Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 9607–9611, September 1994
Genetics

Human apolipoprotein A-I gene expression increases high density lipoprotein and suppresses atherosclerosis in the apolipoprotein E-deficient mouse

ANDREW S. PLUMP, CHRISTOPHER J. SCOTT, AND JAN L. BRESLOW*

The Rockefeller University, 1230 York Avenue, New York, NY 10021

Communicated by Alexander G. Bearn, June 9, 1994

ABSTRACT **Atherosclerosis is a complex disease with both genetic and environmental determinants. Apolipoprotein (Apo) E-deficient mice have been created that are highly susceptible to atherosclerosis. In order to assess the role of human apolipoprotein (hApo) A-I and high density lipoprotein (HDL) in atherosclerosis susceptibility, transgenic mice overexpressing the hApo A-I gene were crossed with Apo E-deficient mice. Apo E −/−, hApo A-I mice with two-fold elevation in HDL cholesterol have markedly diminished atherosclerosis with less fibroproliferative lesions by 8 months of age. A strong reciprocal relationship between HDL cholesterol levels and atherosclerosis was found with HDL levels accounting for 78% of the observed variance in mean lesion area. The effect of HDL on atherosclerosis resistance was independent of non-HDL cholesterol.**

Atherosclerosis is influenced by many genetic and environmental factors. In particular, altered lipoprotein levels caused by diet or mutant genes have been strongly associated with susceptibility to atherosclerotic coronary heart disease. Epidemiological studies have shown a strong inverse correlation between plasma high density lipoprotein (HDL) cholesterol levels and coronary heart disease (1). The principal structural protein of HDL is apolipoprotein (Apo) A-I, a 28-kDa protein found in human plasma at a mean concentration of 125 mg/dl (2). HDL cholesterol levels are highly correlated with plasma Apo A-I levels. Individuals have been described with Apo A-I mutations precluding synthesis of normal Apo A-I (3–6). These individuals have low HDL cholesterol levels and severe premature coronary heart disease. Transgenic mice expressing the human Apo (hApo) A-I gene have increased HDL cholesterol levels (7, 8). These two observations suggest that Apo A-I genetic variation can affect HDL cholesterol levels, which in turn may alter atherosclerosis susceptibility. Although epidemiological studies in humans (1) and studies of diet-induced fatty streak lesions in laboratory animals (8, 9) suggest a role for HDL in mediating atherosclerosis susceptibility, direct proof that genetic variability in HDL cholesterol levels can diminish atherosclerosis susceptibility would require specifically increasing the HDL cholesterol levels and demonstrating a decrease in fibroproliferative atherosclerotic lesions. In addition to documenting the role of HDL in atherosclerosis susceptibility, this system should provide understanding of the mechanism by which HDL acts.

Gene targeting has been used to create Apo E-deficient mice (10–12). These animals develop severe hypercholesterolemia on a low-fat, low-cholesterol, mouse chow diet. The predominant lipoprotein abnormality in the Apo E-deficient mice is the accumulation of β very low density lipoprotein (β-VLDL), a cholesterol ester-enriched atherogenic lipoprotein particle. On the mouse chow diet, Apo E-deficient mice develop extensive atherosclerosis with lesions progressing from endothelial–monocyte adhesions to lipid-laden fatty streaks to advanced fibroproliferative lesions by 20–30 weeks of age (13, 14). This progression of atherosclerotic lesions is seen in humans. The Apo E-deficient mouse model is different from the previous mouse model of atherosclerosis, the C57BL/6 diet-induced model (15). In the latter, an atherogenic, high-cholesterol (1%), cholic acid (0.5%)-containing diet is required to produce foam cells at the base of the aorta. Apo A-I overexpression in this model leads to diminished foam-cell formation (8). Though encouraging, the model is limited because the atherogenic diet is not physiological (16) and has significant hepatotoxicity. Furthermore, lesions resulting from this diet fail to progress through the phases of atherosclerosis to fibroproliferative plaques. Thus, the Apo E-deficient mouse model is a more suitable one for assessing the effect of HDL cholesterol levels on atherosclerosis.

MATERIALS AND METHODS

Mice. The creation of the transgenic hApo A-I mice (line 179) (7) and the Apo E-knockout mice (11) used in this study has been described. To generate the study populations, a C57BL/6 × 129Ola hybrid mouse containing the Apo E −/− mutation was crossed with a C57BL/6 × CBA hybrid mouse containing the hApo A-I transgene. Resulting F_1 Apo E +/− mice containing the hApo A-I transgene were backcrossed to C57BL/6 × 129OlA Apo E −/− hybrid mice. All experiments performed with mice were in accordance with the Rockefeller University Animal Care and Use Committee.

Apolipoprotein and Lipoprotein Analysis. Lipoprotein measurements were made on 10- to 15-week-old chow-fed mice after an 8-hr fast. The lipoprotein and apolipoprotein data in the text and in Table 1 are from pooled mice from the 4- and 8-month-old study groups depicted in Fig. 2. These data were pooled because serial chronological examination of individual mice and comparison of groups of mice of different ages have revealed that mouse age does not affect plasma cholesterol levels. Cholesterol measurements were made by enzymatic assay (Boehringer Mannheim, no. 816302). For HDL cholesterol measurement, individual mouse plasma was first separated at a density (d) = 1.006 g/ml in a Beckman Airfuge. Cholesterol measurement was then made on the $d > 1.006$ g/ml fraction after dextran sulfate precipitation. Human Apo A-I levels were measured by ELISA using a goat anti-human Apo A-I antibody. FPLC (Pharmacia) analysis of plasma lipoproteins was performed as described (11).

Quantitative Atherosclerosis Measurements. Mice were sacrificed at 4 or 6 months of age and perfused at physiologic pressure with 0.9% NaCl by cardiac intraventricular canalization. The heart and proximal aorta were isolated and fixed

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Apo, apolipoprotein; hApo, human apolipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein.
*To whom reprint requests should be addressed.

for 5 days or more in phosphate-buffered formaldehyde. After fixation, hearts were embedded in 25% gelatin and cryostat sectioned at 10-μm thickness. Processing and staining of tissues were carried out according to Paigen *et al.* (17). Lesion area was quantified by the method of Rubin *et al.* (8) with one addition. Because the lesions in the Apo E-deficient mice are not limited to fatty streaks but progress to fibroproliferative lesions, we quantified not only the fatty portion of the lesion but the entire lesion area from the lumen to the point of normal vessel wall.

Qualitative Atherosclerosis Analysis. After intraventricular perfusion with 0.9% NaCl the heart and proximal aorta were removed and quick frozen in liquid N_2. Frozen sections were generated at 6-μm thickness and placed on lysine-coated slides. With standard histologic techniques slides were then stained with one of three stains: oil red O and hematoxylin with a light green counterstain, hematoxylin and eosin, or Masson's trichrome.

Statistical Analysis. Plasma hApo A-I, non-HDL cholesterol, and HDL cholesterol levels are reported as mean ± SD. Comparisons between groups were made by using the Student *t* test with significance at $P < 0.05$. Simple and multiple linear regressions were used to determine the correlation of HDL and non-HDL cholesterol to mean lesion area.

RESULTS

To assess the role of HDL in determining susceptibility to atherosclerosis, a hApo A-I transgene was bred onto the Apo E-deficient mouse background. The line of hApo A-I transgenic mice used to generate the study population, line 179 from Walsh *et al.* (7), has a wide range of plasma hApo A-I levels. Because of this, the study populations were divided into three groups, Apo E −/−; Apo E −/−, low-expressing (low) hApo A-I (plasma hApo A-I < 200 mg/dl); and Apo E −/−, high-expressing (high) hApo A-I (hApo A-I > 200 mg/dl) mice. Control mice and low and high hApo A-I mice were generated within the same litter. hApo A-I levels were 153 ± 26 mg/dl in a representative group of low hApo A-I mice, and 275 ± 50 mg/dl in a group of high hApo A-I mice (Table 1). Sizing FPLC (Fig. 1) showed a significant difference in the plasma lipoprotein pattern between the Apo E −/− mice and the Apo E −/−, high hApo A-I mice. HDL cholesterol levels were significantly elevated in the latter group, whereas non-HDL cholesterol levels were similar. No differences were detected between the Apo E −/−, low hApo A-I mice and the Apo E −/− mice. Control Apo E −/− mice had HDL cholesterol levels of 50 ± 17 mg/dl, while Apo E −/−, high hApo A-I mice had HDL cholesterol levels of 105 ± 32 mg/dl ($P < 0.0001$) (Table 1). Total cholesterol and non-HDL cholesterol were not significantly different between the two groups (Fig. 1, Table 1). In the Apo E −/−, low hApo A-I mice as compared to the Apo E −/− mice there was no significant difference between HDL or non-HDL cholesterol.

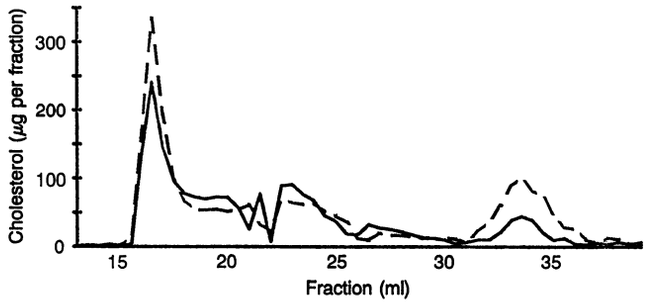

FIG. 1. Sizing FPLC of plasma from Apo E −/− and Apo E −/−, high hApo A-I transgenic mice. As previously described (11), 100 μl of plasma pooled from several mice was loaded onto two Superose 6 columns linked in series. Samples were eluted at a constant flow rate of 0.3 ml/min with 1 mM EDTA/0.15 M NaCl. Fractions of 0.5 ml were collected and cholesterol concentration was measured enzymatically. Cholesterol levels are shown as μg per fraction per 100 μl of plasma. Solid line, Apo E −/−; dashed line, Apo E −/−, high hApo A-I.

To assess the effect of elevated plasma hApo A-I levels on the development of atherosclerosis, groups of chow-fed Apo E −/−; Apo E −/−, low hApo A-I; and Apo E −/−, high hApo A-I mice were analyzed for lesion formation at 4 months of age. On a chow diet Apo E −/− mice are known to develop extensive fatty streak lesions by 4 months of age (13). Animals were analyzed quantitatively for proximal aorta atherosclerosis. Atherosclerosis was quantified by assessing mean lesion area over an ≈350-μm segment of the proximal aorta (17). Unlike previous studies assessing lesion area in the mouse, the current studies made use of the observation that lesions in the Apo E-deficient mice are not limited to fatty streaks, but progress to fibroproliferative lesions. As such, results include quantification of the entire lesion from the lumen to the point of normal vessel wall.

High expression of hApo A-I led to a significant decrease in the development of atherosclerotic lesions. At 4 months of age, Apo E −/− mice had a mean lesion area of 22,964 ± 23,030 μm^2 ($n = 34$) compared with a mean lesion area of 470 ± 825 μm^2 in Apo E −/−, high hApo A-I mice ($n = 12$, $P < 0.0001$) (Fig. 2*A*). Six out of 12 of the Apo E −/−, high Apo A-I mice had no detectable lesions at this time point. At 4 months Apo E −/− mice had well-defined fatty streak lesions (Fig. 3*A*), and the Apo E −/−, high hApo A-I mice that did have detectable pathology had nascent fatty streak lesions (Fig. 3*B*). The Apo E −/−, low hApo A-I mice did not have significantly different mean lesion size from Apo E −/− mice (18,099 ± 10,153 μm^2, $n = 8$). These lesions were predominantly fatty streak lesions resembling those seen in the Apo E −/− mice.

To further assess the role of hApo A-I in atherosclerosis formation and progression, a second group of animals was analyzed for lesion formation at 8 months of age. High hApo A-I expression led to a decrease in lesion area, whereas low

Table 1. Lipoprotein profile of Apo E −/− and Apo E −/−, hApo A-I mice

Mice	Cholesterol, mg/dl			hApo A-I, mg/dl
	Total	Non-HDL	HDL	
Apo E −/− ($n = 34$)	663 ± 245	613 ± 245	50 ± 17	—
Apo E −/−, low hApo A-I ($n = 8$)	533 ± 122	469 ± 110	65 ± 27	153 ± 26 ($n = 11$)
Apo E −/−, high hApo A-I ($n = 14$)	577 ± 137	472 ± 151	105 ± 32*	275 ± 50 ($n = 9$)
Apo E +/+ ($n = 12$)	97 ± 8*	33 ± 3*	64 ± 5**	—

Lipoprotein measurements were made on 10- to 15-week-old chow-fed mice after an 8-hr fast. Cholesterol measurements were made by enzymatic assay (Boehringer Mannheim, no. 816302). For HDL cholesterol measurement, individual mouse plasma was first separated at $d = 1.006$ g/ml. Cholesterol measurement was then made on the $d > 1.006$ g/ml fraction after dextran sulfate precipitation. hApo A-I levels were measured by ELISA using a goat anti-hApo A-I antibody. For reference, plasma cholesterol measurements of Apo E +/+ (C57BL/6 × 129)F_1 mice are shown.
*, $P < 0.0001$; **, $P < 0.01$ as compared with Apo E −/− mice.

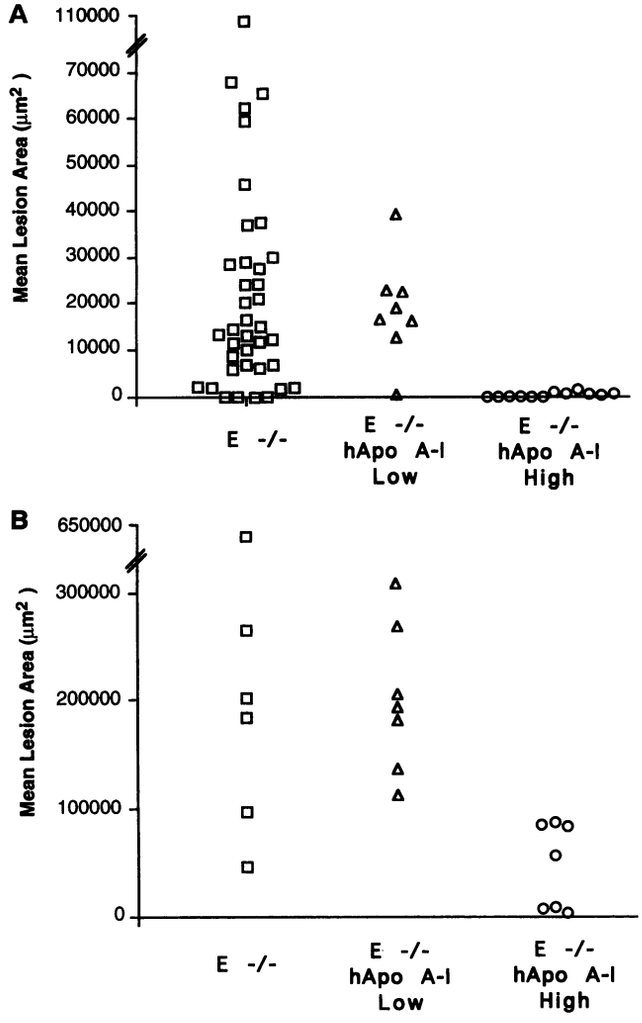

FIG. 2. Mean lesion area in Apo E −/−; Apo E −/−, low hApo A-I; and Apo E −/−, high hApo A-I mice. (*A*) Chow-fed mice were sacrificed at 4 months of age. The proximal aorta was sectioned at 10-μm intervals and stained with oil red O, hematoxylin, and a light green counterstain, and mean lesion area was assessed by calculating lesion area every 80 μm over an ≈350-μm proximal aortic segment (17). Apo E −/− mean lesion area (± SD) = 22,964 ± 23,030 μm² (n = 34); Apo E −/−, low hApo A-I mean lesion area = 18,099 ± 10,153 μm² (n = 8); Apo E −/−, high hApo A-I mean lesion area = 470 ± 825 μm² (n = 12, $P < 0.0001$ compared with Apo E −/− mice). There were no significant weight differences in the animals from each of the groups. (*B*) Chow-fed mice sacrificed at 8 months of age were similarly assessed for mean lesion area. Apo E −/− mean lesion area = 243,200 ± 202,698 μm² (n = 6); Apo E −/−, low hApo A-I mice mean lesion area = 221,536 ± 83,211 μm² (n = 7); Apo E −/−, high hApo A-I mice mean lesion area = 45,222 ± 35,631 μm² (n = 7, $P < 0.05$ compared with Apo E −/− mice). There were no significant weight differences in the animals from each of the groups. Lesion size at both time points was quantified by assessing lipid and nonlipid staining material within the atherosclerotic plaque. Male and female mice were included in the study; there was no difference in mean lesion area between the two.

hApo A-I expression had no effect on mean lesion area (Fig. 2*B*). Apo E −/− mice at 8 months of age developed mean atherosclerotic lesion area of 243,200 ± 202,698 μm² (n = 6). Apo E −/−, low hApo A-I mice had a mean lesion area of 221,536 ± 83,211 μm² (n = 7). Apo E −/−, high hApo A-I mice had a mean lesion area of 45,222 ± 35,631 μm² (n = 7, $P < 0.05$).

Previous studies have demonstrated that by 20–40 weeks of age Apo E −/− mice have developed advanced fibroproliferative lesions (13, 14). As seen by a representative histological section in Fig. 3*C*, 8-month-old Apo E −/− mice in the present study also developed advanced lesions. Compared with the 4-month-old Apo E −/− mice the lesions in the 8-month-old mice were comparatively lipid depleted. Other histological stains have demonstrated that these same lesions are rich in cells and extracellular collagen and elastin. On the other hand, as seen by a representative section in Fig. 3*D*, 8-month-old Apo E −/−, high hApo A-I mice had less advanced lesions. The lesion shown in Fig. 3*D* is a fatty streak lesion with lipid accumulation similar to that seen in the 4-month-old Apo E −/− mice. Additional histological stains of lesions from older Apo E −/−, high hApo A-I mice suggested significantly decreased cell content and extracellular matrix as well.

To determine the effect of lipoprotein patterns on atherosclerosis susceptibility, we correlated mean lesion area with both HDL and non-HDL cholesterol measured in 17 of the 20 eight-month-old mice. A strong inverse relationship was found between HDL cholesterol levels and atherosclerosis (Fig. 4*A*). When fit to a linear model, HDL predicted 49% of the variance in mean lesion area ($r = -0.70$, $P < 0.005$). However, the relationship of HDL cholesterol and atherosclerosis appears hyperbolic, and when mean lesion area was correlated with the inverse of the HDL cholesterol concentration, HDL levels predicted 78% of the observed variance in mean lesion area ($r = 0.88$, $P < 0.0001$). The data were reexamined after removal of the one data point from the mouse with the lowest HDL cholesterol and the highest mean lesion area. As described in the legend to Fig. 4 the correlation remained statistically significant, with a reciprocal model better describing the data. No correlation was found between non-HDL cholesterol levels and atherosclerosis in these animals (Fig. 4*B*). In multiple linear-regression analysis, non-HDL cholesterol levels failed to enhance the predictive value of HDL cholesterol levels.

DISCUSSION

The mechanism by which Apo A-I and HDL cholesterol act to inhibit the development of atherosclerosis is unknown. There are three existing hypotheses to explain the role of HDL in protecting against the development of atherosclerosis: reverse cholesterol transport, direct protective effects of HDL on the vessel wall or on lipoprotein oxidation, and an inverse relationship between HDL and atherogenic Apo B-containing lipoproteins. In the present study the hApo A-I transgene specifically raised HDL cholesterol levels without significantly affecting non-HDL cholesterol levels. Thus, the effect of HDL cholesterol may be a direct one and not a reflection of altered levels of Apo B-containing lipoproteins. This strongly suggests that HDL may act via direct protective effects on the vessel wall or by enhancing reverse cholesterol transport from the vessel wall to the liver. Subsequent studies examining cholesterol flux and various vessel wall parameters, such as oxidative state and expression of endothelial cell adhesion molecules, should provide evidence to support one or both of these hypotheses.

Several factors may influence future studies examining the protective mechanism of elevated HDL cholesterol. The effect of HDL may be modified by its composition, as the expression of the other major HDL apolipoprotein, Apo A-II, in transgenic mice appears to nullify the protective effect of Apo A-I and may in fact be proatherogenic (18, 19); however, this has yet to be tested in the Apo E-deficient mouse model. Furthermore, in addressing mechanism of protection, another point to consider is the mechanism of atherogenesis. There are several means by which a vessel can become atherosclerotic, including plasma abnormalities such as lipoprotein alterations, hemodynamic derangements such as hy-

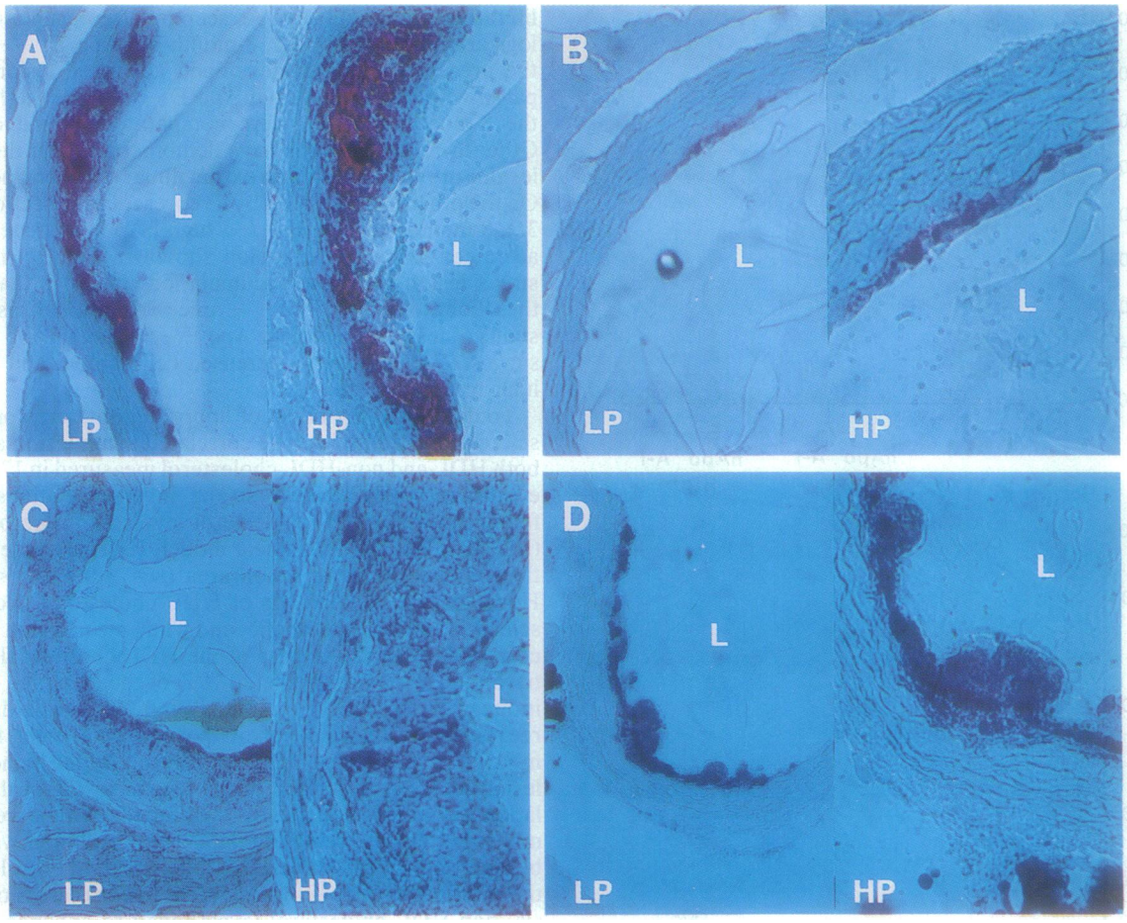

FIG. 3. Oil red O stains of proximal aorta of Apo E −/− (*A* and *C*) and Apo E −/−, high hApo A-I (*B* and *D*) mice from the 4-month (*A* and *B*) and 8-month (*C* and *D*) study groups. After quick freezing, hearts were sectioned at 6-μm thickness in a cryostat. Sections were stained with oil red O and hematoxylin and counterstained with light green. The lesion shown from the 4-month-old Apo E −/− mouse is a pronounced fatty streak lesion (*A*), while that of the 4-month-old Apo E −/−, high hApo A-I mouse is a nascent fatty streak (*B*). This latter lesion was one of the larger lesions detected in this group of mice. At 8 months the Apo E −/− mouse has developed an advanced lesion that is relatively lipid poor (*C*). Other histological stains (not shown) have demonstrated in mice of similar age that these lesions are rich in cells and extracellular matrix. The lesion in the 8-month-old Apo E −/−, high hApo A-I mouse is a fatty streak lesion with no evidence of progression (*D*). The majority of lesions assessed in this group were predominantly fatty streak lesions. Taken together, these data suggest that hApo A-I can inhibit the development of fibroproliferative atherosclerosis in the Apo E −/− mice. LP, low power; HP, high power; L, lumen.

pertension, and vessel wall changes such as those found in diabetes mellitus. In the Apo E-deficient mouse the assumption is that atherosclerosis is caused by elevated β-VLDL. One must, however, consider that the absence of Apo E may alter cellular function and influence lesion development independently of the hypercholesterolemia. Regardless of the mechanism of lesion development in the Apo E-deficient mouse or the mechanism of HDL protection, variation in HDL cholesterol levels in the Apo E-deficient mouse can account for the majority of lesion variability.

Clearly, elevated hApo A-I can inhibit early atherogenesis in Apo E-deficient mice. Although more studies will be needed, it appears that hApo A-I can also delay or inhibit the onset of advanced atherosclerosis. By 4 months, Apo E-deficient mice have well-defined fatty streak lesions, and by 8 months, these lesions increase 10-fold in size and develop into advanced fibroproliferative lesions. In contrast, by 4 months the Apo E −/−, high hApo A-I mice have no or barely detectable fatty streak lesions, and by 8 months these lesions have grown by 80-fold in size into well-defined fatty streaks. The latter observation suggests a delay in the initiation of lesion formation with the majority of lesion growth occurring after 4 months of age. In the Apo E −/− as compared with Apo E −/−, hApo A-I mice there is relatively less growth between 4 and 8 months of age, but there is evolution of the fatty streak into a fibroproliferative lesion. Studies of older Apo E −/−, hApo A-I mice will be necessary to assess whether these animals can develop advanced lesions, and whether hApo A-I can prevent lesion progression as well as diminish lesion size.

The current study has examined atherosclerosis in the Apo E-deficient mouse over a physiological range of HDL cholesterol levels. The reciprocal regression line obtained from the correlation of HDL cholesterol to atherosclerotic lesion area suggests a 2.2-fold effect on atherosclerosis when an HDL cholesterol level of 30 mg/dl is compared with an HDL cholesterol level of 70 mg/dl. This is consistent with the large body of epidemiological evidence showing that HDL cholesterol is a strong inverse predictor of coronary heart disease. Although human population studies have not typically assessed atherosclerosis directly, they do suggest that as in the Apo E-deficient mouse model, HDL cholesterol levels affect susceptibility in a nonlinear fashion (20). This reciprocal correlation also suggests the possible existence of a threshold level of HDL over which protection is strong and under which atherosclerosis susceptibility is high. The fact that the Apo E −/−, low hApo A-I mice did not have diminished atherosclerosis is further suggestive that a threshold level of Apo A-I and/or HDL is needed to achieve resistance. The fact that HDL levels were not significantly greater in the Apo

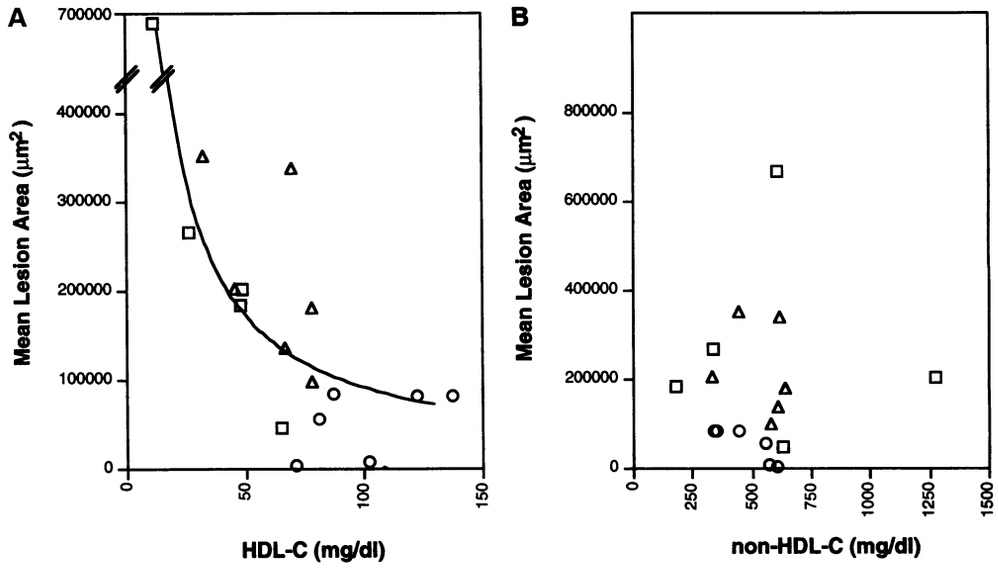

FIG. 4. Mean lesion area plotted against HDL cholesterol (HDL-C) and non-HDL cholesterol (non-HDL-C) for 17 of the 20 mice studied at 8 months of age. (*A*) Mean lesion area versus HDL cholesterol levels in pooled Apo E −/− (□); Apo E −/−, low hApo A-I (△); and Apo E −/−, high hApo A-I (○) mice from the 8-month study group. The data correlate in both a linear and reciprocal model. The curve shown is fit to a reciprocal model. In this model, in which mean lesion area is correlated with 1/HDL cholesterol concentration, $r = 0.88$, $P < 0.0001$, $y = (8.0 \times 10^6)/x + 9.7 \times 10^3$. In the linear model $r = -0.70$, $P < 0.005$, and $y = -3.5 \times 10^3 X + 4.2 \times 10^5$. When considered without the one data point with lesion size of 667,500 μm^2 and HDL cholesterol of 12 mg/dl, the correlation retains its statistical significance when fit to both the reciprocal and linear models. Without this data point, in the reciprocal model $r = 0.68$, $P < 0.001$, and in the linear model $r = -0.62$, $P < 0.01$. (*B*) Mean lesion area versus non-HDL cholesterol levels in the same group of animals. No correlation was found between non-HDL cholesterol levels and mean lesion area. Additionally, no correlation was found between the HDL and non-HDL cholesterol fractions.

E −/−, low hApo A-I mice may, however, implicate the necessity of an Apo A-I and not an HDL threshold level. Though the concept of a threshold Apo A-I and/or HDL level is difficult to assess in these mice because of the presence of mouse Apo A-I and mouse HDL, elevated HDL levels are certainly protective.

During the preparation of this manuscript we became aware of similar data demonstrating that hApo A-I can protect against the development of atherosclerosis in an independently generated line of Apo E-deficient mice (Eddy Rubin, personal communication).

We thank Kathleen Roberts for performing hApo A-I ELISA and Annemarie Walsh for supplying the transgenic hApo A-I mice. This work was supported in part by National Institutes of Health Grants HL33714-10 and HL32435-10.

1. Gordon, D. & Rifkind, B. (1989) *N. Engl. J. Med.* **19**, 1311–1316.
2. Eisenberg, S. (1984) *J. Lipid Res.* **25**, 1017–1058.
3. Norum, R. A., Lakier, J. B., Holdstein, S., Angel, A., Goldberg, R. B., Block, W. D., Noffze, O. K., Dolphin, P. J., Edelglass, J., Bogorad, D. D. & Alqupovic, P. (1982) *N. Engl. J. Med.* **306**, 1513–1519.
4. Schaefer, E., Heaton, W., Wetzel, M. & Brewer, H. B., Jr. (1982) *Arteriosclerosis* **2**, 16–26.
5. Funke, H., von Eckardstein, A., Pritchard, P. H., Karas, M., Albers, J. J. & Assmam, G. (1991) *J. Clin. Invest.* **87**, 371–376.
6. Matsunaga, T., Hiasa, Y., Yanagi, H., Maeda, T., Hattori, N., Yamakawa, K., Yamanouchi, Y., Tanaka, I., Obara, T. & Hamaguchi, H. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 2793–2797.
7. Walsh, A., Ito, Y. & Breslow, J. L. (1989) *J. Biol. Chem.* **264**, 6488–6494.
8. Rubin, E. M., Kraus, R. M., Spangler, E. A., Verstuyft, J. G. & Clift, S. M. (1991) *Nature (London)* **353**, 265–267.
9. Badimon, J. J., Badimon, L. & Fuster, V. (1990) *J. Clin. Invest.* **85**, 1234–1241.
10. Piedrahita, J. A., Zhang, S. H., Jagaman, J. R., Oliver, P. M. & Maeda, N. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4471–4475.
11. Plump, A. S., Smith, J. D., Hayek, T., Aatto-Setäla, K., Walsh, A., Verstuyft, J. G., Rubin, E. M. & Breslow, J. L. (1992) *Cell* **71**, 343–353.
12. Zhang, S. H., Reddick, R. L., Piedrahita, J. A. & Maeda, N. (1992) *Science* **258**, 468–471.
13. Nakashima, Y., Plump, A. S., Raines, E. W., Breslow, J. L. & Ross, R. (1994) *Arterioscler. Thromb.* **14**, 133–140.
14. Reddick, R. L., Zhang, S. H. & Maeda, N. (1994) *Arterioscler. Thromb.* **14**, 141–147.
15. Paigen, B., Morrow, A., Brandon, C., Mitchell, D. & Holmes, P. (1985) *Atherosclerosis* **57**, 65–73.
16. Dietschy, J. M., Turley, S. D. & Spady, D. K. (1993) *J. Lipid Res.* **34**, 1637–1659.
17. Paigen, B., Morrow, A., Holmes, P. A., Mitchel, D. & Williams, R. A. (1987) *Atherosclerosis* **68**, 231–240.
18. Warden, C. H., Hedrick, C. C., Qiao, J. H., Castellani, L. W. & Lusis, A. J. (1993) *Science* **261**, 469–472.
19. Schultz, J. R., Verstuyft, J. G., Gong, E. L., Nichols, A. V. & Rubin, E. M. (1993) *Nature (London)* **365**, 762–764.
20. Gordon, D. J., Probstfield, J. L., Garrison, R. J., Neaton, J. D., Castelli, W. D., Knoke, J. O., Jacobs, O. R., Bangdiwala, S. & Tyroler, H. A. (1989) *Circulation* **79**, 8–15.