rabbit studies, which allow a comparison of olive oil with other oils, are in accordance with the more direct findings in the present study.

It is not known how the addition of fats to the diets of hypercholesterolemic rabbits results in less atherogenic hypercholesterolemia than no addition of fat. Neither is it known why some fats result in a less atherogenic hypercholesterolemia than other fats. It is possible that the fats affect the prostaglandin metabolism in the arterial wall. It has been observed that feeding olive oils to rabbits results in a higher arterial PGI₂ production than feeding corn oil. The effect of these oils may be caused by their fatty acid content but may also be due to one or more of the many other constituents in the oils.

It is noteworthy that people in the Mediterranean countries, and especially Cretan men, have a much lower mortality from ischemic heart disease than do people living in other parts of Europe and the United States who have the same concentrations of plasma cholesterol. One difference between people in the Mediterranean countries and the other regions is their intake of monounsaturated fats, mainly olive oil, which is excessively high in Crete. Assuming that olive oil has the same antiatherogenic effect in humans as in rabbits, that effect may occur in addition to an antiatherogenic effect mediated by beneficial changes in dietary oleic acid on the concentrations of non-HDL and HDL cholesterol in plasma.

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Index Terms: atherogenesis • corn oil • hypercholesterolemia • monounsaturated fats • olive oil • polyunsaturated fats • rabbits
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