Effects of Recombinant Apolipoprotein A–I\textsubscript{Milano} on Aortic Atherosclerosis in Apolipoprotein E–Deficient Mice

Prediman K. Shah, MD; Jan Nilsson, MD, PhD; Sanjay Kaul, MD; Michael C. Fishbein, MD; Hans Ageland, MD, PhD; Anders Hamsten, MD, PhD; Jan Johansson, MD; Frederick Karpe, MD, PhD; Bojan Cercek, MD, PhD

Background—We previously reported marked inhibitory effects of recombinant apolipoprotein (apo) A–I\textsubscript{Milano}/phospholipid complex (A–I\textsubscript{Milano}/PC) on neointimal lesions in balloon-injured iliofemoral arteries of hypercholesterolemic rabbits. In this study, we tested the hypothesis that apo A–I\textsubscript{Milano}/PC would inhibit aortic atherosclerosis in apo E–deficient mice.

Methods and Results—Thirty-five apo E–deficient mice fed a high-cholesterol diet were included in the study. Control mice were killed at 20 (n=8) or 25 (n=7) weeks. Treated mice received 18 injections of either 40 mg/kg apo A–I\textsubscript{Milano}/PC (n=15) or PC only (n=5) intravenously every other day from 20 weeks until death at 25 weeks. Aortic atherosclerosis was identified with Sudan IV staining. Lipid and macrophage contents of the aortic sinus plaques were measured after oil-red O and Mac-1 antibody staining, respectively, and quantified with computed morphometry. In control mice, from 20 to 25 weeks, aortic atherosclerosis increased by 59% (11 ± 1% versus 17 ± 5% of the aortic surface, P=.002), and lipid content increased by 45% (22 ± 8% versus 32 ± 6% of plaque area, P=.02) without a significant change in macrophage content (10.8 ± 2% versus 13.2 ± 6%). Compared with 20-week-old untreated control mice, PC only–treated mice at 25 weeks demonstrated a 32% increase in aortic atherosclerosis (11 ± 1% versus 15 ± 4%, P=.01) and an increase in lipid content (22 ± 8% versus 47 ± 3%, P<.0001) without a change in macrophage content (10.8 ± 2% versus 11 ± 2%). In comparison with 20-week-old untreated control mice, 25-week-old apo A–I\textsubscript{Milano}/PC–treated mice demonstrated no increase in aortic atherosclerosis (11 ± 1% versus 10 ± 4%, P=NS), a 40% reduction in lipid content (22 ± 8% versus 13 ± 8%, P=.01), and a 46% reduction in macrophage content (10.8 ± 2% versus 5.8 ± 2.9%, P=.03). Serum cholesterol levels were markedly elevated in all groups and did not change significantly with apo A–I\textsubscript{Milano}/PC or PC only. In vitro, apo A–I\textsubscript{Milano}/PC stimulated cholesterol efflux from cholesterol-loaded FU5AH hepatoma cell lines in a dose-dependent manner, whereas PC only or PC-free apo A–I\textsubscript{Milano} had no effect.

Conclusions—Recombinant A–I\textsubscript{Milano}/PC prevented progression of aortic atherosclerosis and reduced lipid and macrophage content of plaques in apo E–deficient mice despite severe hypercholesterolemia. Thus, A–I\textsubscript{Milano}/PC may have a role in inhibiting progression and promoting stabilization of atherosclerosis.

Key Words: apolipoproteins ■ atherosclerosis ■ hypercholesterolemia

Several epidemiological and clinical studies have demonstrated an inverse relationship between coronary heart disease and circulating levels of HDL cholesterol and its major apoprotein, apolipoprotein (apo) A-I. Because relationships observed in epidemiological studies do not necessarily prove a cause and effect, it has been debated whether HDL and apo A-I are simply markers of reduced risk or have direct antiatherogenic effects. Several lines of evidence gathered in the past few years have suggested that HDL and apo A-I may have direct antiatherogenic effects.

Apo A–I\textsubscript{Milano} is a naturally occurring mutant of apo A-I, with a cysteine-to-arginine substitution at position 173 that is associated with freedom from vascular disease and longevity in its carriers despite markedly reduced HDL and elevated triglyceride levels. We previously demonstrated that recombinant apo A–I\textsubscript{Milano}/phospholipid complex (A–I\textsubscript{Milano}/PC) significantly reduces neointimal lesions in the balloon-injured iliofemoral arteries of cholesterol-fed rabbits. Similar results have since been reported by Soma et al with a periadventitial carotid injury model in cholesterol-fed rabbits. Because atherosclerotic lesions in rabbits differ from those in humans, we sought to determine the effects of genetically engineered recombinant apo A–I\textsubscript{Milano}/PC on aortic atherosclerosis in apo E–deficient mice, which develop atherosclerotic lesions that more closely resemble the advanced lesions observed in humans.

Methods

Apo E–deficient mice (C57BL/6J strain, aged 5 weeks, 18 to 20 g) obtained from Jackson Laboratory (Bar Harbor, Me) were fed a high-fat, high-cholesterol (atherogenic) diet containing 21% (wt/wt) fat and 0.15%...
cholesterol throughout the duration of the experiment. Control mice were killed at 20 (n=8) and 25 (n=7) weeks of age. With the use of a special mouse restrainer (Scanbur A-S) and a 30-gauge needle, mice were administered 40 mg/kg apo A-I Milano/PC dissolved in 0.5 mL saline (n=15) or PC only (n=5) through the tail vein every other day from 20 weeks until killed at 25 weeks, for a total of 18 injections each. The recombinant apo A-I Milano/PC preparation used in this study has been described previously. Measurements involving the animals was approved by the Institutional Animal Care and Use Committee and conformed to the Guiding Principles in the Care and Use of Laboratory Animals established by the council of the American Physiology Society.

Before death, mice were anesthetized with enflurane inhalation, and 100 of 300 μL of blood was obtained from the retro-orbital plexus through heparin–coated capillaries (Fisher Scientific) and collected in an EDTA-treated Vacutainer tube (Becton Dickinson) for serum through heparin-coated capillaries (Fisher Scientific) and collected in an EDTA-treated Vacutainer tube (Becton Dickinson) for serum. Serum was stored at −70°C until analysis. Serum cholesterol was measured with an enzymatic technique. Lipoproteins were further characterized in 2 mice receiving apo A-I Milano/PC through adjustment of 300 μL of serum to d=1.21 kg/L followed by a 48-hour centrifugation (Beckman 50.3 rotor at 40 000 rpm in an Optima ultracentrifuge at 1°C). The lipoprotein fraction was dialyzed against phosphate-buffered saline and separated with size-exclusion chromatography (Sepharose 6) on an FPLC (Pharmacia-Uppjohn). The cholesterol concentration in the fractions was determined with fluorometry. Peak fractions representative of VLDL, LDL, and HDL were also subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis for visualization of the respective apo contents in the fractions. The elution pattern of isolated VLDL, LDL, and HDL fractions had been determined beforehand to indicate the borders between the respective fractions. The circulating levels of apo A-I Milano and antibodies to apo A-I Milano were determined by ELISA as described previously. Tissue Preparation and Histological Analysis

After anesthesia with enflurane, the mice were killed, and their hearts and aortas were perfusion-fixed with 4% paraformaldehyde, 5% sucrose, and 20 mmol/L EDTA at physiological pH for 10 minutes. The heart and proximal aorta were excised and embedded in OCT compound (Tissue-Tek), frozen on dry ice, and then stored at −70°C until sectioning. Serial 10-μm-thick sections (every fifth section from the middle of the ventricle until the appearance of aortic valve and every second section from the appearance to the disappearance of the aortic valve leaflets) were collected on poly-d-lysine–coated slides, stained with oil-red O and hematoxylin, and counterstained with Fast Green. Quantification of atheromatous lesions in the proximal aorta was performed with computer-assisted morphometry with image analysis software (Optimas 5.1; Bioscan) and expressed as the average of lesion areas from six sections per mouse. The descending thoracic aorta and abdominal aorta (up to bifurcation of common iliac arteries) were removed, stored overnight in formal-sucrose fixative, split open longitudinally, and stained with Sudan IV to visualize the extent of atherosclerosis. Quantification of the percentage of aortic surface covered by atheroma was performed with computer-assisted planimetry of the Sudan IV–positive areas. The interobserver and intraobserver variabilities of these measurements were <1.5% and <0.5%, respectively. In selected sections, immunohistochemical staining was performed using monoclonal rat anti-mouse Mac-1 antibody (Boehringer-Mannheim Biochemicals) as a specific marker for monocyte/macrophages.

Measurement of Cholesterol Efflux In Vitro

To determine the effect of apo A-I Milano/PC on cellular cholesterol efflux, we used Fu5AH hepatoma cell lines preloaded with radiolabeled cholesterol exposed to apo A-I Milano/PC, PC-free apo A-I Milano, or PC only for 4 hours. At the end of incubation, the medium was removed, and the remaining cellular lipids were extracted with isopropanol. Aliquots of cellular extract and medium were measured by liquid scintillation, and the fractional efflux of cholesterol into the medium was determined as a measure of cholesterol efflux–promoting capacity. This method for determination of cholesterol efflux has been described in detail by de La Llera Moya et al. Statistical Analysis

Data are presented as mean ± SD. Group comparisons were made using an unpaired t test or ANOVA followed by Newman-Keuls test with a two-tailed P value considered to be significant.

Results

Extent of Aortic Atherosclerosis

In the untreated control mice, aortic atherosclerosis increased by 59% from 20 to 25 weeks (11 ± 1% versus 17 ± 5% of the aortic surface, P=.004) (Table and Fig 1, top). In comparison with 20-week-old untreated control mice, mice receiving PC only also demonstrated a 36% increase in aortic atherosclerosis at 25 weeks (11 ± 1% versus 15 ± 4%; P=.01). In contrast, aortic atherosclerosis did not progress in mice treated with apo A-I Milano/PC at 25 weeks compared with untreated control mice at 20 weeks (11 ± 1% versus 10 ± 4%). Aortic atherosclerosis at 25 weeks was significantly less in apo A-I Milano/PC–treated mice than in untreated control mice (P<.01; ANOVA) and PC only–treated mice (P<.05; ANOVA).

Lipid Content in Aortic Sinus Atheromatous Plaque

In the untreated control mice, the lipid content in the aortic sinus plaque increased from 22 ± 8% of the plaque area at 20 weeks to 32 ± 6% at 25 weeks (P=.03) (Table and Fig 1, middle). Similarly, in PC only–treated mice, the lipid content

### Table: Lipid Content in Aortic Sinus Atheromatous Plaque

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Mice</th>
<th>PC-Treated Mice</th>
<th>A-I Milano/PC-Treated Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, mmol/L</td>
<td>25.7±7.5</td>
<td>27.3±4.4</td>
<td>29.6±5.6</td>
</tr>
<tr>
<td>Extent of aortic atheroma, % of total aortic surface</td>
<td>11±1</td>
<td>17±5</td>
<td>15±4</td>
</tr>
<tr>
<td>Lipid content in aortic root plaque, % of plaque area</td>
<td>22±8</td>
<td>32±6</td>
<td>47±3</td>
</tr>
<tr>
<td>Macrophage content in aortic root plaque, % of plaque area</td>
<td>10.8±2</td>
<td>13.2±6</td>
<td>11±2</td>
</tr>
</tbody>
</table>

See text for details.
Serum Cholesterol and Apo A-I Milano Levels

Serum cholesterol levels were 25.7±7.5 mmol/L (1029±301 mg/dL) at 20 weeks and 27.3±4.4 mmol/L (1091±179 mg/dL) at 25 weeks in untreated control mice (Table). The serum cholesterol levels were 29.6±5.6 mmol/L (1181±35 mg/dL) and 23±5.9 mmol/L (918±235 mg/dL) in mice receiving PC only and apo A-I Milano/PC, respectively. Serum cholesterol levels did not differ significantly between the groups.

The lipoprotein distribution of cholesterol between untreated controls and apo A-I Milano/PC−treated mice were similar except for a slight increase in the cholesterol content of the HDL fraction in apo A-I Milano/PC−treated mice (Fig 2). The apo A-I band in the apo A-I Milano/PC−treated mice was faint, but a strong band of 60 to 70 kD was detected in these mice, suggesting the presence of apo A-I Milano dimer despite reducing conditions.

Macrophage Content in Aortic Sinus Plaque

From 20 to 25 weeks, there was no significant increase in macrophage content in the untreated control mice (10.8±2% versus 13.2±6% of plaque area) or PC only−treated mice (10.8±2% versus 11±2%) (Table and Fig 1, bottom). In contrast, macrophage content was reduced by 46% in apo A-I Milano/PC−treated mice at 25 weeks compared with untreated control mice (P<.001; ANOVA) as well as PC only−treated mice (P<.001).

Serum cholesterol levels were 29.6±5.6 mmol/L (1181±35 mg/dL) and 23±5.9 mmol/L (918±235 mg/dL) in mice receiving PC only and apo A-I Milano/PC, respectively. Serum cholesterol levels did not differ significantly between the groups.

The lipoprotein distribution of cholesterol between untreated controls and apo A-I Milano/PC−treated mice were similar except for a slight increase in the cholesterol content of the HDL fraction in apo A-I Milano/PC−treated mice (Fig 2). The apo A-I band in the apo A-I Milano/PC−treated mice was faint, but a strong band of 60 to 70 kD was detected in these mice, suggesting the presence of apo A-I Milano dimer despite reducing conditions.

At the time of death, apo A-I Milano was detectable in all mice (n=15) receiving apo A-I Milano/PC but in none of the controls. The apo A-I Milano level averaged 312±124 µg/mL in the 5 mice killed at 8 hours after the last injection and 14±13 µg/mL in 10 mice killed at 24 hours after the last injection. Antibodies against apo A-I Milano were detected in the sera of mice receiving apo A-I Milano (n=3) but in none of the control mice (n=3).

Effect of Apo A-I Milano/PC on Cholesterol Efflux

As shown in Fig 3, apo A-I Milano/PC promoted cholesterol efflux in a dose−dependent fashion (n=6). In contrast, PC only (n=6) or PC−free apo A-I Milano (n=6) had no effect on cholesterol efflux.

Effect of High−Dose Apo A-I Milano/PC

Three additional mice received a higher dose of apo A-I Milano/PC (80 mg/kg per dose) for a total of 18 injections over 5 weeks. The extent of aortic atherosclerosis at 25 weeks was further reduced to 3.6±2.0% of the aortic surface despite a serum cholesterol level of 28.2±5.9 mmol/L (1129±237 mg/dL) with a corresponding further reduction in the macrophage content in aortic sinus plaques to 3.2±0.4% of the plaque area. A comparable dose of PC only (n=3) had no significant effect on aortic atherosclerosis compared with 25−week untreated control mice (16±4.6% versus 17±5% of aortic surface; P=NS).

Discussion

The results of this study demonstrate that recombinant apo A-I Milano/PC prevents progression of aortic atherosclerosis in apo E−deficient mice fed a high−cholesterol diet despite persistent and severe hypercholesterolemia. Preliminary observations involving 3 additional mice receiving a higher dose (80 mg/kg per dose) suggest that apo A-I Milano/PC may also induce regression of aortic atherosclerosis, although this finding needs further confirmation. The present findings extend our previously described protective
effects of recombinant apo A-I Milano/PC on neointimal lesions in balloon-injured iliofemoral arteries of hypercholesterolemic rabbits. In contrast to the present study, we did not detect a change in the total cholesterol content of the aorta in the rabbit model; however, this difference may have resulted from fewer injections (5 versus 18) and lower doses per injection (10 versus 40 mg/kg) used in the rabbit model compared with the mouse model in this study. The results of this study are consistent with the beneficial effects of intravenous injection of homologous HDL/VHDL and apo A-1 in hypercholesterolemic rabbits and the protective effects of apo A-1 transgene expression on diet-induced atherosclerosis in apo E–deficient mice. Taken together, these experimental observations provide compelling evidence for the direct antiatherogenic effects of apo A-I and HDL.

The apo E–deficient mouse is a recently introduced experimental model created through gene targeting and homologous recombination that develops hypercholesterolemia and atherosclerotic lesions on regular mouse chow within a few weeks of birth. In contrast to the present study, we did not detect a change in the total cholesterol content of the aorta in the rabbit model; however, this difference may have resulted from fewer injections (5 versus 18) and lower doses per injection (10 versus 40 mg/kg) used in the rabbit model compared with the mouse model in this study. The results of this study are consistent with the beneficial effects of intravenous injection of homologous HDL/VHDL and apo A-1 in hypercholesterolemic rabbits and the protective effects of apo A-1 transgene expression on diet-induced atherosclerosis in apo E–deficient mice. Taken together, these experimental observations provide compelling evidence for the direct antiatherogenic effects of apo A-I and HDL.

The apo E–deficient mouse is a recently introduced experimental model created through gene targeting and homologous recombination that develops hypercholesterolemia and atherosclerotic lesions on regular mouse chow within a few weeks of birth. In contrast to the present study, we did not detect a change in the total cholesterol content of the aorta in the rabbit model; however, this difference may have resulted from fewer injections (5 versus 18) and lower doses per injection (10 versus 40 mg/kg) used in the rabbit model compared with the mouse model in this study. The results of this study are consistent with the beneficial effects of intravenous injection of homologous HDL/VHDL and apo A-1 in hypercholesterolemic rabbits and the protective effects of apo A-1 transgene expression on diet-induced atherosclerosis in apo E–deficient mice. Taken together, these experimental observations provide compelling evidence for the direct antiatherogenic effects of apo A-I and HDL.

The apo E–deficient mouse is a recently introduced experimental model created through gene targeting and homologous recombination that develops hypercholesterolemia and atherosclerotic lesions on regular mouse chow within a few weeks of birth. In contrast to the present study, we did not detect a change in the total cholesterol content of the aorta in the rabbit model; however, this difference may have resulted from fewer injections (5 versus 18) and lower doses per injection (10 versus 40 mg/kg) used in the rabbit model compared with the mouse model in this study. The results of this study are consistent with the beneficial effects of intravenous injection of homologous HDL/VHDL and apo A-1 in hypercholesterolemic rabbits and the protective effects of apo A-1 transgene expression on diet-induced atherosclerosis in apo E–deficient mice. Taken together, these experimental observations provide compelling evidence for the direct antiatherogenic effects of apo A-I and HDL.

Mechanisms of Action of Apo A-I Milano/PC

The precise mechanisms by which apo A-I Milano/PC produces its antiatherogenic effects are not fully understood. The beneficial effects of HDL and apo A-I have been attributed to promotion of reverse cholesterol transport, inhibition of LDL oxidation, scavenging of lipid peroxides from peripheral tissues, modulation of inflammatory response by inhibition of complement polymerization, and promotion of fibrinolysis. The fact that protective effects of apo A-I Milano/PC on aortic atherosclerosis were observed despite the persistence of hypercholesterolemia indicates that antiatherogenic effects are not mediated by a major reduction in severity of hypercholesterolemia. In this study, apo A-I Milano/PC reduced the lipid and inflammatory cell (monocyte/macrophage) content of the more advanced atheromatous plaques and promoted cellular cholesterol efflux in vitro. In contrast, PC only did not prevent the progression of aortic atherosclerosis, nor did it reduce the lipid or macrophage content in the advanced aortic sinus atherosclerotic lesions. Thus, the favorable effects of apo A-I Milano/PC observed in this study cannot be attributed solely to the PC component of apo A-I Milano/PC. Furthermore, PC only and PC-free apo A-I Milano did not promote cellular cholesterol efflux in vitro. This finding is consistent with the complementary role of apo A-I and PC for stimulation of cholesterol efflux in which apo A-I acts as an acceptor for cellular cholesterol and PC acts as a sink, as proposed by Atger et al. These results are generally consistent with those observed in the rabbit iliofemoral and carotid injury model. A decrease in the lipid and inflammatory cell (macrophage) content in the atheromatous plaques as well as in vitro cholesterol efflux–promoting effects of apo A-I Milano/PC are consistent with reverse cholesterol–promoting effects of apo A-I Milano/PC. The cysteine-for-arginine substitution at position 173 in the amino acid sequence of apo A-I Milano results, among other changes, in a higher kinetic affinity of apo A-I Milano for...
lips and an easier dissociation from lipid/protein complexes, which might contribute to its increased efficiency for uptake of tissue lipids. The precise molecular mechanisms by which apo A–I–containing particles and HDL mediate reverse cholesterol transport in vivo remain incompletely understood. It has recently been demonstrated that scavenger receptor type B class I, also known as SR-BI, acts as a putative HDL receptor mediating selective cellular uptake of HDL cholesterol ester in steroidogenic tissues and liver in vitro and in vivo, suggesting that SR-BI may be involved in HDL metabolism and cholesterol homeostasis. These observations are further supported by the recent demonstration that HDL–dependent cellular cholesterol efflux is markedly enhanced by overexpression of SR–BI in several different cell types in vitro and that SR–BI is expressed in atheroma in apo E–deficient mice.

In experimental animals such as apo E–deficient mice, as well as in humans, oxidative modification of lipoproteins is believed to play an important role in atherogenesis. The proatherogenic effects of oxidized lipoproteins have been attributed to their ability to stimulate inflammatory cell (monocyte) attachment, migration, and recruitment into the arterial wall, transformation of monocytes to macrophages and foam cells through the scavenger receptor pathway, T cell activation, and endothelial dysfunction. The inflammatory cells recruited into the arterial wall have been shown to modulate production of growth factors and cytokines that influence smooth muscle cell migration, proliferation, and matrix secretion. Several in vitro studies have suggested that HDL may inhibit the formation or alter the properties of oxidatively modified lipoproteins. These antioxidant effects have been attributed to the binding of pro–oxidant transition metals by apo A–I and to paraoxonase and platelet-activating factor acetylhydrolase, two enzymes carried by apo A–I– and apo J–containing HDL particles. Furthermore, it has been suggested that HDL may sequester oxidized lipids from LDL, thereby limiting apo B modification. Thus, the favorable effects of apo A–I–Milano/PC observed in this study may have resulted from enhancement of reverse cholesterol transport, antioxidant effects, or other undefined mechanisms.

**Potential Limitations**

Several potential limitations of the present study must be considered. The results in apo E–deficient mice, in which hypercholesterolemia is largely due to elevated levels of triglyceride–rich VLDL, may differ from that in humans, in whom an elevated LDL level is more common. However, several recent studies in humans have highlighted the important prognostic and potential therapeutic implications of triglyceride–rich lipoproteins, including small dense LDL in coronary heart disease. Although LDL-lowering therapy in randomized trials has been shown to reduce coronary events, the continued occurrence of coronary events in a substantial number of subjects despite LDL-lowering therapy strongly suggests the importance of non–LDL lipoproteins and other risk factors in atherosclerotic vascular disease. Moreover, the magnitude of hypercholesterolemia in these mice is considerably greater than that observed in humans. Nevertheless, the atherosclerotic lesions in these mice closely resemble those of human, and it is very likely that an intervention that is effective in the presence of profound hypercholesterolemia may indeed be even more effective in the presence of lesser degrees of hypercholesterolemia. An immune response to apo A–I–Milano/PC could potentially limit the efficacy, but that is less likely to be an issue when human subjects are evaluated. Even in mice, efficacy did not appear to be blunted even though antibodies against apo A–I–Milano were detected at the end of the experiment in apo A–I–Milano/PC–treated animals. Finally, we did not address the issue of whether the antiatherogenic efficacy of apo A–I–Milano/PC differs substantively from that of apo A–I–wild type/PC in this particular model. However, we demonstrated previously that in cholesterol-fed rabbits, reconstituted HDL containing human wild-type apo A–I produced a modest 25% reduction in neointimal lesion formation (n = 10, P = NS versus controls) (unpublished observations) compared with a 70% reduction in neointimal lesion area using recombinant apo A–I–Milano/PC. However, further studies will be necessary to more completely address this issue.

**Potential Clinical Implications**

The favorable effects of recombinant apo A–I–Milano/PC on the extent of atherosclerosis and lipid/macrophage content observed in this study suggest that an antiatherogenic strategy using apo A–I–Milano/PC or possibly other forms of HDL–containing apo A–I holds promise for inhibition of the progression of atherosclerosis and/or stabilization of rupture–prone (lipid and inflammatory cell– or macrophage–rich) atherosclerotic plaques. Although the clinical trials of LDL lowering with statins have been successful in reducing coronary heart disease events by 30% to 40%, many patients continue to experience disease progression and coronary events despite LDL lowering. Further reduction in clinical events will require additional therapies that focus on non–LDL types of lipoproteins and non–lipid–related risk factors for atherosclerosis. Unlike lipid–lowering drugs targeted to reduce cholesterol levels, apo A–I appears to exert its beneficial effects via mechanisms other than a reduction in cholesterol levels. Thus, a therapeutic strategy based on apo A–I may be complementary to LDL lowering. Further exploration of apo A–I and HDL as a potential therapeutic strategy appears warranted.

**Acknowledgments**

The generous support of Pharmacia–Upjohn, United Hostesses, and the Ralph M. Parson Foundation of Los Angeles is deeply appreciated. The technical assistance of Juliana Yano, BS; Jenny Zhu, BS; Helen Xu, BS; Teresa Pan, BS; John Ong, PhD; and Dr. Margarita de la Llera Moya is gratefully acknowledged.

**References**


Effects of Recombinant Apolipoprotein A-I\textsuperscript{Milano} on Aortic Atherosclerosis in Apolipoprotein E–Deficient Mice

Prediman K. Shah, Jan Nilsson, Sanjay Kaul, Michael C. Fishbein, Hans Ageland, Anders Hamsten, Jan Johansson, Frederick Karpe and Bojan Cercek

_Circulation_. 1998;97:780-785
doi: 10.1161/01.CIR.97.8.780

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/97/8/780

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/