11. M. M. Davis and P. J. Bjorkman, *Nature* **334**, 395 (1988); C. Chothia, D. R. Bothwell, A. M. Lesk, *EMBO J.* **7**, 3745 (1988); J.-M. Claverie, A. Prochnicka-Chalufour, L. Bougueleret, *Immunol. Today* **10**, 10 (1989).
12. B.-C. Sim and N. R. J. Gascoigne, data not shown.
13. B. Arden, S. P. Clark, D. Kabelitz, T. W. Mak, *Immunogenetics* **42**, 501 (1995).
14. Mutations are designated by the wild-type residue, followed by its position, and the mutant residue. Abbreviations for the amino acid residues are as follows: F, Phe; P, Pro; S, Ser; and W, Trp.
15. The T cell clone Ar-5, specific for arsonate/I-A^d, expresses V_α3.1J_α31 (AV3S5J31) [K.-N. Tan *et al.*, *Cell* **54**, 247 (1988)]. Nomenclature follows (*13*) and B. F. Koop *et al.* [*Genomics* **19**, 478 (1994)]. This α chain was used to raise the antiserum to V_α3.1 (anti-V_α3.1) and to generate V_α3.1 transgenic mice (*7*). The monoclonal antibody RR3-16 recognizes V_α3.2 (AV3S2) (*8*). The AV3S5J31 genomic clone was mutated by polymerase chain reaction overlap extension [S. N. Ho, H. D. Hunt, R. M. Horton, J. K. Pullen, L. R. Pease, *Gene* **77**, 51 (1989)] and cloned into a genomic α–chain vector [V. Kouskoff, K. Signorelli, C. Benoist, D. Mathis, *J. Immunol. Methods* **180**, 273 (1995)]. Transgenic mice were generated at The Scripps Research Institute (TSRI) with FVB oocytes and backcrossed to C57BL/6J (B6) mice. All animals were treated in accordance with TSRI institutional guidelines.
16. Transgenic mice were screened by fluorescent-activated cell sorting (FACS) on peripheral blood lymphocytes, gating on viable Thy1.2$^+$ cells. The percentage of V_α-expressing cells in CD4 or CD8 populations was determined. Mice expressing greater than threefold above the endogenous level of V_αs were considered transgenic. Antibodies were purchased from PharMingen (San Diego, CA) unless otherwise stated. Biotinylated anti-V_α3.2 (RR3-16) (*8*) and anti-V_α2 (B20.1) (*6*) were used with streptavidin–fluorescein isothiocyanate (FITC). Anti-V_α3.1 (*7*) was used with goat anti-rabbit (H+L)-FITC. Red613-conjugated anti-CD4 (H129.19), anti-CD8α (53-6.7), and anti-Thy1.2-PE (30-H12) were obtained from Gibco BRL (Grand Island, NY).
17. The skewing pattern remained unchanged when analyzed as percent $V_\alpha3^+$ cells expressing CD4 or CD8. In the V_α3.1 transgenics, 20.1 ± 1.2% of $V_\alpha3.1^+$ cells were CD8$^+$ and 47.8 ± 2.4% were CD4$^+$. In the V_α3.1m (line 42) mice, 63.0 ± 6.9% of RR3-16$^+$ cells were CD8$^+$ and 14.9 ± 3.0% CD4$^+$. Peripheral CD4$^-$CD8$^-$ T cells were also present, as in other TCR transgenic lines (*29*). Of the Thy1$^+$ cells in the V_α3.1 transgenic mice, 40.8 ± 2.7% were CD4$^+$ and 24.2 ± 1.8% CD8$^+$, compared with 63.5 ± 6.7% and 30.3 ± 3.0% in the littermate controls. In V_α3.1m transgenic mice, 52.2 ± 5.5% of Thy1$^+$ cells were CD4$^+$ and 33.8 ± 3.4% were CD8$^+$, compared with the littermate values of 54.1 ± 5.8% and 34.3 ± 3.1%. The frequency of $V_\alpha3.1^+$ or RR3-16$^+$ cells within each T cell subset in nontransgenic littermates of each line was comparable to values obtained in wild-type B6 mice (*12*). Each line was second to fourth generation backcross to B6. All mice were homozygous for H-2^b.
18. The V_α3.1m and CDR1m proteins, but not the wild-type V_α3.1 or CDR2m, were detectable with the V_α3.2-specific monoclonal antibody, RR3-16 (*8*). Therefore, the specificity of RR3-16 resides in Phe^{27} in the CDR1 region.
19. Similar results were obtained by calculating the data as percent $V_\alpha3^+$ cells expressing CD4$^+$ or CD8$^+$. The RR3-16$^+$ T cells in CDR1m transgenic line 8 were 46.3 ± 6.1% CD8$^+$ and 29.3 ± 5.8% CD4$^+$. The CDR2m-expressing T cells in line 44 were 56.0 ± 5.0% CD8$^+$ and 22.8 ± 3.4% CD4$^+$. In the CDR1m transgenic mice, 48.7 ± 5.8% of Thy1$^+$ cells were CD4$^+$ and 36.3 ± 3.2% CD8$^+$. In CDR2m transgenic mice, 50.7 ± 4.5% of Thy1$^+$ cells were CD4$^+$ and 32.5 ± 5.7% CD8$^+$.
20. Expression of transgenic V_α3 on CD4$^+$CD8$^+$, CD4$^+$CD8$^-$, and CD4$^-$CD8$^+$ thymocytes, respectively. V_α3.1: 40.7, 43.4, and 39.0%; V_α3.1m (line 7): 10.4, 11.2, and 23.4%; CDR1m (line 8): 26.4, 17.4, and 29.5%; and CDR2m (line 44): 24.9, 16.4, and 31.1%.
21. Radiation bone marrow (BM) chimeras were prepared by standard techniques. Erythrocyte-free BM cells were isolated from the femur and tibia of donor mice and depleted of T cells by killing with anti-CD4, anti-CD8, and anti-Thy1 (clones RL172, 3-168, and T24.2.5, respectively) plus guinea pig complement (Gibco). Donor cells (3.0×10^6 to 1.0×10^7) were injected intravenously into irradiated host B10 mice [11 gray (Gy) of γ-irradiation]. Donor BM cells for V_α3.1m, CDR1m, and CDR2m were extracted from transgenics on the FVB background (Thy1.1, H-2^q). V_α3.1 BM was obtained from BALB/c background (Thy1.2, H-2^d) transgenic mice. Reconstituted splenocytes were examined by FACS after 8 to 10 weeks as described (*16*) with phycoerythrin–anti-mouse Thy1.1 (OX-7) or PE-H-$2K^d$ (SF1-1.1) to distinguish donor cells.
22. We stained these mice for V_β3-8, 10, 11, and 13, but did not see any significant differences.
23. F. R. Carbone, S. J. Sterry, J. Butler, S. Rodda, M. W. Moore, *Int. Immunol.* **4**, 861 (1992); L. J. Berg, B. Fazekas de St. Groth, A. M. Pullen, M. M. Davis, *Nature* **340**, 559 (1989).
24. Three-color FACS analysis was done as described (*16*). Data were analyzed as a percentage of the appropriate V_α3 in the $V_\beta3^+$ (KJ-25$^+$) or $V_\beta5^+$ (MR9-4$^+$) transgenic population of CD4 and CD8 T cells. The frequency of transgenic β-chain expression in the αβ transgenic mice was similar to that in the β-chain transgenics, as was the percentage of T cells in the periphery (*12*), indicating that this αβ combination was selectable by some endogenous peptide-MHC complexes. The V_β transgenic mice were bred at TSRI.
25. C. A. Janeway Jr. and K. Bottomly, *Semin. Immunol.* **8**, 109 (1996).
26. J. H. Brown *et al.*, *Nature* **332**, 845 (1988); J. H. Brown *et al.*, *ibid.* **364**, 33 (1993).
27. U. Dianzani, A. Shaw, B. K. Al-Ramadi, R. T. Kubo, C. A. Janeway Jr., *J. Immunol.* **148**, 678 (1991); I. F. Luescher *et al.*, *Nature* **373**, 353 (1995).
28. E. Jouvin-Marche *et al.*, *EMBO J.* **9**, 2141 (1990); N. R. J. Gascoigne, in *T Cell Receptors*, J. I. Bell, M. J. Owen, E. Simpson, Eds. (Oxford Univ. Press, Oxford, 1995), p. 288.
29. J. H. Russell *et al.*, *J. Immunol.* **144**, 3318 (1990); H. von Boehmer, J. Kirberg, B. Rocha, *J. Exp. Med.* **174**, 1001 (1991).
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Protection Against Atherogenesis in Mice Mediated by Human Apolipoprotein A-IV

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Apolipoproteins are protein constituents of plasma lipid transport particles. Human apolipoprotein A-IV (apoA-IV) was expressed in the liver of C57BL/6 mice and mice deficient in apoE, both of which are prone to atherosclerosis, to investigate whether apoA-IV protects against this disease. In transgenic C57BL/6 mice on an atherogenic diet, the serum concentration of high density lipoprotein (HDL) cholesterol increased by 35 percent, whereas the concentration of endogenous apoA-I decreased by 29 percent, relative to those in transgenic mice on a normal diet. Expression of human apoA-IV in apoE-deficient mice on a normal diet resulted in an even more severe atherogenic lipoprotein profile, without affecting the concentration of HDL cholesterol, than that in nontransgenic apoE-deficient mice. However, transgenic mice of both backgrounds showed a substantial reduction in the size of atherosclerotic lesions. Thus, apoA-IV appears to protect against atherosclerosis by a mechanism that does not involve an increase in HDL cholesterol concentration.

The role of apoA-IV in lipid transport and lipoprotein metabolism is not clear. In humans, apoA-IV is associated with triglyceride-rich lipoproteins and HDL, and also occurs in a lipoprotein-free form (*1–4*). ApoA-IV has been proposed to play a role in reverse cholesterol transport (cholesterol transport from tissues back to the liver for elimination) on the basis of in vitro properties: It activates lecithin cholesterol acyltransferase (*5*, *6*), promotes cholesterol efflux from cholesterol-preloaded cells (*7–9*), and binds to hepatocytes (*10*).

To investigate the function of apoA-IV, we generated transgenic mice that express human apoA-IV in the liver. The transgene comprised an 8.4-kb human genomic DNA fragment encoding apoA-IV linked to the 1.7-kb Pst I–Pst I fragment of the hepatic control region of the apoE/C-I gene (*11*). The DNA fragment was injected into one-cell embryos of C57BL/6 mice (IFFA-Credo, Lyon,

France), a strain that develops fatty streak lesions when maintained on an atherogenic diet (*12*). Two lines (HuA-IVTg5 and HuA-IVTg9) of transgenic animals were obtained, containing 12 and 4 copies, respectively, of the human gene. In both lines, human apoA-IV mRNA was detectable only in the liver. Plasma concentrations of human apoA-IV in HuA-IVTg5 and HuA-IVTg9 mice ranged from 500 to 1200 mg/dl and from 100 to 500 mg/dl, respectively. The HuA-IVTg5 mice were also crossed with apoE-deficient mice, which spontaneously develop severe atherosclerosis (*13*, *14*), to obtain HuA-IVTg5 ApoE$^{-/-}$ hybrid mice.

Total plasma cholesterol concentrations, lipoprotein cholesterol distribution, and mouse apoA-I, apoA-II, and apoA-IV concentrations were similar in transgenic and control mice on a Chow diet (Table 1). The plasma concentration of human apoA-IV doubled in transgenic mice on a high-fat, high-cholesterol diet. A 10-fold increase in non-HDL cholesterol concentration was observed in both transgenic and control mice on the high-fat diet. Transgenic mice on the high-fat diet showed a 35% increase in HDL cholesterol concentration, which was accompanied by a 29% decrease in the concentration of endogenous apoA-I. The correlation between mouse apoA-I and HDL cholesterol concentrations apparent in control mice ($P < 0.0017$) was not observed in transgenic mice. The increase in HDL cholesterol concentration and the decrease in mouse apoA-I in transgenic mice on a high-fat diet, together with the lack of correlation between mouse apoA-I and HDL cholesterol concentrations in these animals, suggest that apoA-IV may functionally substitute for apoA-I and form HDL-like particles.

Expression of human apoA-IV (serum concentration, 160 to 2040 mg/dl) in apoE-deficient mice on a normal diet resulted in a 2.6-fold increase in non-HDL cholesterol concentrations relative to those in nontransgenic apoE-deficient mice [1241 ± 58 versus 471 ± 75 mg/dl (means ± SEM), $P < 0.0002$, $n = 5$ transgenic versus 5 nontransgenic apoE-deficient mice] at 8 months of age; no difference in HDL cholesterol concentrations was apparent (26 ± 5 versus 23 ± 2 mg/dl). Concentrations of apoA-I and apoA-II were similar between transgenic and nontransgenic apoE-deficient mice (apoA-I, 76 ± 5 versus 78 ± 6 mg/dl; apoA-II, 38 ± 8 versus 31 ± 10 mg/dl). The atherogenic lipoprotein profile in human apoA-IV transgenic apoE-deficient mice was therefore even more pronounced than that in nontransgenic apoE-deficient mice, with a 2.75-fold increase in the ratio of non-HDL cholesterol to HDL cholesterol (55 ± 10 versus 20 ± 2, $P < 0.05$).

We also analyzed fatty streak lesions and atherogenic plaques in transgenic and control C57BL/6 mice maintained on an atherogenic diet for 20 weeks, as well as in transgenic and control apoE-deficient mice on a regular diet. Mean lesion areas were 18 and 12 times greater in control mice than in HuA-IVTg9 and HuA-IVTg5 mice, respectively (Fig. 1A), despite the reduction in murine apoA-I concentration in the transgenic animals. Compared with control apoE-deficient mice, transgenic apoE-deficient mice also showed retarded development of atherosclerosis (70% reduction in lesion size) (Fig. 1B), with fewer fibroproliferative lesions, by 8 months of age, despite the more pronounced atherogenic lipoprotein profile. In addition, the apoE-deficient mice allowed us to exclude the possibility that the atherogenic diet was hepatotoxic. A marked reciprocal relation was apparent between total (human + mouse) apoA-IV concentration and atherosclerosis in apoE-deficient mice ($P < 0.0021$) (Fig. 2), whereas the mean lesion

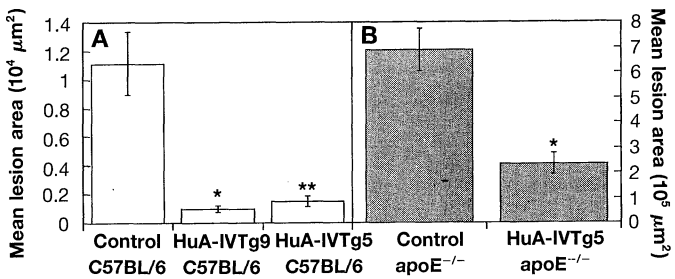

Fig. 1. Area of atherosclerotic lesions (per section) in control mice and transgenic mice expressing human apoA-IV. (**A**) Female C57BL/6 mice were anesthetized and exsanguinated at 30 weeks of age after 20 weeks on a high-fat, high-cholesterol diet. Data are means ± SEM. *$P <$ 0.006, **$P < 0.0003$ versus controls (Student's t test). (**B**) ApoE-deficient mice were examined after 8 months on a Chow diet. Data are means ± SEM. *$P <$ 0.0013 versus control mice (Student's t test). In both (A) and (B), sections were selected as previously described (*15*, *28*). The areas of oil red O staining were evaluated in four 10-μm aortic sections (per mouse) separated by 80 μm for the C57BL/6 mice, and in 21 10-μm aortic sections (per mouse) separated by 50 μm for apoE-deficient mice.

Table 1. Serum concentrations of cholesterol and apolipoproteins in transgenic female C57BL/6 mice expressing human apoA-IV (HuA-IVTg5) (n = 15) and control female littermates (n = 17). Blood was collected from the retro-orbital plexus of animals that had been deprived of food for 4 hours into a tube containing 6 μl of 0.5 M EDTA, gentamycin sulfate (50 μg/ml), and 0.1% NaN_3. Plasma was separated by centrifugation at 2000*g* for 15 min and stored at 4°C until analysis. HDL was separated from apoB-containing lipoproteins by precipitation with dextran sulfate. Cholesterol was assayed enzymatically by incubation of 10 μl of diluted plasma with 200 μl of a cholesterol reagent (Boehringer Mannheim) and measurement of absorbance at 490 nm with a microplate reader (Bio-Teck Instruments EL 311). Human apoA-IV and mouse apoA-I, apoA-II, and apoA-IV were quantified by electroimmunodiffusion with rabbit polyclonal antibodies. The antibodies to human and to mouse apoA-IV showed no cross-species reactivity. Purina laboratory mouse chow 5001 diet (Purina Mills International, St. Louis, MO) contained <0.03% (w/w) cholesterol, 4.5% (w/w) animal fat, and no casein or sodium cholate. Custom high-fat diet (ICN Biomedical, Cleveland, OH) contained 1.25% (w/w) cholesterol, 15% (w/w) fat, 7.5% (w/w) casein, and 0.5% (w/w) sodium cholate. Analyses were performed on mice maintained for 10 weeks on a Chow diet and again after they had been switched for 20 weeks to the high-fat atherogenic diet. Data are means ± SEM.

Analyte (mg/dl)	HuA-IVTg5		Control C57BL/6	
	Chow diet	High-fat diet	Chow diet	High-fat diet
Total cholesterol	68 ± 5	194 ± 7	67 ± 6	171 ± 15*
Non-HDL cholesterol	11 ± 4	117 ± 8	11 ± 6	119 ± 9
HDL cholesterol	57 ± 4	77 ± 5	56 ± 8	52 ± 7†
Mouse apoA-I	93 ± 4	66 ± 2	102 ± 5	85 ± 6‡
Mouse apoA-II	39 ± 7	41 ± 2	49 ± 10	42 ± 3
Mouse apoA-IV	30 ± 10	40 ± 9	27 ± 11	37 ± 8
Human apoA-IV	690 ± 40	1440 ± 70		

*$P < 0.05$, †$P < 0.0002$, ‡$P < 0.004$ versus transgenic mice on the high-fat diet (Student's t test).

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area was independent of HDL cholesterol concentration.

Another human apolipoprotein, apoA-I, also inhibits atherogenesis in mice (*15*) and rabbits (*16*). However, there are important differences between apoA-I and apoA-IV. First, although apoA-IV retards the development of atherosclerosis, it has only a small, if any, effect on HDL cholesterol concentrations. In contrast, HDL cholesterol concentrations are substantially increased and the ratio of non-HDL cholesterol to HDL cholesterol is substantially reduced in human apoA-I transgenic animals (*15*, *16*), even in those with apoE deficiency (*17*, *18*). Second, the distribution of apoA-IV in plasma differs from that of apoA-I. On electrophoresis, most human apoA-IV and the endogenous apoA-IV in the serum of transgenic mice migrates to the preβ position (Fig. 3), in contrast to apoA-I, which migrates mainly to the α position. These electrophoretic mobilities are similar to those observed with human plasma (*19*, *20*). Therefore, most of the apoA-IV in transgenic mice is not associated with apoA-I–containing lipoproteins and thus is not directly related to apoA-I–HDL metabolism.

ApoA-IV–containing lipoproteins may participate in reverse cholesterol transport, the first step of which is cholesterol efflux from peripheral cells to lipoprotein acceptors. Preβ particles are the primary acceptors of cellular cholesterol (*21*). We therefore studied cholesterol efflux from cholesterol-loaded Fu5AH rat hepatoma cells (*22*) in the presence of plasma from transgenic or control mice. Cholesterol efflux after incubation of cells for 2 hours with 2.5% diluted serum from HuA-IVTg5 transgenic mice on an atherogenic diet was 134 ± 5% (mean ± SEM, $n = 5$, $P < 0.03$) of that apparent with serum from control mice on the same diet. These results suggest that an increased capacity to promote cholesterol efflux was established in the serum of the apoA-IV transgenic mice.

The protection against atherogenesis in human apoA-IV transgenic mice could also be related to the observation that the catabolism of apoA-IV occurs more rapidly than that of apoA-I (*23*). We propose that, in addition to its forming large amounts of preβ particles, the rapid catabolism of apoA-IV may explain why apoA-IV–mediated reverse cholesterol transport is more efficient than that mediated by apoA-I. It is also possible that apoA-IV, which is present in high concentrations as lipid-poor protein (*9*), serves as an apolipoprotein reservoir for particle lipid enrichment at the site of plaque formation (*24*). In this respect, it may act similarly to apolipophorin III in insect hemolymph (*25*). Apolipophorin III exists mainly as a lipid-free protein and serves as a reservoir of amphiphilic surface protein capable of reversibly stabilizing lipid-enriched lipoproteins. Specifically, apolipophorin III associates with and stabilizes lipid droplets formed during the delivery of diacylglycerol from the fat body to the muscle (*25*, *26*). Other mechanisms may also contribute to the antiatherogenic action of apoA-IV.

The effects of apoA-IV described here may help to explain the absence of atherosclerosis in apoA-I–deficient mice (*27*). Our results also suggest possible new approaches to the treatment of atherosclerosis that do not require an increase in apoA-I and HDL cholesterol concentrations.

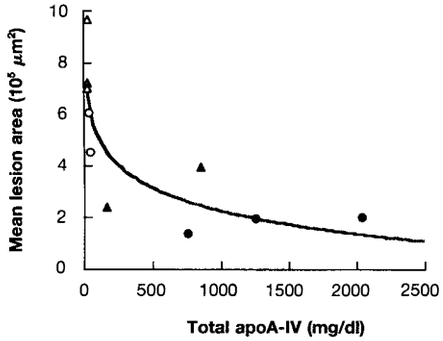

Fig. 2. Mean lesion area plotted against total (human + mouse) apoA-IV concentration in the serum of transgenic and control apoE-deficient mice at 8 months of age. Three male (●) and two female (▲) HuA-IVTg5 transgenic apoE-deficient mice as well as two male (○) and three female (△) control apoE-deficient mice were investigated.

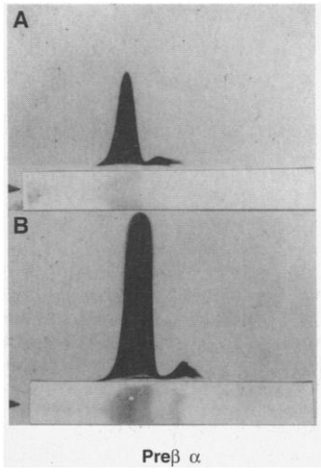

Fig. 3. Crossed immunoelectrophoresis of plasma from HuA-IVTg5 mice on a Chow diet (**A**) or on an atherogenic diet (**B**). Lipoprotein electrophoresis was performed in duplicate for each animal, and the separated lipoproteins were subjected to electroimmunodiffusion in agarose containing antiserum to human apoA-IV as well as to immunoblot analysis with antiserum to rat apoA-IV. The nitrocellulose strips subjected to immunoblot analysis for endogenous apoA-IV (arrowheads) are shown below the respective electroimmunodiffusion patterns for comparison. Most endogenous apoA-IV and the human apoA-IV in transgenic animals comigrated to the same preβ position. A minor fraction of both apoA-IV species migrated more rapidly to the α position; this fraction increased proportionally when the mice were fed an atherogenic diet. The origin for lipoprotein electrophoresis is on the left.

REFERENCES AND NOTES

1. C. L. Bisgaier, O. P. Sachdev, L. Megna, R. M. Glickman, *J. Lipid Res.* **26**, 11 (1985).
2. R. B. Weinberg and M. S. Spector, *J. Biol. Chem.* **260**, 14279 (1985).
3. L. Lagrost, P. Gambert, M. Boquillon, J. Lallemant, *J. Lipid Res.* **30**, 1525 (1989).
4. M. Lefevre and P. Roheim, *ibid.* **25**, 1603 (1984).
5. A. Steinmetz and G. Utermann, *J. Biol. Chem.* **260**, 2258 (1985).
6. C. H. Chen and J. J. Albers, *Biochim. Biophys. Acta* **836**, 279 (1985).
7. O. Stein, Y. Stein, M. Lefevre, P. S. Roheim, *ibid.* **878**, 7 (1986).
8. A. Steinmetz *et al.*, *J. Biol. Chem.* **265**, 7859 (1990).
9. N. Duverger *et al.*, *Arterioscler. Thromb.* **13**, 126 (1993).
10. E. Dvorin, N. L. Gorder, D. M. Benson, A. Gotto, *J. Biol. Chem.* **261**, 15714 (1986).
11. S. W. Simonet, N. Bucay, S. T. Lauer, J. M. Taylor, *ibid.* **268**, 8221 (1993).
12. J. H. Quiao *et al.*, *Arterioscler. Thromb.* **14**, 1480 (1994).
13. A. S. Plump *et al.*, *Cell* **71**, 343 (1992).
14. S. H. Zhang, R. L. Reddick, J. A. Piedrahita, N. Maeda, *Science* **258**, 468 (1992).
15. E. M. Rubin, R. M. Krauss, E. A. Spangier, J. G. Verstuyft, S. M. Gift, *Nature* **353**, 265 (1991).
16. N. Duverger *et al.*, *Circulation* **94**, 713 (1996).
17. C. Paszty, N. Maeda, J. Verstuyft, E. M. Rubin, *J. Clin. Invest.* **94**, 899 (1994).
18. A. S. Plump, C. J. Scott, J. L. Breslow, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 9607 (1994).
19. A. Von Eckardstein *et al.*, *Arterioscler. Thromb. Vasc. Biol.* **15**, 1755 (1995).
20. U. Beisiegel and G. Utermann, *Eur. J. Biochem.* **93**, 601 (1979).
21. G. Castro and C. Fielding, *Biochemistry* **27**, 25 (1988).
22. M. de la Llera *et al.*, *Arterioscler. Thromb.* **14**, 1056 (1994).
23. D. Rader *et al.*, *J. Clin. Invest.* **92**, 1009 (1993).
24. R. B. Weinberg, J. A. Ibdah, M. C. Phillips, *J. Biol. Chem.* **267**, 8977 (1992).
25. R. O. Ryan, *J. Lipid Res.* **31**, 1725 (1990).
26. _____, K. Oikawa, C. M. Kay, *J. Biol. Chem.* **268**, 1525 (1993).
27. H. Li, R. L. Reddick, N. Maeda, *Arterioscler. Thromb.* **13**; 1814 (1993).
28. B. Paigen, A. Morrow, P. A. Holmes, D. Mitchel, R. A. Williams, *Atherosclerosis* **68**, 231 (1987).

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