Intravenous Injection of Rabbit Apolipoprotein A-I Inhibits the Progression of Atherosclerosis in Cholesterol-Fed Rabbits


Abstract The effects of intravenous injection of purified rabbit apoA-I on the progression of aortic atherosclerosis in cholesterol-fed rabbits were examined. In experiment 1, 28 rabbits were equally divided into groups A and B and fed a 0.5% cholesterol diet for 90 days. For the last 30 days, group B received 40 mg apoA-I every week. The fatty streak lesions in group B (23.9±15.6%) were significantly reduced compared with those in group A (46.0±24.9%) (P<.05). In experiment 2, 33 rabbits were divided into four groups (8 or 9 rabbits per group) and fed a 0.5% cholesterol diet. Group A was killed on day 105, while groups B, C, and D were maintained for an additional 60 days on a normal diet, during which time groups C and D received 1 mg apoA-I every other day or 40 mg apoA-I every week, respectively. The lesions in group C (70.2±15.4%) and group D (65.7±20.0%) were significantly suppressed compared with those in group B (80.2±13.7%) (P<.05) but were not reduced to the level of group A (50.0±22.9%). Although apparent regression was not observed under these conditions, the present study provided the first evidence for the antiatherogenic effect of homologous apoA-I on the progression of atherosclerosis in cholesterol-fed rabbits. (Arterioscler Thromb Vasc Biol. 1995;15:1882-1888.)

Key Words • apoA-I • HDL • diet-induced atherosclerosis • cholesterol-fed rabbits • experimental atherosclerosis

Several epidemiological studies have demonstrated that plasma levels of HDL and apoA-I are inversely correlated with the incidence of coronary heart disease.1-4 Patients with congenital apoA-I deficiency are reported to show low HDL-C levels with precocious atherosclerotic disease.5,6 In contrast, individuals with CE transfer protein deficiency show high levels of HDL-C due to the inhibition of CE transfer from HDL to apoB-containing lipoproteins.7 Inazu et al have suggested that individuals with CE transfer protein deficiency might be resistant to atherogenic diseases and have longer life spans than normal individuals. These observations suggest that HDL may play a protective role in atherogenesis.

The antiatherogenic properties of HDL or apoA-I have also been demonstrated in experimental animals. Transgenic mice overexpressing human apoA-I are much more resistant to diet-induced atherosclerosis than nontransgenic mice.8 Moreover, when human apoA-I gene is overexpressed in apoE knock-out mice, which exhibit severe hypercholesterolemia and atherosclerosis on a normal diet, the development of atherosclerotic lesions is markedly inhibited.9,10 Badimon et al11,12 first demonstrated that intravenous administration of exogenous HDL could suppress experimental atherosclerosis in cholesterol-fed rabbits. In their first report,11 rabbits were fed a 0.5% cholesterol diet for 8 weeks while receiving injections (50 mg/wk IV) of the homologous HDL-VHDL plasma fraction (d=1.063 to 1.25 g/mL). Atherosclerotic lesions covered 37.9% of the luminal surface area of the aortas in the control rabbits, which was significantly greater than the percentage in rabbits that were treated with the HDL-VHDL fraction (14.9%). In their second report,12 rabbits were divided into three groups and fed a 0.5% cholesterol diet. After 60 days, group 1 was killed, while groups 2 and 3 remained on the same diet until they were killed on day 90. For the last 30 days, group 3 was treated with the HDL-VHDL fraction (50 mg/wk IV). The aortic area occupied by atherosclerotic lesions in group 3 (17.8%) was significantly less than those in groups 1 (34%) and 2 (38.8%), which suggests the possibility that injection of the HDL-VHDL fraction might not only suppress the progression of atherosclerosis but may also induce its regression.

Since apoA-I is thought to play a key role in the antiatherogenic effects of HDL, we considered that purified apoA-I would be effective in suppressing atherosclerosis. To test this hypothesis, we examined whether purified rabbit apoA-I, instead of the rabbit HDL-VHDL fraction, might have a similar antiatherogenic effect on atherosclerosis in cholesterol-fed rabbits. We found that apoA-I inhibited the progression of atherosclerosis in these experimental animals, suggesting that apoA-I has therapeutic potential as an agent for controlling atherosclerosis.
Materials

Large-Scale Purification of Rabbit ApoA-I

Rabbit apoA-I was purified by using the methods of Carson13 and Ross and Carson14 with some modifications. They purified human apoA-I from human plasma by column chromatography by using phenyl-Sepharose followed by gel filtration with Sephacryl S-300. We modified this method for large-scale purification of rabbit apoA-I as follows.

Plasma (20 L) obtained from 500 New Zealand White rabbits was applied to a phenyl-Sepharose CL-4B column (6×25 cm) and delipidated twice with 3 L of the same solvent. The column containing 40% propylene glycol (buffer B) and then with 10 L of buffer A containing 6 mol/L urea (buffer C). The peak fractions were precipitated with 15 L ethanol/ethyl ether (3:2, vol/vol) and delipidated twice with 3 L of the same solvent. The precipitates were further delipidated twice with 2 L ethyl ether and evaporated to dryness.

The precipitates were dissolved in 500 mL buffer A containing 3 mol/L guanidine and dialyzed against 10 mmol/L Tris, 0.1 mmol/L NaCl, and 1 mmol/L EDTA (pH 7.6) (buffer D). The solution (800 mL) was applied to a Q-Sepharose Fast Flow column (11×10 cm) that had been preequilibrated with buffer D. After washing with 5 L buffer D, the bound fraction was eluted stepwise at a flow rate of 250 mL/min (500 mL/fraction). The column was first washed with 10 L buffer A containing 40% propylene glycol (buffer B) and then with 10 L buffer A containing 6 mol/L urea (buffer C). The peak fractions eluted with buffer C (4 L), which contained concentrated apoA-I, were precipitated with 15 L ethanol/ethyl ether (3:2, vol/vol) and delipidated twice with 3 L of the same solvent. The precipitates were further delipidated twice with 2 L ethyl ether and evaporated to dryness.

Methods

Cholesterol Efflux From Macrophage Foam Cells

The capacity of rabbit apoA-I to induce cholesterol efflux from macrophage foam cells was determined by the method of Hara and Yokoyama.20 Mouse resident peritoneal macrophages were collected from nonstimulated male DDY mice (20 to 25 g) and suspended in 2×10⁶ cells/mL in Dulbecco's modified Eagle's medium containing 0.2% bovine serum albumin, 10 mmol/L HEPES (pH 7.4), 0.1 mg/mL streptomycin, and 100 U/mL penicillin (medium A). Cell suspension (1 mL) was added to each 35-mm dish, and the cells were incubated at 37°C in 5% CO₂ for 2 hours. Cell monolayers thus formed were washed three times with 1 mL medium A. Macrophages were first converted to foam cells by incubation for 24 hours with 25 µg/mL [3H]cholesterol oleate-labeled ac-LDL. The cells were washed three times with 1 mL medium A and incubated for an additional 24 hours with 5, 10, or 20 µg/mL rabbit apoA-I or human apoA-I. The medium was collected and centrifuged to remove detached cells, and the radioactivity released into the medium from cells was determined by liquid scintillation counting. The cells were washed three times with 1.5 mL phosphate-buffered saline, and cellular lipids were extracted and run on thin-layer chromatography, followed by determination of the radioactivities of [3H]cholesterol and [3H]CE.21

Animals

Male New Zealand White rabbits (2.5 kg body weight, 12 weeks old) were housed in the Animal Research Center of Chemo-Sero-Therapeutic Institute, Kumamoto, Japan. The temperature and humidity were controlled at 24±2°C and 55±15%, respectively, with a 12-hour light/dark cycle. To induce experimental atherosclerosis, the rabbits were fed a 0.5% cholesterol diet at 150 g/d. A standard rabbit chow (LABO R stock) and one containing 0.5% cholesterol were purchased from Nihon Nosan Industrial Co Ltd. Both chows contained 17.3% protein, 17% diet fibers, and 3% fat with standard fatty acid composition. Experimental protocols were approved by the Experimental Animal Care Committee of Kumamoto University School of Medicine, and procedures were in accordance with the animal care guidelines of the committee.

Plasma Clearance of Intravenously Injected Rabbit ApoA-I

Purified rabbit apoA-I (240 mg) was labeled with 37 MBq of [125I] by using the method of McFarlane23 to a specific radioactivity of 72·10⁴ cpm/µg. [125I]-Labeled rabbit apoA-I (40 mg) in 5 mL phosphate-buffered saline was infused into each of three normolipidemic rabbits by a bolus injection. Labeled apoA-I was also injected into each of three hyperlipidemic rabbits that had been fed a 0.5% cholesterol diet for 90 days. Blood (5 mL) was sampled at the indicated times, and the radioactivities remaining in the plasma were determined.

Determination of ApoA-I (Experiment 1) and Combined Effects of ApoA-I and Change to Normal Diet (Experiment 2) on the Progression of Atherosclerosis in Cholesterol-Fed Rabbits

For experiment 1, normolipidemic rabbits were randomly divided into group A (n=14) and group B (n=14). All the rabbits in both groups were fed an atherogenic diet containing 0.5% cholesterol for 90 days (Fig 1). For the last 30 days (from days 60 through 90) of the experiment, group B received an injection of purified rabbit apoA-I (40 mg IV) in 5 mL saline once a week. Each rabbit in group A was injected with an equal volume of saline.

For experiment 2, 33 normolipidemic rabbits were randomly divided into four groups (Fig 1). Rabbits in group A (n=9) were fed a 0.5% cholesterol diet for 105 days and then fed a diet containing a normal composition of animal fat. Groups B, C, and D (n=8 for each) were fed the same diet for 105 days and then normal chow for 60 days (Fig 1). From day 105
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Morphometric Evaluation of Atherosclerotic Lesions

Animals were killed under deep anesthesia with sodium pentobarbital 25 mg/kg IV. The entire aorta from the aortic valve to the iliac bifurcation was removed from each rabbit and opened longitudinally. The vessel was fixed with 10% buffered formaldehyde (pH 7.4). Atheromatous lesions were measured independently by using a standard enzymatic method (cholesterol E-test, Wako). Significant amounts of radioactivities were measured by measuring the cholesterol contents of rabbit plasma after precipitating apoB- and apoE-containing lipoproteins with dextran sulfate.29

Statistical Analysis

Data were evaluated by Student's t test; P<.05 was judged as significant.

Results

Capacity of Rabbit ApoA-I to Enhance Cholesterol Efflux From Macrophage Foam Cells

To examine the capacity of purified rabbit apoA-I to promote cholesterol efflux from macrophage foam cells, mouse macrophages were converted to foam cells with [3H]cholesterol oleate-labeled ac-LDL and then exposed to rabbit apoA-I. Significant amounts of radioactivities were released into the medium in the presence of rabbit apoA-I (Fig 2). Similar studies were performed with human apoA-I. The capacity of the rabbit apoA-I for cholesterol efflux was indistinguishable from that of human apoA-I that had been purified by a conventional method.18 Similarly, rabbit apoA-I and human apoA-I were equally effective in reducing cellular [3H]CE (20% reduction from the control). These results indicate that the purification procedure for rabbit apoA-I employed in the present study may not significantly affect its cholesterol efflux capacity when compared with a widely used method for the purification of human apoA-I.

Plasma Clearance of Intravenously Injected Rabbit ApoA-I

Plasma clearance of rabbit apoA-I was examined by bolus injection of 40 mg labeled apoA-I since plasma clearance of a higher dose of apoA-I in rabbit has not been reported. The time required for a 50% reduction in plasma radioactivity in normal lipoprotein rabbits was approximately 20 hours, which is close to that for the trace amount of apoA-I reported by Badimon et al.31 (Fig 3). When 40 mg labeled apoA-I was injected into cholesterol-fed hypercholesterolemic rabbits, its plasma clearance rate was much faster than that in normal lipoprotein rabbits (P<.05 at 24 and 44 hours) (Fig 3).
Atherosclerosis in Cholesterol-Fed Rabbits was sampled at the indicated times, and radioactivities was also injected into three hypercholesterolemic rabbits (○). Saline was injected into three normolipidemic rabbits (●). ApoA-I and HDL-VHDL fraction could suppress the progression of atherosclerosis in cholesterol-fed rabbits, the present experimental conditions. Therefore, in experiment 2, we changed the experimental protocol so that the effect of apoA-I on the regression of atherosclerosis could be more easily observed with a change from the cholesterol diet to a normal diet during apoA-I treatment. In addition, since some rabbits showed negligible aortic lesions even on day 60, the duration of cholesterol feeding before apoA-I treatment was prolonged from 60 days in experiment 1 to 105 days in experiment 2 to induce moderately extensive aortic lesions (around 50%). Thus, in experiment 2, rabbits were fed normal chow during apoA-I-treatment (days 105 through 165) after being fed the atherogenic diet for 105 days.

Plasma cholesterol levels rose to 2000 mg/dL with the 0.5% cholesterol diet and gradually decreased after the change in diet on day 105 (Fig 6). Plasma cholesterol (Fig 6) and HDL-C (Table 2) levels in the apoA-I-treated rabbits did not significantly differ from those in the control groups during the experiment.

Cholesterol contents in vascular walls were also determined (Table 3). The amount of TC increased from 36.1 mg cholesterol/g wet wt on day 105 (group A) to 59.3 mg cholesterol/g wet wt on day 165 (group B) (P<.01), indicating that cholesterol accumulation in vascular walls progressed during this period even with a normal diet. The amount of CE also increased during the last 60 days. With a higher dose of apoA-I (group D), TC levels decreased from 59.3 (group B) to 41.9 (group D) mg/g wet wt (P<.05). CE content also decreased from 37.7 (group B) to 23.5 (group D) mg/g wet wt (P<.05). When rabbits were treated with an even lower dose of apoA-I, CE content significantly decreased, from 37.7 (group B) to 26.8 (group C) mg/g wet wt (P<.05), whereas the reduction in TC was not significant.

Morphometric analysis of aortic lesions showed that the area of atherosclerosis progressed from 50.0±22.9% on day 105 (group A) to 86.2±13.7% on day 165 (group B) (Fig 7), indicating that the progression of atherosclerosis was not inhibited by changing the diet alone. When the rabbits were injected with 1 mg apoA-I every other day (group C) from day 105 through 165, the extent of aortic fatty streak formation (70.2±15.4%) was significantly suppressed compared with that in group B (P<.05) (Fig 7). Group D was injected with a higher dose (40 mg) of apoA-I every week, and atheroma formation (65.7±20.0%) was also significantly suppressed compared with that in group B (P<.05) (Fig 7). However, the areas of atheromatous plaques in the apoA-I-treated groups (C and D) were not lower than those in group A, indicating that the regression of atherosclerosis could not be induced even though the rabbits were treated with apoA-I and a change to a normal diet.

**Discussion**

The present study directly demonstrated the antiatherogenic effect of purified apoA-I on experimental atherosclerosis. Although Badimon et al have demonstrated that intravenous injection of the homologous HDL-VHDL fraction could suppress the progression of atherosclerosis in cholesterol-fed rabbits, the present study provides the first evidence that purified apoA-I has an antiatherogenic effect in experimental atherosclerosis.

**FIG 3.** Line graph showing plasma clearance of rabbit apoA-I. Labeled rabbit apoA-I (40 mg IV) in 5 mL phosphate-buffered saline was injected into three normolipidemic rabbits (○). ApoA-I was also injected into three hypercholesterolemic rabbits (●) after 90 days' feeding of a 0.5% cholesterol diet. Blood (5 mL) was sampled at the indicated times, and radioactivities remaining in plasma were determined. Data are presented as percent of the radioactivity determined 10 minutes after injection; bars show SD. a indicates P<.05.

**FIG 4.** Line graph showing plasma cholesterol levels in cholesterol-fed rabbits. Combined Effects of ApoA-I and Change to Normal Diet on the Progression of Atherosclerosis in Cholesterol-Fed Rabbits (Experiment 1) Both the control (A) and the apoA-I-treated (B) groups were fed a 0.5% cholesterol diet throughout the experiment (90 days). For the last 30 days of the experiment, group B received an intravenous injection of purified rabbit apoA-I, and the effect on the progression of atherosclerosis was examined. The plasma level of TC increased to 2500 mg/dL with the atherogenic diet and remained constant throughout the remainder of the experiment (Fig 4). The cholesterol (Fig 3) and HDL-C (Table 1) levels in the two groups were indistinguishable. However, aortic fatty streaks in apoA-I-treated rabbits (23.9±15.6%) were significantly inhibited compared with those in control rabbits (46.0±24.9%) (P<.05) (Fig 5).

**FIG 5.** Combined Effects of ApoA-I and Change to Normal Diet on the Progression of Atherosclerosis in Cholesterol-Fed Rabbits (Experiment 2) In a preliminary experiment similar to experiment 1, some of the rabbits were killed on day 60 to examine whether apoA-I could induce regression of atherosclerosis during apoA-I treatment (from days 60 through 90). Atheroma formation in the apoA-I-treated group killed on day 90 was significantly suppressed compared with that in the 90-day control. However, it was not lower than that in the 60-day control (data not shown), indicating that apparent regression did not occur under the present experimental conditions. Therefore, in experiment 2, we changed the experimental protocol so that the effect of apoA-I on the regression of atherosclerosis could be more easily observed with a change from the cholesterol diet to a normal diet during apoA-I treatment.
One of the important findings in the present study was that apoA-I did not affect plasma cholesterol levels but did significantly reduce cholesterol contents in the vascular walls (Table 3), thus inhibiting the progression of atherosclerosis (Fig 7). A reasonable interpretation of these results may be as follows. ApoA-I might enhance cholesterol removal from vascular walls to plasma and subsequent transfer to the liver, thereby increasing net cholesterol transport from peripheral tissue to liver (reverse cholesterol transport), whereas plasma cholesterol levels might not be altered under dynamic equilibrium. In this context, intravenous injection of apoE into Watanabe heritable hyperlipidemic rabbits is also effective in suppressing the progression of atherosclerosis without affecting plasma cholesterol levels. It is likely that apoE as well as apoA-I enhances a reverse cholesterol transport system and inhibits the progression of atherosclerosis.

According to Badimon et al, the plasma clearance rate of a trace amount of apoA-I in normal rabbits is similar to that in cholesterol-fed rabbits. In the present study, although the plasma clearance rate of a higher dose of rabbit apoA-I (40 mg) in normal rabbits was similar to their values, the corresponding rate in cholesterol-fed rabbits was significantly faster than that in normal lipoprotein (Fig 3). The reason for the discrepancy is unknown. The hypercholesterolemic rabbits used for this experiment were studied after 90 days' feeding of a 0.5% cholesterol diet, while the plasma clearance of apoA-I (40 mg) in normal rabbits was significantly lower than that in normolipidemic rabbits (Fig 3). According to Badimon et al., the corresponding rate in cholesterol-fed rabbits was significantly faster than that in normal lipoprotein rabbits (Table 1). A similar result was obtained in cholesterol-fed rabbits that were treated with the HDL-VHDL fraction. These observations are somewhat inconsistent with the result obtained from transgenic mice overexpressing human apoA-I, whose HDL-C level was twofold higher. This could be because intravenous injection of apoA-I in rabbits might have a relatively small effect on total apoA-I level compared with the genetic expression of apoA-I in mice, which dramatically increased their total apoA-I level. The discrepancy could perhaps be ascribed to the method of determining HDL-C. HDL particles that received apoE from other plasma fractions might have been precipitated as apoE-containing lipoproteins during HDL-C determination.

Intravenous injection of exogenous apoA-I inhibited the progression of atherosclerosis in cholesterol-fed rabbits (Figs 5 and 7). However, no apparent regression of established lesions was achieved even when the diet

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**TABLE 1. Plasma Levels of HDL-C in Experiment 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 59</th>
<th>Day 89</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>71±19</td>
<td>2516±582</td>
<td>2457±539</td>
</tr>
<tr>
<td>Group B</td>
<td>66±25</td>
<td>2600±843</td>
<td>2549±785</td>
</tr>
<tr>
<td>Group C</td>
<td>19±6</td>
<td>493±80</td>
<td>582±143</td>
</tr>
<tr>
<td>Group D</td>
<td>40±14</td>
<td>2126±762</td>
<td>1895±409</td>
</tr>
<tr>
<td>Group E</td>
<td>50±19</td>
<td>523±513</td>
<td>1966±612</td>
</tr>
</tbody>
</table>

Values are expressed in milligrams per deciliter and are mean±SEM.

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**Fig 5.** Plot showing effect of apoA-I on the extent of aortic fatty streaks in cholesterol-fed rabbits from experiment 1. Both groups A and B were fed a 0.5% cholesterol diet for 90 days. For the last 30 days of the experiment, group B was injected with apoA-I (40 mg IV) once a week. On day 90 both groups were killed, and the luminal surface area of the aortas that was covered with fatty streaks was determined as described in Methods.

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**Fig 6.** Line graph showing plasma cholesterol levels in cholesterol-fed rabbits from experiment 2. Rabbits were divided into four groups (A-D) and fed a 0.5% cholesterol diet. Group A was killed on day 105, and groups B, C, and D were fed a normal diet for an additional 60 days. For the last 60 days, group C was injected with 1 mg apoA-I every other day, and group D was injected with 40 mg apoA-I once a week. The results in group A were indistinguishable from those in groups B, C, and D (not shown).
was changed to normal chow during treatment with apoA-I (Fig 7). There could be some explanations for these results. Plasma cholesterol levels as well as atherogenic β-VLDL levels remain high in cholesterol-fed rabbits for more than 10 weeks, even after cessation of cholesterol feeding, during which time cholesterol accumulation in aortic walls is still in progress. The present results were consistent with this observation; the lesion area (Fig 7) as well as cholesterol contents in vascular walls (Table 3) continued to increase after a normal diet was instituted (from days 105 through 165). Such an atherogenic predisposition of cholesterol-fed rabbits might have counteracted the antiatherogenic effect of injected apoA-I even after the change in diet. Alternatively, apoA-I treatment may have been started too late to induce a regression of atherosclerosis in the present protocol. We started apoA-I injection 105 days after cholesterol feeding (Fig 1). In contrast, Badimon et al. began HDL injection 60 days after cholesterol loading and observed significant regression even when the atherogenic diet was continued during the therapeutic period (days 60 through 90). All these results strongly suggest that it would be practically difficult for us to achieve a significant regression of atherosclerotic lesions in cholesterol-fed rabbits within the time period of this study. Although the present study did not show any regression of atherosclerosis, this does not necessarily imply that the antiatherogenic function of purified apoA-I is weaker than that of the HDL-VHDL fraction. Further studies are needed to compare the antiatherogenic properties of purified apoA-I with those of the HDL-VHDL fraction.

Another important issue that remains to be addressed is whether an apoA-I complex with phospholipids may have a stronger antiatherogenic effect than free apoA-I in vivo. An in vitro study has demonstrated that the capacity of apolipoproteins for cholesterol efflux is increased by forming complexes with phospholipids. Phospholipids are also known to reduce the atherogeneity of atherogenic lipoproteins. The plasma obtained from cholesterol-fed rabbits injected with phospholipid liposomes shows a much weaker ability to induce CE accumulation in macrophages in vitro than that from control rabbits. This is explained by the transfer of apoE from β-VLDL to liposomes, which reduces the ligand activity of β-VLDL. Moreover, the apoE liposomes thus formed could compete with β-VLDL for its binding to macrophages and reduce cellular uptake of β-VLDL. When modified LDLs such as ac-LDL and oxidized LDL are incubated with apoA-I complexed with dimyristoylphosphatidylcholine (DMPC), DMPC was transferred to modified LDLs, which resulted in a decrease in their net negative charge, thus reducing their ligand activity for the macrophage scavenger receptors.

The significant antiatherogenic effect observed with a low dose of apoA-I for group C (1 mg x 30 times) in experiment 2 gave us an important suggestion. When assessed by the lesion area (Fig 7) and the CE content in vascular walls (Table 3), the antiatherogenic effect of a lower dose of apoA-I for group C was indistinguishable from that of a higher dose of apoA-I for group D (40 mg x 8 times). This suggests that the therapeutic efficiency of apoA-I could be improved by modifying the treatment regimen. It is possible that a lower dose of apoA-I, when injected frequently, might be efficiently converted to an HDL subfraction such as pre-β-HDL.

### Table 2. Plasma Levels of HDL-C in Experiment 2

<table>
<thead>
<tr>
<th></th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>185±19</td>
<td>170±55</td>
<td>135±62</td>
</tr>
<tr>
<td>FC</td>
<td>39±17</td>
<td>37±12</td>
<td>30±15</td>
</tr>
<tr>
<td>CE</td>
<td>146±59</td>
<td>133±43</td>
<td>105±48</td>
</tr>
<tr>
<td>HDL-C</td>
<td>25±5</td>
<td>23±6</td>
<td>24±4</td>
</tr>
<tr>
<td>TG</td>
<td>78±57</td>
<td>86±52</td>
<td>76±68</td>
</tr>
</tbody>
</table>

The data of group A, which was killed on day 105, showed no significant difference from those of groups B, C, and D (data not shown). Values are expressed in milligrams per deciliter and are mean±SEM. TG indicates triglycerides.

### Table 3. Combined Effects of ApoA-I and a Change to a Normal Diet on Cholesterol Contents of Vascular Walls in Cholesterol-Fed Rabbits (Experiment 2)

<table>
<thead>
<tr>
<th>Cholesterol Contents of Vascular Walls, mg cholesterol/g wet wt</th>
<th>TC</th>
<th>FC</th>
<th>CE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (n=9)</td>
<td>36.1±15.7</td>
<td>9.8±5.3</td>
<td>26.3±10.8</td>
</tr>
<tr>
<td>Group B (n=8)</td>
<td>59.3±14.2</td>
<td>21.6±6.0</td>
<td>37.7±12.7†</td>
</tr>
<tr>
<td>Group C (n=8)</td>
<td>47.1±12.6</td>
<td>20.3±4.8</td>
<td>26.8±9.12</td>
</tr>
<tr>
<td>Group D (n=8)</td>
<td>41.9±11.31</td>
<td>18.4±6.9</td>
<td>23.5±6.31</td>
</tr>
</tbody>
</table>

Rabbits were fed a 0.5% cholesterol diet for 105 days and then normal chow for an additional 60 days (see experimental protocols in Fig 1). As controls, groups A and B were killed on days 105 and 165, respectively. For the last 60 days, group C was injected with 1 mg apoA-I every other day; group D was injected with 40 mg apoA-I once a week. Lipids were extracted from vascular walls (both intima and media), and the cholesterol contents were determined as described in "Methods."
which serves as an effective cholesterol acceptor from vascular walls. To further elucidate this point, more specific studies are needed to examine the effects of various doses of apoA-I on lipoprotein compositions and atherosclerotic lesions in cholesterol-fed rabbits.

Acknowledgments

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