Expression of human apolipoprotein A-II in apolipoprotein E-deficient mice induces features of familial combined hyperlipidemia

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Abstract **Familial combined hyperlipidemia (FCHL) is a common inherited hyperlipidemia and a major risk factor for atherothrombotic cardiovascular disease. The cause(s) leading to FCHL are largely unknown, but the existence of unidentified "major" genes that would increase VLDL production and of "modifier" genes that would influence the phenotype of the disease has been proposed. Expression of apolipoprotein A-II (apoA-II), a high density lipoprotein (HDL) of unknown function, in transgenic mice produced increased concentration of apoB-containing lipoproteins and decreased HDL. Here we show that expression of human apoA-II in apoE-deficient mice induces a dose-dependent increase in VLDL, resulting in plasma triglyceride elevations of up to 24-fold in a mouse line that has 2-fold the concentration of human apoA-II of normolipidemic humans, as well as other well-known characteristics of FCHL: increased concentrations of cholesterol, triglyceride, and apoB in very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low density lipoprotein (LDL), reduced HDL cholesterol, normal lipoprotein lipase and hepatic lipase activities, increased production of VLDL triglycerides, and increased susceptibility to atherosclerosis. However, FCHL patients do not have plasma concentrations of human apoA-II as high as those of apoE-deficient mice overexpressing human apoA-II, and the apoA-II gene has not been linked to FCHL in genome-wide scans. Therefore, the apoA-II gene could be a "modifier" FCHL gene influencing the phenotype of the disease in some individuals through unkown mechanisms including an action on a "major" FCHL gene. We conclude that apoE-deficient mice overexpressing human apoA-II constitute useful animal models with which to study the mechanisms leading to overproduction of VLDL, and that apoA-II may function to regulate VLDL production.**—Escolà-Gil, J. C., J. Julve, À. Marzal-Casacuberta, J. Ordóñez-Llanos, F. González-Sastre, and F. Blanco-Vaca. **Expression of human apolipoprotein A-II in apolipoprotein E-deficient mice induces features of familial combined hyperlipidemia.** ***J. Lipid Res.*** **2000.** 41: **1328–1338.**

Supplementary key words apoB • atherosclerosis • high density lipoprotein • transgenic mice • very low density lipoprotein

High density lipoprotein (HDL) particles are classified according to the content of their major apolipoproteins, namely apolipoprotein A-I (apoA-I) and apoA-II. ApoA-I is required to maintain HDL structure, induces specific and nonspecific cholesterol efflux, activates lecithin: cholesterol acyltransferase (LCAT), is an in vivo ligand of the scavenger receptor B-I, and plays an antiatherogenic role, as has been clearly established by studies in both transgenic and knockout mice (1, 2). In contrast, the physiologic role of apoA-II is poorly defined. Studies in mouse apoA-II transgenic mice revealed an increase in atherosclerosis susceptibility that was consistent only when the mice were on a regular chow diet (3), whereas double human apoA-I/apoA-II transgenic mice lost part of the protection against atherosclerosis shown by human apoA-I transgenic mice fed an atherogenic diet (4). Mouse and human apoA-II present significant differences in structure that result in different effects in HDL size and concentration (5–7). For this reason we expressed different levels of human apoA-II in transgenic mice, without concomitant expression of human apoA-I, and found a dose-related increase in plasma triglycerides and decreased HDL due, at least partially, to an LCAT functional deficiency (8). These mice showed increased atherosclerosis susceptibility, but this was seen exclusively when they were fed an atherogenic diet (9); however, this diet has proinflammatory and hepatotoxic effects that could interfere with studies of atherosclerosis (1). To rule out this possibility

Abbreviations: ALT, alanine aminotransferase; apo, apolipoprotein; AST, aspartate aminotransferase; FCHL, familial combined hyperlipidemia; FFA, free fatty acid; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoprotein; LDP, lipoprotein-depleted plasma; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; VLDL, very low density lipoprotein.

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and to study a possible species-specific interaction with diet, in the context of gaining insight into the pathophysiological role of human apoA-II as a goal, we cross-bred human apoA-II transgenic mice with apoE-deficient mice (apoE$^{-/-}$). ApoE$^{-/-}$ mice are susceptible to massive atherosclerosis, even when fed a regular chow diet with only 4% fat (10, 11). To our surprise, apoE$^{-/-}$ mice overexpressing human apoA-II and fed a regular chow diet showed a 24-fold increase in plasma triglyceride concentration compared with apoE$^{-/-}$ mice. This and the results of subsequent experiments performed to investigate the mechanism(s) underlying this hypertriglyceridemia demonstrated that human apoA-II gene expression in mice caused effects that would be expected from a familial combined hyperlipidemia (FCHL) gene. Plasma apoA-II concentration is known to be similar or lower in patients with hypertriglyceridemia and/or myocardial infarction than in control subjects (12). However, our results are consistent with those of investigations that illustrate the pathophysiological role of apoA-II (7, 12, 13). Also, it is of note that genome-wide scans seeking genes causing FCHL detected a "major" locus causing this disease in a region containing the apoA-II gene, even though this locus was outside the maximum interval of linkage (14) and established the LCAT locus as a potential "modifier" FCHL gene (15).

MATERIALS AND METHODS

Mice

Mice were maintained in a temperature-controlled (20°C) room with a 12-h light/dark cycle and food and water ad libitum. All animal procedures were in accordance with published recommendations for the use of laboratory animals (16). Human apoA-II transgenic mice (lines 25.3 and 11.1, which in this study presented, respectively, plasma human apoA-II <20 mg/dL and >40 mg/dL) were created in the C57BL/6 background by injection of a 3-kilobase pair fragment containing the human apoA-II gene, which was isolated after digestion of genomic DNA with *Msp*I (8). Homozygous apoE$^{-/-}$ mice were created in the 129/Ola background (17). The apoE$^{-/-}$ mice were crossed with C57BL/6 control mice for four generations and, thereafter, crossed with C57BL/6 human apoA-II transgenic mice to produce offspring with the human apoA-II transgene and heterozygous for the apoE null allele (apoE$^{-/+}$). To produce study populations, these mice were inbred to generate the apoE$^{-/-}$ mice overexpressing human apoA-II (line 11.1), apoE$^{-/-}$ mice that expressed low concentrations of human apoA-II (line 25.3), and apoE$^{-/-}$ mice (also with 97% genetic background of C57BL/6). ApoE$^{-/-}$ mice were distinguished from apoE$^{+/-}$ as described (17) and genotype assignment of the mice was confirmed by plasma cholesterol levels. Regular chow-fed (Rodent Toxicology Diet; B&K Universal, N. Humberside, UK) mice were used for studies at 12–13 weeks of age.

Plasma lipids, lipoproteins, and apolipoproteins

Plasma total cholesterol, free cholesterol, and triglycerides were determined enzymatically with commercial kits adapted to a BM/HITACHI 911 autoanalyzer (Boehringer GmbH, Mannheim, Germany). Triglyceride determinations were corrected for the free glycerol present in plasma (Sigma, St. Louis, MO). HDL cholesterol was measured after precipitation with phosphotungstic acid and magnesium ions (Boehringer GmbH). For this purpose, lipidemic plasmas were diluted 1:2 with saline prior to precipitation of apoB-containing lipoproteins and, in all cases, the supernatant was clear. Non-HDL cholesterol was calculated as the difference between total cholesterol and HDL cholesterol.

The lipoproteins of 0.2 mL of pooled filtered plasma were fractionated by fast performance liquid chromatography (FPLC), using a Superosa 6HR column (Pharmacia Biotech, Uppsala, Sweden) and their content in cholesterol and triglycerides was measured (8). When required, larger quantities of isolated lipoproteins were isolated by sequential ultracentrifugation (8). Lipoprotein protein concentrations were determined by the method of Bradford (Bio-Rad Laboratories, Hercules, CA) (18). Plasma human apoA-II concentrations were measured by a commercial single radial immunodiffusion method (Daiichi Pure Chemicals, Tokyo, Japan).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to study the content of apoB-100 and apoB-48 in lipoprotein families of $d < 1.063$ g/mL.

Free fatty acids, glucose, and insulin determinations

Glucose and free fatty acid (FFA) concentrations in plasma were measured with commercial kits (Boehringer GmbH and Wako [Neuss, Germany], respectively) adapted to the automated analyzer BM/HITACHI 911. Insulin levels were measured by a commercial radioimmunoassay that incorporates an antibody to rat insulin (WAK-Chemie Medical GmbH, Bad Homburg, Germany).

Enzyme activities

Lipoprotein lipase (LPL) and hepatic lipase (HL) activities against exogenous substrates were measured in postheparin plasmas, using a radiolabeled glycerol tri[^{3}H]oleate emulsion (Amersham Life Science, Bristol, UK) as described previously (19). Paraoxonase/arylesterase activity was assayed using 1.0 mM phenylacetate as substrate (20) in plasma obtained from blood collected in lithium–heparin tubes.

In vivo removal of labeled VLDL

The very low density lipoprotein (VLDL) fraction isolated by ultracentrifugation from each type of mouse was radiolabeled with [^{3}H]triolein or [^{3}H]cholesteryl oleoyl ether as described (7). In both cases, approximately 250,000 cpm was injected intravenously into fasted anesthetized mice and serial blood samples were collected for ^{3}H radioactivity counting. The average radioactivity observed 2 min after injection was defined as 100% of injected radioactivity. At the end of the experiments, organs were extracted, homogenized in chloroform–methanol, and counted.

In vivo triglyceride production rate

Triglyceride production rates in plasma were measured as described (21). Briefly, mice were bled to measure baseline plasma triglyceride. Anesthetized mice were then subjected to an intravenous injection of Triton WR-1339 (Sigma) in a dose of 500 mg/kg, dissolved in a 15% solution of 0.9% NaCl. Blood was collected after Triton injection and plasma triglycerides and FFA were measured and compared with baseline results.

Liver lipid content and plasma hepatic enzymes

Liver lipids were extracted with isopropyl alcohol–hexane as described (22), dried with nitrogen, reconstituted with isopropyl alcohol–0.5% sodium cholate, and sonicated for 10 min (50 Hz) on ice, prior to lipid measurements. Plasma alanine transferase (ALT) and aspartate transferase (AST) activities were measured with commercial kits (Boehringer GmbH) adapted to a BM/HITACHI 911 autoanalyzer.

Evaluation of atherosclerosis

After an overnight fast, mice were anesthetized with isofluorane, exsanguinated, and killed by cervical dislocation. The heart and proximal aorta were removed, embedded in O.C.T. compound (Tissue-Tek, Sakura Finetechnical, Tokyo, Japan), sectioned, and stained. Lesioned areas were quantified as previously described (9).

Statistical analysis

All values are given as means ± SEM. Comparison of data for two groups was performed by Student's *t*-test or Mann-Whitney U test, depending on whether the distribution of data was Gaussian or not. Analysis of atherosclerosis incidence in coronary arteries was analyzed by chi-square test. A value of $P < 0.05$ was considered significant.

RESULTS

Lipids, lipoproteins, and apolipoproteins

ApoE$^{-/-}$ mice overexpressing human apoA-II exhibited dramatically elevated plasma triglycerides, 18- and 30-fold higher, respectively, than male and female apoE$^{-/-}$ mice (**Table 1**). ApoE$^{-/-}$ mice expressing low levels of human apoA-II (hA-II) had moderate increases in plasma triglycerides (1.2- and 1.5-fold in male and female mice, respectively, compared with apoE$^{-/-}$ mice). Total cholesterol levels in plasma of male and female apoE$^{-/-}$ mice overexpressing human apoA-II were 2.4- and 2.7-fold elevated, respectively, compared with apoE$^{-/-}$ mice. This elevation was 1.2-fold, both in males and females, in apoE$^{-/-}$ mice expressing low levels of apoA-II. Hypercholesterolemia in mice expressing human apoA-II was, in all cases, the result of a substantial increase in non-HDL cholesterol, because HDL cholesterol remained stable in mice expressing low concentrations of human apoA-II and was greatly decreased in mice overexpressing human apoA-II.

To confirm and expand the data obtained by the study of the lipid profile, we separated the lipoproteins of these mice by FPLC and sequential ultracentrifugation and analyzed their lipid and protein content (**Fig. 1**). These analyses (Fig. 1A), consistent with the results of Table 1, revealed that mice expressing human apoA-II exhibited a dose-related increase in apoB-containing lipoproteins. Only 7% (in males) and 18% (in females) of plasma triglycerides of apoE$^{-/-}$ mice overexpressing apoA-II floated as chylomicrons, with the bulk of triglycerides associated with VLDL. The elevation of apoB-containing lipoproteins was due to an increase in both lipids and proteins (Fig. 1B and C). ApoB-48 and apoB-100, associated with VLDL, intermediate density lipoprotein (IDL), and low density lipoprotein (LDL), were increased in mice expressing human apoA-II, as judged by SDS-PAGE analysis (Fig. 1D). Interestingly, a major proportion of human apoA-II (66% in males and 53% in females) was associated with apoB-containing lipoproteins in apoE$^{-/-}$ mice overexpressing the human transgene.

TABLE 1. Plasma lipids of 12- to 13-week-old fasted chow-fed apoE$^{-/-}$ mice with low (apoE$^{-/-}$ low hA-II) or high (apoE$^{-/-}$ high hA-II) plasma human apoA-II concentrations

	ApoE$^{-/-}$	ApoE$^{-/-}$ Low hA-II	ApoE$^{-/-}$ High hA-II
Males	n = 15	n = 13	n = 9
Total cholesterol	294 ± 13	353 ± 22[a]	701 ± 69[a]
Non-HDL cholesterol	264 ± 15	320 ± 22[a]	693 ± 68[a]
HDL cholesterol	30 ± 3	33 ± 3	8 ± 2[a]
% Free cholesterol	30 ± 1	29 ± 1	40 ± 1[a]
Triglycerides	37 ± 3	46 ± 8	668 ± 126[a]
Human apoA-II	0 ± 0	12 ± 1[a]	66 ± 7[a]
Females	n = 14	n = 11	n = 8
Total cholesterol	325 ± 10	389 ± 20[a]	892 ± 52[a]
Non-HDL cholesterol	306 ± 10	368 ± 20[a]	881 ± 53[a]
HDL cholesterol	19 ± 1	20 ± 2	11 ± 2[a]
% Free cholesterol	29 ± 1	28 ± 1	36 ± 1[a]
Triglycerides	25 ± 3	38 ± 5[a]	757 ± 129[a]
Human apoA-II	0 ± 0	10 ±1[a]	78 ± 4[a]

Results are shown as means ± SEM. The units used are mg/dL.
[a]Significantly different ($P < 0.05$) from apoE$^{-/-}$ mice.

Weight and plasma concentration of FFA, glucose, and insulin

FFA plasma concentrations were slightly or clearly elevated in apoE$^{-/-}$ mice expressing low levels or high levels of human apoA-II, respectively. Weight and fasting glucose and insulin concentrations did not differ significantly between apoE$^{-/-}$ mice overexpressing human apoA-II and apoE$^{-/-}$ mice (**Table 2**).

Correlations of human apoA-II and lipid parameters

Human apoA-II correlated with plasma triglycerides (n = 70, 37 males and 33 females) in both a linear and a polynomial model. In the linear model, human apoA-II predicted 88 and 85% of the variance of triglycerides (r = 0.94 and 0.92, $P < 0.01$) of male and female mice, respectively. When fit to a second order polynomial model, human apoA-II enhanced the predicted percentage of the variance in triglycerides to 96 and 94% in male ($y = 0.11x^2 + 1.7x + 25$; $r = 0.98$, $P < 0.01$) and female ($y = 0.18x^2 + 5.7x + 48$; $r = 0.97$, $P < 0.01$) mice, respectively. A strong relationship was also found between human apoA-II and non-HDL cholesterol levels. When fit to a linear model, human apoA-II predicted 86 and 90% of the variance of non-HDL cholesterol in male ($y = 6.7x + 258$; $r = 0.93$, $P < 0.01$) and female ($y = 7.2x + 302$; $r = 0.95$; $P < 0.01$) mice. Other significant ($P < 0.01$) Pearson correlation coefficients were found between human apoA-II and FFA ($r = 0.76$ and $r = 0.87$ in male and female mice, respectively), between human apoA-II and percentage of free cholesterol ($r = 0.74$ in both male and female mice) and between human apoA-II and HDL cholesterol ($r = -0.57$ and -0.49 in male and female mice, respectively).

Lipolytic activities and in vivo VLDL triglyceride metabolism

Activities of LPL and HL toward exogenous substrates were measured in postheparin plasma. No significant differences in the activity of either enzyme were observed when the different groups of mice were compared (**Fig. 2A** and **B**). To rule out that VLDL containing human apoA-II was a poor substrate for one or both enzymes,

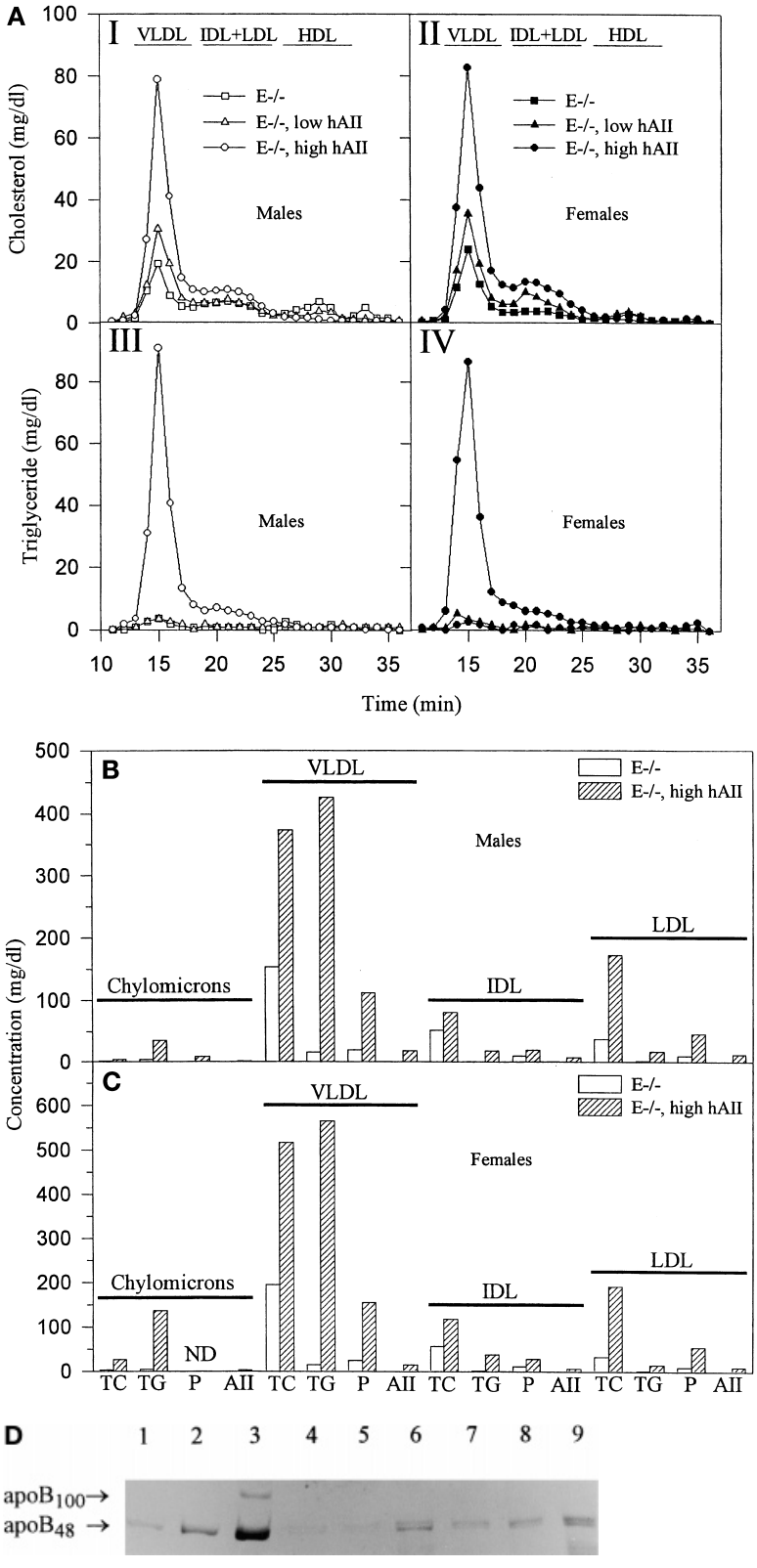

Fig. 1. Analysis of plasma lipoproteins. (A) Cholesterol and triglyceride concentrations of 1-mL lipoprotein fractions isolated by FPLC from pooled plasma (6–8 fasted animals from each group). The positions of elution of the VLDL, IDL/LDL, and HDL are represented by horizontal lines. (B) Concentrations of total cholesterol (TC), triglycerides (TG), proteins (P), and apoA-II (AII) of apoB-containing lipoproteins isolated by sequential ultracentrifugation from male mice. Concentrations expressed are those found in each lipoprotein family in plasma. Notice that the human apoA-II concentration in apoB-containing lipoproteins represented ~60% of the total human apoA-II in plasma. ND, Not determined. (C) Same as (B), but for female mice. (D) SDS-PAGE analysis of the apoB-100 and apoB-48 contained in lipoprotein plasma fractions of mice. Plasma (1 mL) from 4–6 female and male mice from each group was pooled and apoB-containing lipoprotein fractions were separated by sequential ultracentrifugation, desalted, and concentrated. Samples equivalent to 10 μL of original plasma were denatured and electrophoresed. Lanes 1, 4, and 7, apoE$^{-/-}$ mice; lanes 2, 5, and 8, apoE$^{-/-}$ mice expressing low levels of human apoA-II; lanes 3, 6, and 9, apoE$^{-/-}$ mice overexpressing human apoA-II. Lanes 1–3, VLDL; lanes 4–6, IDL; lanes 7–9, LDL. ApoB-100 and apoB-48 bands are indicated by arrows. In all panels, 12- to 13-week-old fasted chow-fed apoE$^{-/-}$ mice with low (apoE$^{-/-}$ low hA-II) or high (apoE$^{-/-}$ high hA-II) human apoA-II plasma concentrations are presented.

VLDL of each type of mouse was isolated and radiolabeled with triolein or cholesteryl oleoyl ether. [^{3}H]Triolein-VLDL clearance in apoE$^{-/-}$ mice was similar to that of apoE$^{-/-}$ mice expressing low concentrations of human apoA-II or that of apoE$^{-/-}$ mice overexpressing human apoA-II (Fig. 2C and D). In addition, no differences were found in the tissue content of [^{3}H]triolein in liver, adipose tissue, skeletal muscle, and heart (data not shown).

In vivo triglyceride production rates were determined by the accumulation of triglycerides in the fasting plasma of mice after intravenous injection of Triton WR-1339 (**Fig. 3A** and **B**), which, as we confirmed in our experi-

TABLE 2. Body weight and plasma concentrations of fasting glucose, insulin, and FFA of 12- to 13-week-old fasted chow-fed apoE$^{-/-}$ mice with low (apoE$^{-/-}$ low hA-II) or high (apoE$^{-/-}$ high hA-II) plasma human apoA-II concentrations

	ApoE$^{-/-}$	ApoE$^{-/-}$ Low hA-II	ApoE$^{-/-}$ High hA-II
Males	n = 15	n = 13	n = 9
Body weight (g)	25.5 ± 0.5	25.3 ± 0.6	25.7 ± 0.9
Glucose (mg/dL)	171 ± 11	ND	150 ± 11
Insulin (ng/mL)	0.24 ± 0.04	ND	0.25 ± 0.08
FFA (mg/dL)	41 ± 3	47 ± 5	54 ± 6[a]
Females	n = 14	n = 11	n = 8
Body weight (g)	19.3 ± 0.3	18.7 ± 0.6	19.4 ± 0.4
Glucose (mg/dL)	165 ± 7	ND	180 ± 26
Insulin (ng/mL)	0.25 ± 0.08	ND	0.23 ± 0.07
FFA (mg/dL)	37 ± 1	45 ± 3[a]	71 ± 5[a]

Data are from the same experiment shown in Table 1. Results are shown as means ± SEM. ND, not determined.

[a] Significantly different ($P < 0.05$) from apoE$^{-/-}$ mice.

mental conditions, completely blocks plasma triglyceride clearance (21). Triglyceride production rates were increased in male and female apoE$^{-/-}$ mice overexpressing human apoA-II (60 min: 271 ± 35 and 287 ± 16 mg/dL; 120 min: 648 ± 72 and 597 ± 30 mg/dL, respectively) compared with those of male and female apoE$^{-/-}$ mice (60 min: 195 ± 50 and 195 ± 22 mg/dL; 120 min: 374 ± 97 and 406 ± 43 mg/dL, respectively). Male apoE$^{-/-}$ mice expressing low concentrations of human apoA-II had triglyceride production rates similar to those of male apoE$^{-/-}$ mice, whereas female apoE$^{-/-}$ mice expressing low concentrations of human apoA-II had a moderately increased triglyceride production compared with female apoE$^{-/-}$ mice. Because part of the triglyceride synthesized in the liver is formed from FFA extracted from plasma (12), FFA in plasma was simultaneously determined (Fig. 3C and D). FFA levels were decreased at 60 and 120 min in male and female apoE$^{-/-}$ mice overexpressing human apoA-II mice (60 min: −15 ± 4.2 and −14.4 ± 6.8 mg/dL; 120 min: −18.5 ± 5.6 and −15.1 ± 8.3 mg/dL, respectively) compared with apoE$^{-/-}$ mice (60 min: −4 ± 2.2 and −1.2 ± 3.2 mg/dL; 120 min: −1.8 ± 1.7 and −2.9 ± 4 mg/dL in male and female mice, respectively). No differences were found between apoE$^{-/-}$ mice expressing low levels of human apoA-II and apoE$^{-/-}$ mice.

Other biochemical parameters

Absence of kidney failure in all mouse lines was verified by plasma creatinine measurement (data not shown). Absence of liver disease and lipid content of livers of the different mouse lines were assessed and compared by several measurements (**Table 3**). Liver weight and plasma ALT and AST activities did not differ when the various animal lines were compared. A tendency toward a higher content of total cholesterol was observed in the livers of mice expressing human apoA-II. In contrast, liver triglyceride content was clearly reduced (7.4-fold and 2.25-fold, $P <$

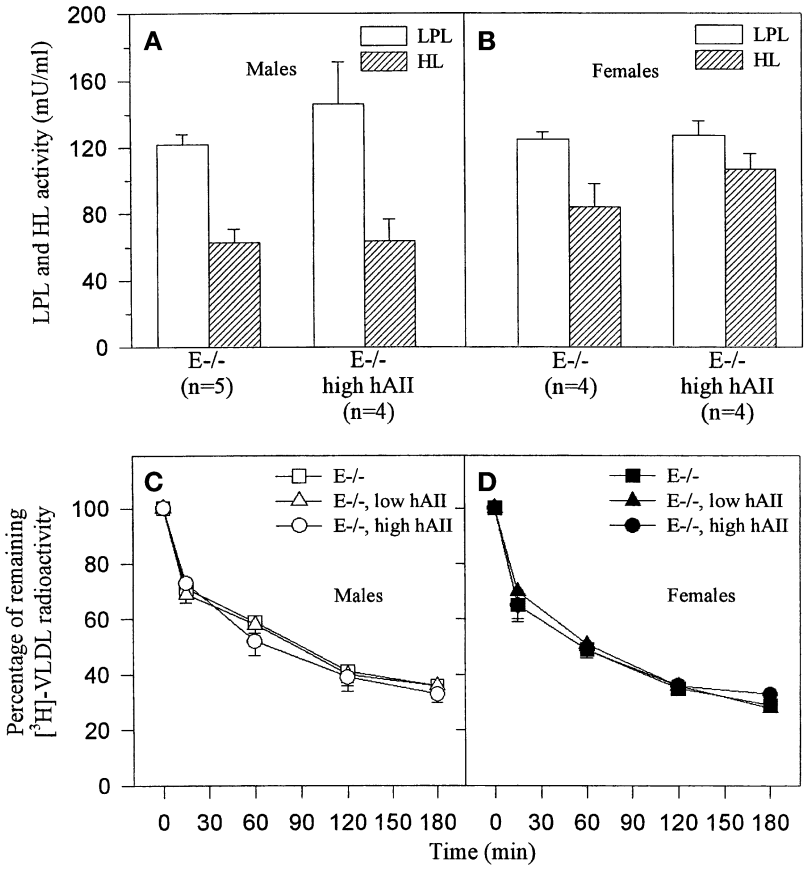

Fig. 2. Postheparin plasma lipase activities and VLDL catabolism. (A and B) Postheparin plasma lipoprotein lipase (LPL) and hepatic lipase (HL) activities. Numbers in parentheses represent the number of animals. (C and D) Plasma clearance of injected [^{3}H]triolein-VLDL (n = 3 or 4 animals for each line and gender). The small errors for some of the data are obscured by the symbol. Both experiments were performed in fasted mice. In all cases, results are shown as means ± SEM. No significant differences were found between the various mice groups. In (A–D), 12- to 13-week-old fasted chow-fed apoE$^{-/-}$ mice with low (apoE$^{-/-}$ low hA-II) or high (apoE$^{-/-}$ high hA-II) plasma human apoA-II concentrations are presented.

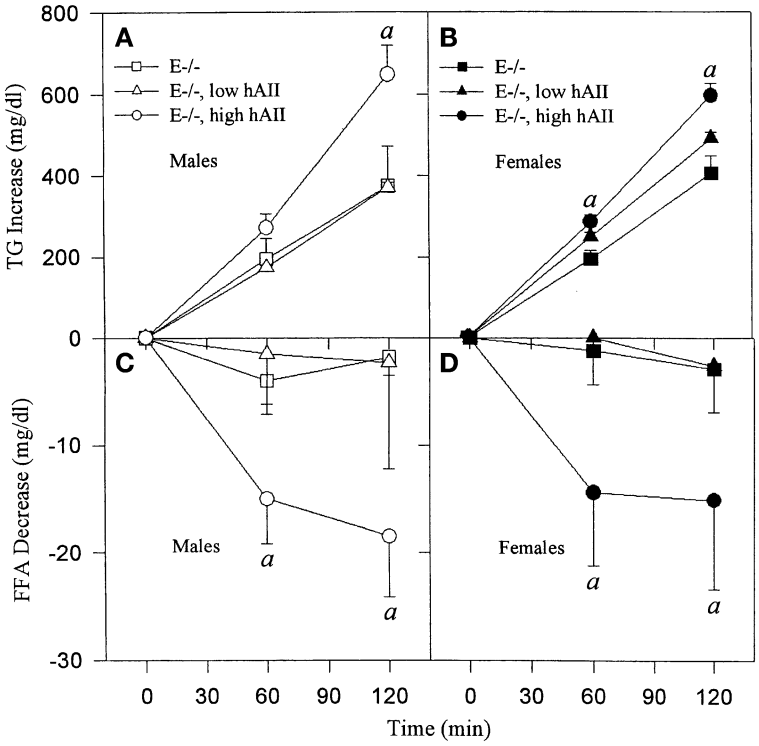

Fig. 3. In vivo production of triglyceride (TG). Fasted mice were bled immediately prior to Triton WR-1339 injection and 60 and 120 min afterward. TG (A and B) and FFA (C and D) were measured and their changes with respect to the baseline result are shown. Each value represents the mean ± SEM of data from 4 to 6 mice. *a*, Significantly different ($P < 0.05$) from apoE$^{-/-}$ mice of the same gender. In (A–D), 12- to 13-week-old fasted chow-fed apoE$^{-/-}$ mice with low (apoE$^{-/-}$ low hA-II) or high (apoE$^{-/-}$ high hA-II) plasma human apoA-II concentrations are presented.

0.01) in male and female apoE$^{-/-}$ mice overexpressing human apoA-II compared with apoE$^{-/-}$ mice.

Susceptibility to atherosclerosis

Expression of human apoA-II in apoE$^{-/-}$ led to a significant increase in the development of atherosclerotic lesions in the various groups of mice (**Fig. 4**). At 12–13 weeks of age, male and female apoE$^{-/-}$ mice had aortic lesion areas of 19,022 ± 1,391 and 47,305 ± 3,331 μm^2/section. The lesion areas of male and female apoE$^{-/-}$ mice expressing low concentrations of human apoA-II were 1.47- and 1.35-fold higher (27,996 ± 2,891 and 63,929 ± 8,420 μm^2), respectively, than their apoE$^{-/-}$ counterparts ($P < 0.05$). The lesion areas of male and female apoE$^{-/-}$ mice overexpressing human apoA-II mice were 3.5- and 3.0-fold higher (66,595 ± 9,226 and 140,258 ± 8,846 μm^2), respectively, than their apoE$^{-/-}$ counterparts ($P < 0.01$). In addition, the incidence of atherosclerosis in large coronary arteries was significantly higher ($P < 0.05$) in apoE$^{-/-}$ mice overexpressing human apoA-II (89% and 88% in males and females, respectively) than in apoE$^{-/-}$ mice (60 and 50%).

TABLE 3. Liver parameters measured in 12- to 13-week-old fasted chow-fed apoE$^{-/-}$ mice with low (apoE$^{-/-}$ low hA-II) or high (apoE$^{-/-}$ high hA-II) plasma human apoA-II concentrations

	ApoE$^{-/-}$	ApoE$^{-/-}$ Low hA-II	ApoE$^{-/-}$ High hA-II
Males	n = 7	n = 4	n = 4
Liver weight (g)	1.03 ± 0.06	1.08 ± 0.07	0.96 ± 0.04
Liver cholesterol (mg/g)	2.2 ± 0.1	1.9 ± 0.2	2.6 ± 0.02[a]
Liver triglycerides(mg/g)	52 ± 5	55 ± 16	7 ± 6[a]
ALT (U/L)	38 ± 5	ND	34 ± 3
AST (U/L)	93 ± 14	ND	76 ± 5
Females	n = 6	n = 3	n = 6
Liver weight (g)	0.77 ± 0.01	0.76 ± 0.01	0.87 ± 0.07
Liver cholesterol (mg/g)	2.8 ± 0.42	3.1 ± 0.33	3.6 ± 0.24
Liver triglycerides(mg/g)	67.5 ± 13	55 ± 2	30 ± 4[a]
ALT (U/L)	38 ± 1	ND	36 ± 4
AST (U/L)	91 ± 7	ND	95 ± 4

Data are from the same experiment shown in Table 1. Results are shown as means ± SEM. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ND, not determined.

[a] Significantly different ($P < 0.05$) from apoE$^{-/-}$ mice.

To study the contribution of different plasma lipid variables to aortic atherosclerosis susceptibility, we correlated them with area of aortic atherosclerosis. The best relationship was found between non-HDL cholesterol and atherosclerosis when fit to a linear model (Fig. 4A and B, insets). Non-HDL cholesterol levels predicted 61 and 69% of the variance in mean lesion area of male and female mice, respectively ($r = 0.78$ and 0.83, $P < 0.01$). Triglycerides correlated with mean lesion area in both a linear and a polynomial model. When fit to a second order polynomial model, plasma triglycerides predicted the percentage of the variance in the mean lesion area of atherosclerosis at 59 and 62% ($r = 0.77$ and 0.79, $P < 0.01$) in male and female mice, respectively. Other significant values ($P < 0.01$) for Pearson correlation coefficients were those between percentage of free cholesterol and mean lesion area ($r = 0.62$ and $r = 0.66$, in male and female mice, respectively) and between FFA and mean lesion area ($r = 0.53$ and $r = 0.68$, in male and female mice, respectively). No significant correlation was found between mean lesion area and HDL cholesterol levels.

Previous studies with transgenic mice overexpressing mouse apoA-II (20) and in apoE$^{-/-}$ mice (23) revealed significant correlations between serum paraoxonase activ-

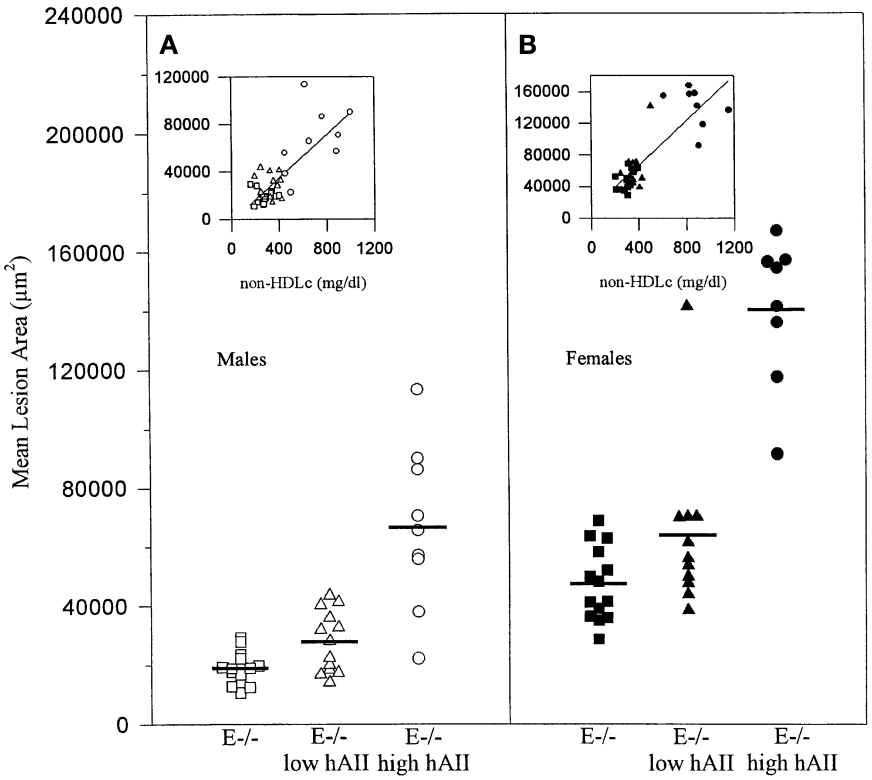

Fig. 4. Susceptibility to atherosclerosis. Atherosclerotic lesion areas in male (A) and female (B) mice. Each point represents the average area of lesion of four proximal aortic sections from each animal. Group means are indicated by horizontal bars. Insets of (A) and (B): The lipid parameter that correlated best with the lesion area was non-HDL cholesterol (see text for more details).

ity and atherosclerosis. We determined paraoxonase/arylesterase activities in EDTA-free plasmas. Male and female apoE$^{-/-}$ mice overexpressing human apoA-II (n = 5) had activities of 68 ± 2 and 75 ± 3 U/mL, respectively, whereas male and female apoE$^{-/-}$ mice (n = 6) had activities of 61 ± 4 and 71 ± 5 U/mL.

Comparison of the characteristics of apoE$^{-/-}$ mice overexpressing apoA-II and the characteristics defining FCHL

Table 4 shows that apoE$^{-/-}$ mice overexpressing human apoA-II presented in this study had almost all the characteristics of FCHL (24). However, other features of these mice differ from FCHL (see Discussion for details).

DISCUSSION

In previous studies with these transgenic mice overexpressing human apoA-II (line 11.1) the increase in plasma triglycerides, compared with controls, ranged from 3-fold to 6-fold, depending on the diet and age of the animals (8, 9, 25). It was, therefore, puzzling to find that apoE$^{-/-}$ mice, having plasma concentrations of human apoA-II that were 2-fold that of normolipidemic humans, presented a 24-fold increase in plasma triglycerides. Because hypertriglyceridemia is common in patients with inherited LCAT deficiency (26), in previous studies (8, 9, 25) we attributed the increase in triglycerides in mice overexpressing human apoA-II to their functional LCAT deficiency, which is due to decreased plasma apoA-I concentration. However, when apoA-I-deficient mice were crossed with human apoB transgenic mice (27) or with apoE-deficient mice (28), and fed a regular chow diet, they presented plasma triglycerides of 175 and 78 mg/dL, respectively. LCAT-deficient mice generated in two independent laboratories presented plasma triglycerides that

TABLE 4. Comparison of the phenotype characteristic of human familial combined hyperlipidemia (FCHL) and that of apoE$^{-/-}$ mice overexpressing human apoA-II

Phenotype	FCHL[a]	ApoE$^{-/-}$ High hA-II
Increased VLDL cholesterol	+	+
Increased LDL cholesterol	+	+
Low HDL cholesterol	+/−	+
Increased VLDL triglyceride	+	+
Increased IDL triglyceride	+	+
Increased VLDL secretion	+	+
Increased apolipoprotein B	+	+
Age-dependent phenotype	+	ND
Normal lipase activity	+/−	+
Atherosclerosis risk factor	+	+

[+] Indicates observation of the mentioned phenotype; [+/−] indicates equivocal or modest results; ND, not determined.

[a] Taken from Ref. 24.

were, in both cases, 1.7-fold those of control mice (2, 29). Consequently, the dramatic increase in triglycerides found in this study seems largely not to be due to the decrease in LCAT activity in mice overexpressing human apoA-II, and the concomitant apoE deficiency, which severely impairs recognition of apoB-containing lipoproteins by different tissues, contributed greatly to raising plasma triglycerides to the levels observed in this investigation.

Analyses after FPLC and ultracentrifugation (Fig. 1) demonstrated that, in apoE$^{-/-}$ mice overexpressing human apoA-II, triglycerides were mainly associated with VLDL rather than chylomicrons. Type V hyperlipidemia and hypertriglyceridemia secondary to Tangier disease present VLDL with apoB–apoA-II complexes that are relatively resistant to the action of LPL (30). Consequently, it seemed feasible that the hypertriglyceridemia of apoE$^{-/-}$ mice overexpressing human apoA-II was due to a slow VLDL catabolism. However, postheparin lipase activities and [^{3}H]triolein-VLDL clearances of apoE$^{-/-}$ mice overexpressing human apoA-II were similar to those of apoE$^{-/-}$ mice. Similar results were obtained when VLDL was labeled with [^{3}H]cholesteryl oleoyl ether, when these experiments were repeated in apoE$^{+/+}$ mice overexpressing human apoA-II or when in these mice the reactivity of VLDL from each type of line was tested against their postheparin plasma LPL (data not shown). These results, together with the increase in FFA in fasting plasma, increased triglyceride production, and the lower triglyceride liver content, strongly suggest that the dramatic hypertriglyceridemia found in apoE$^{-/-}$ mice overexpressing human apoA-II was due to increased synthesis and secretion of VLDL and not to its delayed catabolism. The concomitant increase in non-HDL cholesterol (Table 1) and apoB (Fig. 1D) found in the plasma of these mice is also consistent with increased VLDL production secondary to increased FFA arrival in the liver (31). We did not study the lipolysis of triglyceride-rich lipoproteins under postprandial conditions and an impairment of this process could theoretically contribute, as demonstrated in independently generated human apoA-II transgenic mice (32), to the dramatic degree of hypertriglyceridemia observed in our study. However, this possibility appears unlikely because we have not found such differences when comparing fed apoE$^{+/+}$ mice overexpressing human apoA-II and control mice (data not shown).

A moderate increase in triglycerides and non-HDL cholesterol was found in apoE$^{-/-}$ mice with low concentrations of human apoA-II (12 mg/dL, ~30% of that found in normolipidemic humans), which supports the concept of a dose-related effect of human apoA-II on plasma VLDL. It is of note that the human apoA-II plasma concentrations found in two mouse lines used in this study (12 and 66 mg/dL) are lower than the ~22 and ~85 mg/dL obtained in previous studies using the same methods (8, 9, 25). It is possible that an increased catabolism in apoE$^{-/-}$ mice decreases the plasma concentration of the human protein without affecting its hepatic synthesis. The liver is one of the sites where human apoA-II may function to increase VLDL synthesis and secretion. Conversely, human apoA-II may act, directly or indirectly, on the adipose tissue, causing an increased efflux of fatty acids to the liver and a rise in VLDL synthesis and secretion (24, 31). Current studies in our laboratory are being conducted to distinguish between the two possibilities and to try to define the molecular mechanisms involved. It is of note that, as we have previously shown the existence of a deficiency in mouse apoA-II in our transgenic mice overexpressing human apoA-II fed a regular chow diet (8), the effects described in this article are most likely due to expression of the human protein.

FCHL is one of the most common and atherogenic forms of inherited hyperlipidemia (0.5–2% in Western populations), being present in up to 20% of patients with premature myocardial infarction (24, 33). Affected individuals have increased production of apoB-containing lipoproteins, resulting in elevated VLDL, LDL, or both (24, 33). The inherited basis of FCHL is largely unknown, but is currently thought to be complex and heterogeneous (24). Several studies have proposed the existence in FCHL of unidentified genes with "major" effects on VLDL triglyceride production and "modifier" genes that would affect the phenotype shown by the disease (i.e., LPL, the apoA-I/apoC-III/apoA-IV cluster) (24). In 1998, a study conducted with Finnish FCHL patients revealed a strong linkage to a locus situated in chromosome 1q21-q23, a region that includes the apoA-II gene even though this specific locus appeared to reside outside the maximal linkage interval (14). Furthermore, a similar study performed with mice found a synteneic genomic region that segregated with an FCHL-like trait (34). Similar to our findings, this locus in mice was highly related to triglycerides and non-HDL cholesterol (LOD scores of 30.5 and 22.4). However, this locus was in chromosome 3 and, thus, excludes the mouse apoA-II locus, which is in chromosome 1 (34). Therefore, current studies do not support the apoA-II locus as a "major" gene that causes FCHL. This is in contrast with the findings made in apoE$^{-/-}$ mice overexpressing human apoA-II summarized in Table 4 and that, apart from the aspects already discussed, include an increased atherosclerosis susceptibility. These findings indicate that these mice present the features typical of FCHL. However, the apoE$^{-/-}$ mice overexpressing human apoA-II also present several important characteristics that are not found in FCHL, such as a high plasma ratio of apoB-48/apoB-100, a high content of human apoA-II in apoB-containing lipoproteins, a low HDL apoA-I/apoA-II ratio, a high percentage of plasma free cholesterol, a low content of hepatic triglycerides, and a lack of insulin resistance and central obesity which even though are not included in the definition of FCHL are frequently associated to the disease. Several of these features not characteristic of FCHL could be changed to improve apoE$^{-/-}$ mice overexpressing human apoA-II as a mouse model of FCHL. The increased apoB-48/apoB-100 ratio could be reverted if these mice were bred with mice deficient in the editing enzyme, especially if they are also human apoB-100 transgenic mice (35). Similarly, insulin resistance and central obesity could be induced by cross-breeding with other

mouse models (36). Other findings not characteristic of FCHL are exaggerated in the mouse model but not completely alien to this disease. First, even though signs of LCAT deficiency, which usually requires impairment of the product of the two alleles, are not present in FCHL, the LCAT locus has been linked to FCHL as a "modifier" gene (15). Second, it is likely that the lower content of hepatic triglycerides in apoE$^{-/-}$ mice overexpressing human apoA-II, with respect to apoE$^{-/-}$ mice, is due to the high rate of VLDL synthesis and secretion concomitant with the impairment of receptor-mediated reuptake of apoB-containing lipoproteins lacking its main ligand, which is apoE. This explanation would be consistent with the fact that apoE$^{+/+}$ mice overexpressing human apoA-II have an increased hepatic content of triglyceride with respect to that of control mice (data not shown). A similar case was observed in SREBP-1a transgenic mice, in which disruption of the LDL receptor prevented liver enlargement and unmasked hyperlipidemia (37).

Taken as a whole, this information suggests that the apoA-II gene could be a "modifier" gene rather than a "major" FCHL-causing gene in the human populations. It is therefore possible that in subjects susceptible to developing the disease, a high production of apoA-II influences the production of triglyceride and, thus, the phenotype of FCHL. ApoA-II could directly control VLDL overproduction through action in other genes or, indirectly, through regulation of a "major" FCHL gene (14, 15). However, both possibilities are equally surprising because apoA-II plasma concentration is usually lower in patients with hypertriglyceridemia and/or myocardial infarction than in control subjects (12). A possible explanation for this paradox could be the coexistence of a relatively high rate of apoA-II synthesis in the liver and a normal/low plasma apoA-II concentration, the latter being due to enhanced plasma apoA-II clearance that would cause increased concentration of apoA-II in the interstitial and/or cellular compartment(s). In this context it is noteworthy that there have been reports of a positive correlation ($r = 0.71$, $n = 14$) between the synthesis of apoA-II and VLDL-apoB in human beings, whereas the latter parameter did not correlate with plasma apoA-II concentrations (38). Also, increased concentrations of apoA-II have been found in interstitial fluid of patients with myocardial infarction compared with those of control subjects (13). Consistent with this possibility, and as stated previously, plasma apoA-II levels in the mice used in this study were lower than those of human apoA-II transgenic mice with wild-type apoE, even though the latter had a much more moderate hypertriglyceridemia (8, 9, 25).

Other studies exist that could be reinterpreted to support apoA-II as a candidate gene for FCHL. First, apoA-II- and apoE/apoA-II-deficient mice have lower plasma FFA, a tendency to lower plasma triglycerides and non-HDL cholesterol, and accelerated remnant clearance (7). Second, the apoA-II locus (followed by segregation of a CA repeat in intron 2) has been linked to a locus controlling plasma levels of apoA-II and FFA in both mice and humans (12). Third, most although not all studies performed in mice with increased plasma concentration of mouse or human apoA-II showed increases in plasma triglycerides and non-HDL cholesterol (3, 6, 8, 9, 20, 32, 39, 40). Fourth, hypertriglyceridemia, decreased HDL cholesterol, and/or an altered apoA-I/apoA-II ratio in HDL had been associated in several population studies with a common *Msp*I site polymorphism within an Alu sequence 528 base pairs 3′ to the human apoA-II structural gene, although not in others (see refs. 41 and 42 for a complete list of these studies). It is possible that the *Msp*I-resistant form is in linkage disequilibrium with the CA repeat of intron 2 and that the latter influences the translational efficiency of apoA-II mRNA (43). However, the *Msp*I polymorphism determines only 7.2% of the variation of plasma apoA-II in the population and this may be too low to obtain consistent results in different studies (44). Fifth, in one study elevated apoA-II-containing VLDL was related to the progression of coronary artery disease in patients treated with lovastatin (45) and in another the apoA-II/HDL cholesterol ratio was elevated in patients with this disease (46). High plasma levels of mouse or human apoA-II, such as those found in congenic and transgenic mice, have also been found to consistently promote atherosclerosis (3, 4, 9, 47).

In summary, this study shows that mice overexpressing human apoA-II constitute a model of VLDL overproduction and suggests that the apoA-II gene may be involved, directly or indirectly, in the expression of FCHL in some individuals. The interaction of human apoA-II expression and apoE deficiency is an interesting example of the increasingly recognized heterogeneity of FCHL. Finally, these findings could indicate an apoA-II function related to the regulation of VLDL production.

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REFERENCES

1. Breslow, J. L. 1996. Mouse models of atherosclerosis. *Science.* **272:** 685–688.
2. Ng, D. S., O. L. Francone, T. M. Forte, J. Zhang, M. Haghpassand, and E. M. Rubin. 1997. Disruption of the murine lecithin:cholesterol acyltransferase gene causes impairment of adrenal lipid delivery and up-regulation of scavenger receptor class B type I. *J. Biol. Chem.* **272:** 15777–15781.
3. Warden, C. H., C. C. Hedrick, J-H. Qiao, L. W. Castellani, and A. J. Lusis. 1993. Atherosclerosis in transgenic mice overexpressing apolipoprotein A-II. *Science.* **261:** 469–471.
4. Schultz, J. R., J. G. Verstuyft, E. L. Gong, A. V. Nichols, and E. M. Rubin. 1993. Protein composition determines the anti-atherogenic properties of HDL in transgenic mice. *Nature.* **365:** 762–764.

5. Deeb, S. S., K. Takata, R. Peng, G. Kajiyama, and J. J. Albers. 1990. A splice-junction mutation responsible for familial apoA-II deficiency. *Am. J. Hum. Genet.* **46:** 822–827.
6. Gong, E. L., L. J. Stolzfus, C. M. Brion, D. Murugesh, and E. M. Rubin. 1996. Contrasting in vivo effects of murine and human apolipoprotein A-II. *J. Biol. Chem.* **271:** 5984–5987.
7. Weng, W., and J. L. Breslow. 1996. Dramatically decreased high density lipoprotein cholesterol, increased remnant clearance, and insulin hypersensivity in apoA-II knock-out mice suggest a complex role for apolipoprotein A-II in atherosclerosis susceptibility. *Proc. Natl. Acad. Sci. USA.* **93:** 14788–14794.
8. Marzal-Casacuberta, À., F. Blanco-Vaca, B. Y. Ishida, J. Julve-Gil, S. Shen, S. Calvet-Márquez, F. González-Sastre, and L. Chan. 1996. Functional lecithin:cholesterol acyltransferase deficiency and high density lipoprotein deficiency in transgenic mice overexpressing human apolipoprotein A-II. *J. Biol. Chem.* **271:** 6720–6728.
9. Escolà-Gil, J. C., À. Marzal-Casacuberta, J. Julve-Gil, B. Y. Ishida, J. Ordóñez-Llanos, L. Chan, F. González-Sastre, and F. Blanco-Vaca. 1998. Human apolipoprotein A-II is a pro-atherogenic molecule when it is expressed in transgenic mice at a level similar to that in humans: evidence of a potentially relevant species-specific interaction with diet. *J. Lipid. Res.* **39:** 457–462.
10. Plump, A. S., J. D. Smith, T. Hayek, K. Aalto-Setälä, A. Walsh, J. G. Verstuyft, E. M. Rubin, and J. L. Breslow. 1992. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell.* **71:** 343–353.
11. Zhang, S. H., R. L. Reddick, J. A. Piedrahita, and N. Maeda. 1992. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science.* **258:** 468–471.
12. Warden, C. H., A. Daluiski, X. Bu, D. A. Purcell-Huynh, C. De Meester, D. L. Puppione, S. Teruya, B. Lokensgard, S. Daneshman, J. Brown, R. J. Gray, J. I. Rotter, and A. J. Lusis. 1993. Evidence for linkage of the apolipoprotein A-II locus to plasma apolipoprotein A-II and free fatty acid levels in mice and humans. *Proc. Natl. Acad. Sci. USA.* **90:** 10886–10890.
13. Luc, G., Z. Majd, P. Poulain, L. Elkhalil, and J-C. Fruchart. 1996. Interstitial fluid apolipoprotein A-II: an association with the occurrence of myocardial infarction. *Atherosclerosis.* **127:** 131–137.
14. Pajukanta, P., I. Nuotio, J. D. Terwilliger, K. V. K. Porkka, K. Ylitalo, J. Pihlajamäki, A. J. Suomalainen, A-C. Syvänen, T. Lehtimäki, J. S. A. Viikari, M. Laakso, M-R. Taskinen, C. Ehnholm, and L. Peltonen. 1998. Linkage of familial combined hyperlipidaemia to chromosome 1q21–q23. *Nature Genet.* **18:** 369–373.
15. Aouzierat, B. E., H. Allayee, R. M. Cantor, G. M. Dallinga-Thie, C. D. Lanning, T. W. A. de Bruin, A. J. Lusis, and J. I. Rotter. 1999. Linkage of a candidate gene locus to familial combined hyperlipidemia. Lecithin:cholesterol acyltransferase on 16q. *Arterioscler. Thromb. Vasc. Biol.* **19:** 2730–2736.
16. Committee on Care and Use of Laboratory Animals. 1985. Guide for the Care and Use of Laboratory Animals. Institute of Laboratory Animal Resources, National Research Council, Washington, D.C.
17. Piedrahita, J. A., S. H. Zhang, J. R. Hagaman, P. M. Oliver, and N. Maeda. 1992. Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. *Proc. Natl. Acad. Sci. USA.* **89:** 4471–4475.
18. Bradford, M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye-binding. *Anal. Biochem.* **72:** 248–254.
19. Peinado-Onsurbe, J., C. Soler, M. Soley, M. Llobera, and I. Ramírez. 1992. Lipoprotein lipase and hepatic lipase activities are differentially regulated in isolated hepatocytes from neonatal rats. *Biochim. Biophys. Acta.* **1125:** 82–89.
20. Castellani, L. W., M. Navab, B. J. Van Lenten, C. C. Hedrick, S. Y. Hama, A. M. Goto, A. M. Fogelman, and A. J. Lusis. 1997. Overexpression of apolipoprotein A-II in transgenic mice converts high density lipoproteins to proinflammatory particles. *J. Clin. Invest.* **100:** 464–474.
21. Otway, S., and D. S. Robinson. 1967. The use of a non-ionic detergent (Triton WR 1339) to determine rates of triglyceride entry into the circulation of the rat under different physiological conditions. *J. Biol. Chem.* **244:** 4406–4412.
22. Hara, A., and N. S. Radin. 1978. Lipid extraction of tissues with a low-toxicity solvent. *Anal. Biochem.* **90:** 420–426.
23. Navab, M., S. Hama-Levy, B. J. Van Lenten, G. C. Fobarow, C. J. Cardinez., L. W. Castellani, M-L. Brennan, A. J. Lusis, and A. M. Fogelman. 1997. Mildly oxidized LDL induces an increased apolipoproteinJ/paraoxonase ratio. *J. Clin. Invest.* **99:** 2005–2019.
24. Aouizerat, B. E., H. Allayee, J. Bodnar, K. L. Krass, L. Peltonen, T. W. A. de Bruin, J. I. Rotter., and A. J. Lusis. 1999. Novel genes for familial combined hyperlipidemia. *Curr. Opin. Lipidol.* **10:** 113–132.
25. Julve-Gil, J., E. Ruiz-Pérez, R. P. Casaroli-Marano, À. Marzal-Casacuberta, J. C. Escolà-Gil, F. González-Sastre, and F. Blanco-Vaca. 1999. Free cholesterol deposition in the cornea of human apolipoprotein A-II transgenic mice with functional lecithin: cholesterol acyltransferase deficiency. *Metabolism.* **48:** 415–421.
26. Blanco-Vaca, F., S-J. Qu, C. Fiol, H-Z. Fan, Q. Pao, À. Marzal-Casacuberta, J. J. Albers, I. Hurtado, V. Gracia, X. Pintó, T. Martí, and H. J. Pownall. 1997. Molecular basis of fish-eye disease in a patient from Spain. *Arterioscler. Thromb. Vasc. Biol.* **17:** 1382–1391.
27. Voyiaziakis, E., I. Goldberg, A. S. Plump, E. M. Rubin, J. L. Breslow, and L-S. Huang. 1998. ApoA-I deficiency causes both hypertriglyceridemia and increased atherosclerosis in human apoB transgenic mice. *J. Lipid Res.* **39:** 313–321.
28. Zhang, S. H., R. L. Reddick, E. Avdievich, K. Surles, R. G. Jones, J. B. Reynolds, S. H. Quarfordt, and N. Maeda. 1997. Paradoxical enhancement of atherosclerosis by probucol treatment in apolipoprotein E-deficient mice. *J. Clin. Invest.* **99:** 2858–2866.
29. Sakai, N., B. L. Vaisman, C. A. Koch, R. F. Hoyt, Jr., S. M. Mayn, G. D. Talley, J. A. Paiz, H. B. Brewer, Jr., and S. Santamarina-Fojo. 1997. Targeted disruption of the mouse lecithin:cholesterol acyltransferase (LCAT) gene. *J. Biol. Chem.* **272:** 7506–7510.
30. Alaupovic, P., C. Knight-Gibson, C-S. Wang, D. Downs, E. Koren, H. B. Brewer, Jr., and R. E. Gregg. 1991. Isolation and characterization of an apoA-II-containing lipoprotein (LP-A-II:B complex) from plasma very low density lipoproteins of patients with Tangier disease and type V hyperlipoproteinemia. *J. Lipid Res.* **32:** 9–19.
31. Sniderman, A. D., Z. Zhang, and K. Cianflone. 1998. Divergent responses of the liver to increased delivery of glucose or fatty acids: implications for the pathogenesis of type IV hyperlipoproteinemia. *Atherosclerosis.* **137:** 291–301.
32. Boisfer, E., G. Lambert, V. Atger, N. Q. Tran, D. Pastier, C. Benetollo, J-F. Trottier, I. Beaucamps, M. Antonucci, M. Laplaud, S. Griglio, J. Chambaz, and A-D. Kalopissis. 1999. Overexpression of human apolipoprotein A-II in mice induces hypertriglyceridemia due to a defective very low density lipoprotein hydrolysis. *J. Biol. Chem.* **274:** 11564–11572.
33. Goldstein, J. L., H. G. Schrott, W. R. Hazzard, E. L. Bierman, and A. G. Motulsky. 1973. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. *J. Clin. Invest.* **52:** 1544–1568.
34. Castellani, L. W., A. Weinreb, J. Bodnar, A. M. Gotto, M. Doolittle, M. Mehrabian, P. Demant, and A. J. Lusis. 1998. Mapping a gene for combined hyperlipidemia in a mutant mouse strain. *Nature Genet.* **18:** 374–377.
35. Nakamuta, M., B. H-J. Chang, E. Zsigmond, K. Kobayashi, H. Lei, B. Y. Ishida, K. Oka, E. Li, and L. Chan. 1996. Complete phenotypic characterization of apobec-1 knockout mice with a wild-type genetic background and a human apolipoprotein B background, and restoration of apolipoprotein mRNA editing by somatic gene transfer of apobec-1. *J. Biol. Chem.* **271:** 25981–25988.
36. Ranheim, T., C. Dumke, K. L. Schueler, G. D. Cartee, and A. D. Attie. 1997. Interaction between BTBR and C57BL/6J genomes produces an insulin resitance syndrome in (BTBR × C57BL/6J) F_1 mice. *Arterioscler. Thromb. Vasc. Biol.* **17:** 3286–3293.
37. Horton, J. D., H. Shimano, R. L. Hamilton, M. S. Brown, and J. L. Goldstein. 1999. Disruption of LDL receptor gene in transgenic SREBP-1a mice unmasks hyperlipidemia resulting from production of lipid-rich VLDL. *J. Clin. Invest.* **103:** 1067–1076.
38. Magill, P., S. N. Rao, N. E. Miller, A. Nicoll, J. Brunzell, J. St Hilaire, and B. Lewis. 1982. Relationships between the metabolism of high density and very-low density lipoproteins in man: studies of apolipoprotein kinetics and adipose tissue lipoprotein lipase activity. *Eur. J. Clin. Invest.* **12:** 113–120.
39. Schultz, J. R., E. L. Gong, M. R. McCall, A. V. Nichols, S. M. Clift, and E. M. Rubin. 1992. Expression of human apolipoprotein A-II and its effect on high density lipoproteins in transgenic mice. *J. Biol. Chem.* **267:** 21630–21636.
40. Hedrick, C. C., L. W. Castellani, C. H. Warden, D. L. Puppione, and A. J. Lusis. 1993. Influence of mouse apolipoprotein A-II on

plasma lipoproteins in transgenic mice. *J. Biol. Chem.* **268:** 20676–20682.
41. Vohl, M-C., B. Lamarche, J. Bergeron, S. Moorjani, D. Prud'homme, A. Nadeau, A. Tremblay, P. J. Lupien, C. Bouchard, and J-P. Després. 1997. The *Msp* I polymorphism of the apolipoprotein A-II gene as a modulator of the dyslipidemic state found in visceral obesity. *Atherosclerosis.* **128:** 183–190.
42. Hong, S. H., B. Y. Kang, W. H. Park, J. Q. Kim, and C. C. Lee. 1998. Association between apolipoprotein A2 MspI polymorphism and hypertriglyceridemia in Koreans. *Hum. Biol.* **70:** 41–46.
43. Thorn, J. A., J. Stocks, D. Reichl, J. C. Alcolado, J. C. Chamberlain, and D. Galton. 1993. Variability of plasma apolipoprotein (apo) A-II levels associated with an apoA-II gene polymorphism in monozygotic twin pairs. *Biochim. Biophys. Acta.* **1180:** 299–303.
44. Hannis, C. L., R. Chakraborty, and D. Hewett-Emmett. 1985. How much of variability in apolipoprotein A-II concentration is explained by polymorphism adjacent to apoA-II gene? *Lancet.* **1:** 1339–1340.
45. Alaupovic, P., W. J. Mack, C. Knight-Gibson, and H. N. Hodis. 1997. The role of triglyceride-rich lipoprotein families in the progression of atherosclerotic lesions as determined by sequential coronary angiography from a controlled clinical trial. *Arterioscler. Thromb. Vasc. Biol.* **17:** 715–722.
46. Kawagushi, A., Y. Miyao, T. Noguchi, H. Nonogi, M. Yamagishi, K. Miyatake, Y. Kamikubo, K. Kumeda, M. Tsushima, A. Yamamoto, and H. Kato. 2000. Intravascular free tissue factor pathway inhibitor is inversely correlated with HDL cholesterol and postheparin lipoprotein lipase but proportional to apolipoprotein A-II. *Arterioscler. Thromb. Vasc. Biol.* **20:** 251–258.
47. Mehrabian, M., J-H. Qiao, R. Hyman, D. Ruddle, C. Laughton, and A. J. Lusis. 1993. Influence of the apoA-II gene locus on HDL levels and fatty streak development in mice. *Arterioscler. Thromb.* 13: 1–10.