Overexpression of Human Apolipoprotein A-II in Mice Induces Hypertriglyceridemia Due to Defective Very Low Density Lipoprotein Hydrolysis*

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Elisabeth Boisfer‡§, Gilles Lambert‡§, Véronique Atger¶, Nhuan Quang Tran‡, Danièle Pastier‡, Claire Benetollo‡, Jean-François Trottiere‡, Isabelle Beaucamps‡, Micheline Antonucci‡, Michel Laplaud, Sabine Griglio, Jean Chambaz‡, and Athina-Despina Kalopissis‡‡**

From ‡Unité 505 INSERM, Institut des Cordeliers, 15, rue de l’Ecole de Médecine, 75006 Paris, the ¶Laboratoire de Biochimie Hôpital Broussais, 96, rue Didot, 75014 Paris, and |Unité 321 INSERM, Hôpital de la Pitié-Salpêtrière, 83, boulevard de l’Hôpital, 75013 Paris, France

Two lines of transgenic mice, hAIItg-δ and hAIItg-λ, expressing human apolipoprotein (apo)A-II at 2 and 4 times the normal concentration, respectively, displayed on standard chow postprandial chylomicronemia, large quantities of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) but greatly reduced high density lipoprotein (HDL). Hypertriglyceridemia may result from increased VLDL production, decreased VLDL catabolism, or both. Post-Triton VLDL production was comparable in transgenic and control mice. Postheparin lipoprotein lipase (LPL) and hepatic lipase activities decreased at most by 30% in transgenic mice, whereas adipose tissue and muscle LPL activities were unaffected, indicating normal LPL synthesis. However, VLDL-triglyceride hydrolysis by exogenous LPL was considerably slower in transgenic compared with control mice, with the apparent V_{max} of the reaction decreasing proportionately to human apoA-II expression. Human apoA-II was present in appreciable amounts in the VLDL of transgenic mice, which also carried apoC-III. The addition of purified apoA-II in postheparin plasma from control mice induced a dose-dependent decrease in LPL and hepatic lipase activities. In conclusion, overexpression of human apoA-II in transgenic mice induced the proatherogenic lipoprotein profile of low plasma HDL and postprandial hypertriglyceridemia because of decreased VLDL catabolism by LPL.

Low plasma HDL levels are negatively correlated with the risk of atherosclerosis. Although a number of metabolic functions of HDL have been identified, no direct link has been established between HDL functions and its antiatherogenic effect (1). In vitro studies have shown that apolipoprotein (apo)A-I, the major HDL apolipoprotein, activates reverse cholesterol transport from extrahepatic tissues to the liver (2). However, conflicting results have been reported concerning the role of apoA-II, the second most abundant HDL apolipoprotein (3, 4).

Studies of transgenic mice overexpressing human apoA-I and apoA-II reported that apoA-I protected more against aortic lesions than apoA-II (3). Furthermore, HDL from transgenic mice overexpressing mouse apoA-II lost the ability of HDL to protect against low density lipoprotein (LDL) oxidation (5) and was even proinflammatory (6). Deficiency of either apoA-I (7, 8) or apoA-II (9) obtained by gene targeting technology resulted in very low plasma HDL, showing the critical importance of both apolipoproteins in maintaining the normal structure and metabolism of HDL. At present, apoA-II has been linked with HDL metabolism only, but its exact role remains to be elucidated.

Studies of transgenic mice expressing human (10–13) or murine (14, 15) apoA-II, alone or in combination with human apoA-I, apoC-III, and cholesterol ester transfer protein (16), have provided interesting information. When human apoA-II was expressed at normal levels, overall lipoprotein metabolism was not markedly modified, except for the appearance of a smaller HDL population containing solely human apoA-II (10). At greater expression levels of human (12) or murine (15) apoA-II, plasma HDL was increased in mice overexpressing murine apoA-II (14, 15) but decreased in mice overexpressing human apoA-II (12, 16). Surprisingly, mouse apoA-II overexpression resulted in increased atherosclerotic lesions under standard chow (14), whereas human apoA-II overexpression increased fatty streaks only after long term feeding with an atherogenic diet (13). Two of the above studies reported mildly increased plasma triglyceride (TG), which was associated with the VLDL fraction when mice expressed high levels of human (12) or murine (15) apoA-II. However, comparisons among the various studies are rendered difficult by the different nutritional status of transgenic mice, namely whether they were fasted overnight (10–15) or fed ad libitum (16).

In the present work we were interested in studying the metabolic effects of human apoA-II overexpression under ad libitum feeding conditions. The two transgenic lines we generated were characterized by marked hypertriglyceridemia that was proportional to the level of human apoA-II expression (2 and 4 times the normal in human and mouse serum, respectively). To elucidate the mechanisms underlying this drastic VLDL increase, we focused on the two possible causes for VLDL accumulation in the circulation: greater hepatic VLDL production and/or decreased catabolism by lipoprotein lipase (LPL).

**EXPERIMENTAL PROCEDURES**

*Generation of Transgenic Mice—Transgenic mice were generated by microinjection of the 3-kilobase genomic clone of the human apoA-II gene (−911/+2045), subcloned into the HindIII site of pUC19 (17), into...
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one-cell embryos of (C57BL/6J × CBA/2J) F1 female mice (IFFA-CREDO, Lyon, France). The two founder mice were then backcrossed to strain C57BL/6J, and the majority of the transgenic mice used in this study were obtained after four or five backcrosses. Southern blot analysis of genomic DNA was performed using as a probe a 1,273-base pair fragment of mouse apoA-II cDNA. The probe was digested with EcoRI, which is specific for mouse apoA-II gene. Approximately 7 and 25 copies of the human apoA-II gene were integrated, respectively, into the genome of lines hAIItg-δ and -λ.

Animals—The animals were housed in animal rooms with alternating 12-h periods of light (7 a.m.–7 p.m.) and dark (7 p.m.–7 a.m.) and were fed a chow diet (UAR, Villemeizon-sur-Orge). The presence of the transgene was detected by dot-blot hybridization of tail-derived DNA, and human apoA-II was also measured in plasma by immunoenzyme assay using an antibody (IMMUNO-AG) specific for human apoA-II and not recognizing mouse apoA-II. All transgenic mice were hemizygous for the transgene and were more than 8 weeks old. Because most studies were performed with pooled plasma, we discarded non-transgenic littermates to exclude misidentifications and used as controls C57BL/6J mice (IFFA-CREDO) of the same age and maintained under the same nutritional conditions. Male and female mice were used in equal proportions. In all experiments, mice with free access to food and water were bled between 9 and 12 a.m., either from the retroorbital venous plexus or from the abdominal vein.

RNA Isolation and Northern Blot Analysis—Total RNA was isolated from several tissues using RNA Instapure kit (Eurogentech). RNA samples (15 μg) were separated in 3% formaldehyde-containing agarose gels and transferred to nylon membranes (Hybond N+). DNA samples were hybridized to the human apoA-II cDNA probe. The 18 S RNA probe was used as an internal standard. Autoradiograms were scanned and analyzed with the NIH Image program.

Lipoprotein Analysis—Blood was collected into EDTA-containing tubes on ice and plasma supplemented with 0.05% gentamicin, 1 mM EDTA, and 0.04% sodium azide and protease inhibitors. Chylomicrons were prepared by ultracentrifugation at 10,000 × g for 30 min at 20 °C and washed once by ultracentrifugation for 18 h at 100,000 × g and 10 °C to eliminate albumin. Then, sequential ultracentrifugations were performed at 100,000 × g and 10 °C to isolate VLDL, intermediate density lipoprotein (IDL), and LDL (for 18 h at densities 1.006, 1.020, and 1.063 g/ml, respectively) and HDL (for 40 h at density 1.21 g/ml). LDL and HDL were dialyzed against phosphate-buffered saline. The protein content of lipoproteins was measured (18), and triglyceride (Biotrol A 01548), total cholesterol (Biotrol A 01368), free cholesterol (Biotrol A 01371), and phospholipid (BioMérieux PAP 150) contents were determined with commercial kits.

Fast protein liquid chromatography was performed on 0.2 ml of pooled plasma, using two Superose 6 columns operating in series. The elution rate was 0.4 ml/min. 0.2-ml fractions were collected into 96-well microplates, and the TG and total cholesterol contents were determined in each tube.

The size distribution of HDL was analyzed as follows: 10% of the HDL from each subject was electrophoresed in 4–15% polyacrylamide gels (precast, Bio-Rad), and the gel was scanned, analyzed with NIH Image program, and the percentages of the various apolipoproteins were calculated.

The isoforms of human apoA-II and the percentages of apoC-II and apoC-III were determined by isoelectric focusing (IEF) electrophoresis. The gel was stained, scanned, and analyzed with NIH Image program.

In Vivo TG Production—Control and transgenic mice fed ad libitum were studied between 10:00 a.m. and noon. An equal number of control, hAIItg-δ, and hAIItg-λ mice were studied on any given day. Mice were injected into the jugular vein with Triton WR 1339 (500 mg/kg, as described in Table 1) and 5 min later, with 4 μCi of [1-14C]oleic acid complexed to albumin, to label newly synthesized VLDL-TG (22). Four blood samples were taken from each mouse: at 0 min (before Triton) and 30, 50, and 70 min after Triton. Plasma VLDL clearance in mice is essentially completely inhibited under these conditions, so that the accumulation of [%14C]TG in plasma between 0 and 70 min post-Triton reflects VLDL-TG production rate. Plasma samples were extracted for lipids (23), and lipid classes were separated by TLC and counted (22). Total TG was determined with a commercial kit.

LPL and Hepatic Lipase (HL) Activities in Postheparin Plasma and Tissue Homogenates—Heparin (Choay, France) was injected into the jugular vein of fed transgenic mice (500 units/kg). Blood was collected 5 min later into EDTA-containing tubes on ice, and plasma was stored at −80 °C until the assays. Lipolytic activities were assayed in triplicate using a radiolabeled trilinolein emulsion (24). Total and LPL activities were measured in the absence of 1 μM NaCl and in the presence of human heat-inactivated serum as a source of apoC-II. HL was measured separately on another trilinolein emulsion optimal for this enzyme (24) in the presence of 1 μM NaCl to inhibit LPL activity. LPL was calculated by subtraction. One milliunit of activity is defined as the amount that generates 1 nmol of free fatty acids/min at 37 °C. Plasma LPL and HL activities were expressed as milliunits/ml of plasma.

LPL activity was measured directly in muscle (heart, diaphragm, and gastrocnemian muscle) and adipose tissues (brown and interscapular), which were immediately frozen in liquid nitrogen and stored at −80 °C until the assays. Tissues were homogenized at 4 °C in 0.223 M Tris, 0.2% sodium deoxycholate, 0.008% Nonidet P-40, 0.25 M sucrose, 0.05% heparin, 1% albumin, pH 8.3, centrifuged at 13,000 rpm for 15 min at 4 °C, and LPL activity was measured on supernatants as described above (24).

In Vivo Labeling of VLDL-TG in Vitro Lipolysis by Commercial LPL—Mice were injected in the jugular vein with 5 μCi of [1-14C]oleic acid.
HDL Decrease in Transgenic Mice—HDL decreased substantially in both transgenic lines (Fig. 2). The HDL-cholesterol peak is not apparent in hAIItg-δ mice because of the large scale used due to their high VLDL-TG values.

Lipoprotein isolation by sequential ultracentrifugation confirmed the 3-fold decrease in plasma HDL of transgenic mice (Table III). Surprisingly, this HDL had an increased TG/protein ratio, whereas the ratios of free cholesterol, cholesterol ester, and phospholipid to HDL-protein were 2 times lower. The net result was a lower ratio of HDL-total lipid/HDL-protein in transgenic mice, indicative of decreased particle size. Indeed, nondenaturing gradient gel electrophoresis showed a strong correlation between plasma apoA-II and TG concentrations in normal human plasma (30 mg/dl) or in plasma of control mice.

Statistical Analysis—Results are given as the mean ± S.E., and differences were determined by the t test for nonpaired samples after analysis of variance, using GraphPad Prism. Linear regression analyses for post-Triton TG production rates were performed using GraphPad Prism and the slopes of the regression lines were compared by unpaired t test.
that HDL of our transgenic mice comprised two populations, both smaller than control mouse HDL (Fig. 3A), as reported for other human apoA-II-expressing transgenic mouse lines (10, 12). Mouse apoA-I accounted for 80% of total apolipoproteins in control HDL and drastically decreased down to 12 and 1.5% in HDL from δ and λ mice, respectively (Fig. 3B). The presence of dimeric human apoA-II in HDL from transgenic mice, migrating as a triplet, was confirmed by immunoblotting (Fig. 3C).

**Apparent Chylomicronemia**—The milky appearance of several plasma samples from transgenic animals led us to isolate a chylomicron-like fraction in both transgenic lines, which was absent from control mouse plasma (Table III). Chylomicrons are synthesized in the intestine and carry TG of dietary origin. Because all mice were fed standard chow containing 5% lipid, very few chylomicron particles should have been produced. Apolipoprotein analysis (Fig. 4, A and B) showed that the chylomicron-like lipoproteins from transgenic mice carried approximately equal amounts of apoB-48 and apoB-100, which is solely synthesized in the liver, thus demonstrating that they were actually very large VLDL of mainly hepatic origin. The very low cholesterol/TG ratio also indicates that these large VLDL were little catabolized.

Large amounts of human dimeric apoA-II were present in all apoB-containing lipoproteins of transgenic mice (as confirmed by immunoblotting in Fig. 4C), whereas apoA-II was absent from VLDL of control mice (Fig. 4B). All lipoproteins from λ mice transported consistently more apoA-II than those from δ mice. Regarding apolipoprotein composition, control VLDL transported 48% apoB48, 30% apoE, and 22% apoC3. VLDL from λ mice comprised 45% apoB48, 11% apoE, 33% human apoA-II, and 11% apoC3. On the other hand, more apoB48 than apoB100 was present in VLDL from transgenic compared with control mice (ratios of apoB48:B100 of 1.7 and 0.9, respectively).

The concomitant increase of all VLDL and LDL constituents in transgenic mice (Table III) indicated an increased particle number, whereas their greater TG/protein ratios, suggestive of larger size, further suggested accumulation caused by decreased lipolysis. However, the ratio of cholesterol to TG decreased in VLDL of λ mice only, suggesting that decreased lipolysis may not be the only process leading to VLDL accumulation.

**Influence of Human ApoA-II Overexpression on VLDL Production**—VLDL production was assessed in vivo after Triton WR 1339 injection, which blocks VLDL-TG hydrolysis by LPL (21). Thus, VLDL-TG accumulation in plasma after Triton serves as a measure of VLDL production rate. Total plasma TG of hAIItg-δ and -λ mice was already dramatically elevated before Triton injection, and the increase after Triton did not allow adequate estimation of total VLDL-TG secretion (Fig. 5), except in control mice (2.40 mg TG/ml/h). Therefore, mice were injected with [1-14C]oleic acid to label newly synthesized TG in the liver, which is partly incorporated into VLDL-TG and exported into the circulation from 20 min onward (22). This method allows estimation of secretion rates of newly formed VLDL, the majority of which originates from the liver under standard, high carbohydrate feeding (25). We thus showed that production of [14C]TG was similar in transgenic and control mice.

**LPL and HL Activities**—LPL and HL activities measured in postheparin plasma using an emulsion-based assay were decreased by at most 30% in transgenic mice (Table IV). Because LPL activity in postheparin plasma is assessed indirectly from the difference between total lipase and HL activities, we measured LPL activity in homogenates directly from five tissues in which LPL is most abundant and HL is absent. Table IV shows that muscle and adipose tissue LPL activities were very comparable with those of control mice, and it is usually assumed that tissue LPL activity is proportional to the enzyme mass synthesized in tissues. Thus, transgenic mice probably synthesized LPL at normal rates.

**In Vitro VLDL Hydrolysis by Exogenous LPL**—To account for the hypertriglyceridemia of transgenic mice, we hypothesized that their VLDL might not be a good substrate for LPL. This hypothesis was tested by incubating in vivo labeled TG-rich lipoproteins of transgenic and control mice with commercial LPL. In control mice, chylomicrons were absent, and 90% of the injected 14C label was in the VLDL fraction. In transgenic mice, approximately 8% of the label was present in the chylomicron-like fraction, 72% in VLDL, and 20% in LDL. Hydrolysis of chylomicron-TG and VLDL-TG of hAIItg-δ and -λ mice was considerably slower than hydrolysis of control mouse VLDL-TG (Table V). VLDL of transgenic mice gave results similar to those obtained with chylomicrons and VLDL (not shown). No significant differences in apparent K_m were observed, whereas the apparent V_max of the reaction was decreased 2 and 4 times in lines hAIItg-δ and -λ, respectively, proportionately to human apoA-II expression.

**Human ApoA-II Isoforms and ApoC Content of VLDL**—The
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FIG. 3. Electrophoretic analysis of HDL. Three separate plasma pools from each group of mice were ultracentrifuged to isolate HDL, and characteristic gels are shown. Panel A, non-denaturing gradient gel electrophoresis using 4–15% gels. Lane 1, human HDL; lanes 3–5, HDL from control, hAIItg-8, and hAIItg-λ mice, respectively. Panel B, 15% SDS-PAGE of HDL apolipoproteins. Lane 1, low molecular weight standards (Bio-Rad), with the corresponding sizes of the markers in kDa; lane 2, human HDL; lanes 3–5, HDL from control, hAIItg-8, and hAIItg-λ mice, respectively. Panel C, Western blot after 15% SDS-PAGE of HDL apolipoproteins probed with a specific rabbit anti-human apoA-II antiserum. Lane 1, human HDL; lanes 2–4, HDL from control, hAIItg-8, and hAIItg-λ mice, respectively.

FIG. 4. Electrophoretic analysis of lipoproteins isolated by sequential ultracentrifugation. Panel A, 5% SDS-PAGE. Lane 1, chylomicrons of λ mice; lanes 2 and 3, VLDL from control and λ mice, respectively; lane 4, LDL from λ mice; lane 5, human LDL. Panel B, 4–20% SDS-PAGE, with constant protein amounts loaded on the gels to compare relative apolipoprotein contents between transgenic and control mice. Lane 1, low molecular weight standards (Bio-Rad), with the corresponding sizes of the markers in kDa; lane 2, chylomicrons from λ mice; lanes 3 and 4, VLDL from control and λ mice, respectively; lane 5, human VLDL; lanes 6 and 7, HDL from control and λ mice, respectively; lane 8, human HDL. Panel C, Western blot after 15% SDS-PAGE of VLDL and LDL apolipoproteins probed with a specific rabbit anti-human apoA-II antiserum. Lanes 1 and 2, VLDL from δ and λ mice, respectively; lanes 3 and 4, LDL from δ and λ mice, respectively; lane 5, purified human apoA-II (Sigma). Apolipoproteins from VLDL, LDL, and HDL from control mice did not react with anti-human apoA-II antiserum (not shown).

The observation that postheparin LPL activity was significantly lower in transgenic compared with control mice, whereas tissue LPL activities were very similar, suggested that apoA-II transported in VLDL in postheparin plasma of transgenic mice may have interfered with LPL activity in the enzymatic assay. We therefore measured lipolytic activity of pooled postheparin plasma from control mice in the absence or presence of increasing amounts of purified human apoA-II, up to the concentration of human apoA-II in the plasma of hAIItg-λ mice. The addition of increasing apoA-II amounts resulted in concomitant inhibition of LPL activity, down to 21% of controls in the presence of 7.5 µg of apoA-II in the assay medium (Fig. 7). HL activity likewise decreased as a function of human apoA-II addition into the assay medium, down to 23% of controls.

ApoA-II may inhibit LPL activity either directly, or indirectly by altering the apolipoprotein composition of the lipoproteins present in postheparin plasma, in turn changing the activator properties of the emulsion. A direct inhibitory effect of apoA-II on LPL can only be shown using purified components (apoC-II/LPL/HL).

DISCUSSION

Hypertriglyceridemia is a common metabolic disorder caused by an array of nutritional and genetic factors, most of which are not identified clearly. In this paper we present an unusual mouse model of hypertriglyceridemia resulting solely from overexpression of human apoA-II, which is normally an apolipoprotein component of HDL. Hypertriglyceridemia was proportional to human apoA-II expression in two transgenic lines fed standard chow diet and occurred concomitantly with a decrease in HDL. Excess apoA-II was carried mostly in VLDL of transgenic mice and hindered the action of LPL so that VLDL accumulated in plasma for several hours.
The hypertriglyceridemia of transgenic mice was very marked under ad libitum feeding and was abolished after an overnight fast in line d but persisted at a lower level in the higher expressing line l. The plasma apoA-II concentration was lowered concomitantly by fasting. The near normalization of plasma TG after fasting indicates that VLDL in our transgenic animals has a longer residence time in plasma but is ultimately catabolized and the remnants taken up by tissues. This is supported further by the absence of overt hypercholesterolemia, which is the hallmark of defective lipoprotein uptake resulting from apoE (27, 28) or LDL receptor deficiency (29).

In our study, an increase in plasma apoA-II was consistently accompanied by proportional increases in the TG and apoA-II content of VLDL. A careful survey of the literature shows slight increases in plasma TG proportionately to apoA-II expression in other transgenic lines that also exhibited increased VLDL and LDL levels, even after an overnight fast (12, 15). However, apoA-II has never been detected in the VLDL fraction of these transgenic mice.

Conversely, apoA-II overexpression resulted in markedly decreased plasma HDL, which contained little apoA-I, in accord with the study by Marzal-Casacuberta et al. (12). These au-

**TABLE IV**

| Group        | Lipolytic activity | Adipose tissue |
|--------------|--------------------|----------------|
|              | Total LPL | HL | Brown | Interscapular | Epididymal | Heart | Gastrocnemian muscle |
|              | milliunits/ml | | | | | | |
| Controls     | 270.8 ± 9.58 (9) | 186.6 ± 7.34 (9) | 103.7 ± 4.30 (9) | | | | |
| hAIItg-d     | 248.3 ± 9.12 (16) | 150.7 ± 9.40 (15*) | 98.8 ± 4.50 (16) | 1.07 ± 0.31 (6) | 0.25 ± 0.03 (6) | 0.24 ± 0.03 (5) | 2.24 ± 0.22 (6) | 0.09 ± 0.01 (6) |
| hAIItg-l     | 211.2 ± 15.6 (15 *) | 134.9 ± 15.9 (15*) | 82.5 ± 4.74 (15*) | 0.99 ± 0.17 (6) | 0.19 ± 0.03 (5) | 0.26 ± 0.04 (5) | 2.10 ± 0.29 (6) | 0.12 ± 0.01 (6) |

* p < 0.05 between transgenic and control mice.

**FIG. 5.** Post-Triton VLDL-TG production in fed transgenic and control mice. Blood was drawn at 0 min (before Triton WR 1339) and 30, 50, and 70 min after Triton WR 1339 injection (500 mg/kg of body weight). [1-14C]Oleic acid was injected 5 min after Triton. Linear regression analysis showed that total TG production was linear in control mice only, whereas 14C-TG production was linear in all groups. Panel A, total TG production (mg/ml plasma); panel B, [14C]TG production (cpm/ml of plasma). ●, control mice; ■, δ mice; ▲, λ mice.

**TABLE V**

| Group and lipoprotein | K<sub>m</sub> mg TG | V<sub>max</sub> nsc FFA/min |
|-----------------------|-------------------|--------------------------|
| Controls VLDL         | 1.38 ± 0.12 (5)   | 95.83 ± 11.64 (5)        |
| hAIItg-d CM           | 0.87 ± 0.41 (3)   | 46.30 ± 12.96 (3*)        |
| hAIItg-l VLDL         | 1.05 ± 0.34 (5)   | 43.65 ± 5.21 (5)*        |
| hAIItg-δ CM           | 1.13 ± 0.41 (4)   | 22.62 ± 1.48 (4)         |
| hAIItg-l VLDL         | 0.64 ± 0.31 (6)   | 19.96 ± 1.78 (6)*        |

* p < 0.05 between transgenic and control mice.

**In vivo kinetic parameters of triglyceride-rich lipoproteins for LPL-mediated lipolysis in vitro**

In vivo labeled TG-rich lipoproteins were tested as lipolytic substrates of exogenous LPL. Three distinct plasma pools from each group of mice were used for lipoprotein preparation. Numbers in parentheses represent different incubations of each lipoprotein fraction with exogenous LPL. Apparent Michaelis constants (K<sub>m</sub>) and maximal enzyme activities (V<sub>max</sub>) were calculated from Lineweaver-Burk plots. Statistical analysis was as in Table I. FFA, free fatty acids.
Human VLDL and HDL apolipoproteins were run in parallel to spot the positions of mouse apoC-II and apoC-III relative to the isoforms of human apoA-II. At least four different lipoprotein preparations from control and transgenic mice were used, and characteristic gels are shown.

The presence of chylomicron-like lipoproteins in transgenic, but not in control mice, was unexpected because all animals were fed a standard chow diet containing only 5% lipid so that very few chylomicrons would be produced by the intestine. Analysis of the apoB content of these lipoproteins clearly showed the presence of apoB-100 as well as apoB-48. Because apoB-100 is exclusively synthesized in the liver (32), the chylomicron-like fraction corresponded to very large hepatic VLDL. The mainly hepatic origin of VLDL under high carbohydrate feeding has long been known from experiments in rats (25).

The large increase in the circulation of VLDL and LDL may have been caused either by VLDL overproduction or by defective VLDL hydrolysis by LPL, or both. Post-Triton VLDL secretion was similar in control and transgenic mice, arguing against VLDL overproduction. Anyway, the presence of chylomicron-like particles in transgenic mice was a strong argument for defective VLDL-TG hydrolysis by LPL because large TG-rich lipoproteins have a very short half-life in plasma, and chylomicronemia results from congenital LPL deficiency in humans (33). The modest 30% decrease in postheparin LPL activity in transgenic mice relative to controls could not account for the apparent chylomicronemia. On the contrary, the drastically decreased hydrolysis of VLDL-TG from transgenic mice by exogenous LPL, strongly suggesting that this VLDL was not a good substrate for LPL, may explain their accumulation in a near native form.

Defective VLDL hydrolysis by LPL may be caused by the absence of apoC-II, the obligatory cofactor of the enzyme (34). Because apoC-II is less hydrophobic than apoA-II it may have been displaced from the surface of VLDL by apoA-II (35). However, the presence of apoC-II in VLDL from transgenic mice in greater amounts than in control VLDL should have been sufficient for LPL activity because very little apoC-II is needed for LPL activation (34).

We therefore tested the hypothesis of whether the presence of apoA-II on the surface of VLDL was responsible for the defective VLDL catabolism by LPL. We demonstrated for the first time, to our knowledge, a dose-dependent inhibition of
postheparin LPL activity by apoA-II. Whether apoA-II exerts a direct inhibitory effect on LPL activity could not be established in this study because of the presence of lipoproteins in the postheparin plasma used in our LPL assay. Only an assay using purified components could address the intrinsic inhibitory activities of apoA-II. Thus, the mechanism of LPL inhibition by apoA-II remains at present unclear. By analogy to HL (36), apoA-II carried on VLDL may saturate the surface of the particle and impede binding of LPL to VLDL and thus TG hydrolysis. Such a mechanism would be consistent with the decrease in \( V_{\text{max}} \) measured for LPL assayed with VLDL of transgenic mice.

The addition of apoA-II in postheparin plasma from control mice also inhibited HL activity in a dose-dependent manner. Inhibition of HL by apoA-II has been reported in several studies in vitro (37, 38) and in vivo (8, 16), although in vitro activation of HL by apoA-II has also been measured (39). Two observations in the present study indicate that HL was inhibited in our transgenic mice in vivo. First, the marked TG enrichment of their HDL was proportional to human apoA-II expression. Second, hypertriglyceridemia in fasted \( \lambda \) mice persisted, which may have been the result of partial HL inhibition because HL hydrolyzes TG and phospholipid of chylomicron and VLDL remnants and HDL.

Concerning remnant TG hydrolysis, the respective roles of HL and LPL remain to be determined. Contrary to humans with functional HL deficiencies, mice lacking HL through homologous recombination only displayed increased HDL, whereas remnant TG clearance was affected only after a massive oral fat load (40). On the other hand, adult mouse liver has a significant amount of LPL activity, in contrast to humans and rats (41), suggesting that mice under normal conditions may depend more on LPL for facilitating clearance of TG-rich particles by the liver than do humans and rats.

The following metabolic cascade is proposed to explain the lipoprotein profile of fed transgenic mice overexpressing human apoA-II. Human apoA-II produced at high levels in liver becomes associated in the circulation with HDL and newly secreted VLDL. ApoA-II on the VLDL surface hinders hydrolysis of VLDL-TG by LPL, the first obligatory step of VLDL catabolism, thereby inducing accumulation of very large VLDL, which may have been the result of partial HL inhibition because HL hydrolyzes TG and phospholipid of chylomicron and VLDL remnants and HDL.

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