Expression of Human ApoAII in Transgenic Rabbits Leads to Dyslipidemia
A New Model for Combined Hyperlipidemia

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Objective—Apolipoprotein AII (apoAII) is the second major apolipoprotein in high-density lipoprotein (HDL). However, the physiological functions of apoAII in lipoprotein metabolism have not been fully elucidated.

Methods and Results—We generated human apoAII transgenic (Tg) rabbits, a species that normally does not have an endogenous apoAII gene. Plasma levels of human apoAII in Tg rabbits were ≈30 mg/dL, similar to the plasma levels in healthy humans. The expression of human apoAII in Tg rabbits resulted in increased levels of plasma triglycerides, total cholesterol, and phospholipids accompanied by a marked reduction in HDL-cholesterol levels compared with non-Tg littermates. Analysis of lipoprotein fractions showed that hyperlipidemia exhibited by Tg rabbits was caused by elevated levels of very-low-density lipoproteins (VLDL) and intermediate-density lipoproteins. Furthermore, postheparin lipoprotein lipase activity significantly decreased in Tg rabbits compared with non-Tg rabbits.

Conclusions—These results indicate that apoAII plays an important role in both VLDL and HDL metabolism, possibly through the inhibition of lipoprotein lipase activity. ApoAII Tg rabbits may become a new model for the study of human familial combined hyperlipidemia. (Arterioscler Thromb Vasc Biol. 2009;29:2047-2053.)

Key Words: apolipoprotein ■ lipase ■ transgenic rabbits ■ hyperlipidemia ■ HDL

High levels of plasma high-density lipoproteins (HDL) are associated with a low incidence of cardiovascular disease.1 HDL contains 2 major apolipoproteins (apo): apoAI and apoAII. It is generally accepted that apoAI plays a central role in reverse cholesterol transport and protects against atherosclerosis2,3; however, apoAII functions have not been clearly characterized.4–6

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Clinical and epidemiological studies have yielded conflicting results regarding the relationship between plasma apoAII levels and coronary heart disease: apoAII is either proatherogenic7 or atheroprotective.8 The −265C polymorphism in the apoAII promoter region was shown to be associated with decreased plasma apoAII concentration and enhance postprandial metabolism of large very-low-density lipoproteins (VLDL).9 Nevertheless, apoAII has long been considered to be of physiologically minor importance in lipoprotein metabolism because apoAII deficiency is not associated with a high susceptibility to coronary heart disease.10

ApoAII transgenic (Tg) mice along with knock-out (KO) mice have revealed multiple functions of apoAII.4,5 Both human and mouse apoAII in Tg mice are involved in VLDL metabolism, but mouse apoAII is also associated with obesity and insulin resistance.11–13 In addition, mouse apoAII is proatherogenic in chow-fed Tg mice, whereas human apoAII is either atheroprotective or proatherogenic in Tg mice dependent on an atherogenic diet.14–17 Although the cause of these discrepancies in different mice expressing different transgenes is unclear, it seems that there is a species difference in apoAII functions between human and mouse, and that the precise physiological functions of apoAII in vivo remain to be elucidated. Studies using Tg mice are often complicated by additional factors such as the effect of human homodimer apoAII versus murine monomer apoAII, transgenic apoAII (either human or murine) versus endogenous murine apoAII,

Received May 7, 2009; revision accepted August 21, 2009.
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Arterioscler Thromb Vasc Biol is available at http://atvb.ahajournals.org
DOI: 10.1161/ATVBAHA.109.190264

2047
and the absence of cholesteryl ester transfer protein (CETP), a critical modulator of lipoprotein metabolism, in the plasma of mice. To overcome these problems, we investigated the functions of human apoAII in lipid and lipoprotein metabolism using rabbits. Like humans but unlike mice, rabbits have abundant plasma CETP and exhibit hepatic apoB100 and intestinal apoB48 synthesis, and their lipoprotein profiles are low-density lipoprotein (LDL)-rich. Interestingly, wild-type rabbits are genetically deficient in an apoAII analogous gene; therefore, they can be considered as an “apoAII-KO” model. To gain insight into the in vivo functional roles of human apoAII, we generated and characterized Tg rabbits expressing human apoAII and examined the effect of human apoAII on lipid and lipoprotein metabolism. We found that the expression of human apoAII led to combined hyperlipidemia and marked reduction of plasma HDL in Tg rabbits.

Materials and Methods

Production of Human ApoAII Transgenic Rabbits
Tg rabbits were generated by the methods established in our laboratory as reported previously. The DNA construct used for microinjection was a 3-kb human apoAII genomic fragment with 4 copies of the chicken β globin insulator, which prevents the position effect of transgenes (Figure 1A). Tg founders were identified by Southern blotting and mated with non-Tg rabbits to produce F1 progeny. All animal experiments were performed with the approval of the Animal Care Committee of the universities of Yamanashi and Saga and conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health. Rabbits were fed with a chow diet (RM-4, Funabashi Farmer) containing 16.5% protein, 4.2% fat, and 13% crude fiber. To examine the tissue expression of human apoAII in Tg rabbits, total RNA was isolated from various tissues and Northern blotting was performed as described previously. Plasma lipids and apolipoproteins along with enzymes were analyzed using the methods described in the supplemental materials (available online at http://atvb.ahajournals.org).

Statistical Analysis
All data were expressed as mean ± SEM and assessed for significance with the Student t test. In all cases, statistical significance was set at P < 0.05.

Results
We created 3 independent Tg founders as shown by Southern blotting, and they were designated as A-192(L), A-222(M), and A-230(H) (Figure 1B). These Tg founders were bred to provide F1 progeny for our study. Northern blotting analysis showed that the human apoAII transgene in Tg rabbits was predominantly expressed in the liver, with a small amount of apoAII expression in the bone marrow and lung (approximately 1% and 6% of the liver expression as determined by real-time RT-PCR; Figure 1C). Plasma levels of human apoAII in the founder Tg rabbits were 6.8 mg/dL (A-192), 13.6 mg/dL (A-222), and 30 mg/dL (A-230); A-230 Tg rabbit thus had a plasma level of apoAII similar to that of healthy humans (31 to 35 mg/dL). Plasma lipids and apolipoproteins along with enzymes were analyzed using the methods described in the supplemental materials (available online at http://atvb.ahajournals.org).

Analysis of Human ApoAII in the Plasma and Liver
Rabbit plasma was separated by 10% SDS-PAGE under either nonreducing or reducing conditions, and then immuno blotting was performed using anti–human apoAII mAb. Plasma apoAII protein isolated from Tg rabbit showed the same molecular weight as human plasma apoAII: as a homodimer (approx. 17.4 kDa) under the nonreducing condition and a monomer (approx. 7.8 kDa) under the reducing condition (Figure 1D). Histological and immunohistochemi-
cal staining revealed that the liver histology of Tg rabbits was normal compared to non-Tg rabbits, and that human apoAII immunoreactive proteins were concentrated along the sinus lumens (Figure 1E), which is similar to apoAII staining pattern of human liver (data not shown).

ApoAII Tg Rabbits Exhibited Hyperlipidemia

We first analyzed the plasma lipids of 3 lines of Tg rabbits and found that expression of human apoAII resulted in a dose-dependent changes in plasma TG and HDL-C (Figure 1F). In the current study, we performed all subsequent analyses using the A-230 line of Tg rabbits because they had a level of plasma apoAII (male: 28±1 mg/dL, n=10; female: 36±2 mg/dL, n=14) similar to that of healthy humans and showed apparent hyperlipidemia as described below. We compared plasma lipid and lipoprotein levels of Tg rabbits in both fasting and postprandial states with those of non-Tg littermates (Figure 2A). Compared with non-Tg littermates, there were 2 striking changes in the plasma lipid levels of Tg rabbits. First, both male and female Tg rabbits had higher plasma levels of triglycerides (TG; 2.4- to 2.6-fold increase in males and 3.0- to 4.5-fold increase in females), Total cholesterol (TC; 1.6- to 1.8-fold increase in both males and females), and phospholipids (PL; 1.7-fold increase in males and 1.8-fold increase in females), which was more prominent in female than male Tg rabbits. Second, Tg rabbits showed a remarkable reduction in HDL-C levels: a 50% decrease in males and a 65% decrease in females. Increased plasma levels of lipids and decreased HDL-C levels were found in both fasting and postprandial Tg rabbits. There was no change in plasma free fatty acids, glucose, or insulin levels between Tg and non-Tg rabbits (data not shown).

Effect of Expression of ApoAII on Plasma Lipoproteins

To analyze the effect of apoAII expression on lipoproteins, plasma lipoprotein profiles were initially examined by Fat Red 7B after agarose gel electrophoresis. There were 3 marked changes in plasma lipoprotein levels of Tg rabbits compared with non-Tg rabbits: (1) a marked increase in β-migrating lipoproteins (VLDLs), (2) a marked decrease in α-migrating lipoproteins (HDLs), and (3) a noticeable increase in remnant lipoproteins in the postprandial state (Figure 2B). Note that “fast” α-migrating HDLs in Tg rabbits decreased markedly compared with those in non-Tg rabbits.

Analysis of the density fractions of lipoproteins further confirmed the findings described above and showed that the increased levels of β-migrating lipoproteins in Tg rabbits was attributable to high levels of VLDL (d<1.006 g/mL), intermediate-density lipoproteins (IDL) (d=1.006 to 1.02
g/mL), and large LDL (d=1.02 to 1.04 g/mL), but a reduced level of fast-migrating HDL2-HDL3 compared to non-Tg rabbits (Figure 3A). In addition, HDL present in d=1.04 to 1.06 g/mL fractions (so-called HDL1) was prominent in Tg rabbits. Almost all HDLs in Tg rabbits migrated to the pre-β position. SDS-PAGE Western blotting analysis using the same density fractions showed that human apoAII was mainly associated with HDLs, whereas a certain amount of apoAII is also seen in apoB-containing particles. B, The quantitation of cholesterol and triglyceride (TG) contents in lipoproteins. Cholesterol and TG contents in each density fraction were quantified. The total recovery for each animal averaged ~80% of the total amount in the plasma. Data are expressed as mean±SEM. *P<0.05, **P<0.01 vs non-Tg rabbits. Note that different scales are used for illustrating TG content in HDLs.

C, Analysis of apolipoproteins in lipoprotein density fractions. An equal volume of each fraction (5 μL) was resolved by electrophoresis on 10% SDS-PAGE. Apolipoproteins were visualized using Coomassie brilliant blue. Compared with non-Tg (left), apoB and apoE levels increased but the level of apoAI was markedly reduced in Tg rabbits. ApoAII was only present in the lipoproteins of Tg rabbits. Apolipoproteins are indicated by arrows. D, Comparison of apoB100 and apoB48 in VLDLs in fasting and postprandial states. The density fractions of d<1.006 g/mL (VLDLs) were separated by ultracentrifugation and resolved by 3.5% SDS-PAGE followed by immunoblotting with apoAb.

Each density fraction of lipoproteins was quantified by measuring TC and TG contents. There was a marked increase in VLDL-C (11-fold) and VLDL-TG (5.1-fold), and IDL-C (6-fold) and IDL-TG (7-fold) and a prominent reduction in HDL2 and HDL3-C (Figure 3B). To analyze the apolipoproteins, we fractionated each sample using 4% to 20% SDS-PAGE. This revealed that increased VLDL and IDL levels had more apoB and apoE, whereas a reduced level of HDLs was associated with a marked reduction in apoAI content, although HDL1 was apoE-rich (Figure 3C). ApoAI proteins were mainly distributed in HDL fractions, and some were associated with apoB-containing particles (Figure 3C). We also compared the apoB100 and apoB48 levels of Tg rabbits with those of non-Tg rabbits in the fasting and postprandial conditions. In non-Tg rabbits, apoB48 was only visible in the VLDLs in the postprandial state. In contrast, Tg rabbits showed a more prominent increase in both apoB100 and apoB48 levels in Tg rabbits further increased compared with those of non-Tg rabbits (Figure 3D).

We further analyzed lipoprotein particles by negative-staining electron microscopy and showed that the average particle sizes of VLDL and IDL of Tg rabbits were smaller than those of non-Tg rabbits, possibly due to the increased accumulation of small particles (supplemental Figure I).

In addition to the above analyses, 3 HDL subfractions (fast-, intermediate-, and slow-migrating HDLs), 2 TG-rich lipoprotein (TRL) subfractions (fast- and slow-migrating TRL), and 2 LDL subfractions (fast- and slow-migrating LDLs) were separated by ultracentrifugation and resolved by 10% SDS-PAGE followed by immunoblotting with apoAb.
LDLs) were analyzed using cITP (Figure 4A). The levels of fast- and intermediate-migrating HDLs of Tg rabbits were significantly decreased by 57% and 37%, respectively, whereas the level of slow-migrating HDLs increased 1.7-fold compared with those of non-Tg rabbits (Figure 4B). Consistent with the ultracentrifugation results, the levels of fast- and slow-migrating TRL subfractions (equivalent to chylomicron remnants and VLDL/IDL) of Tg rabbits increased 23- and 5.9-fold, respectively, compared with those of non-Tg rabbits, and fast- and slow-migrating LDLs both increased 2.5-fold (Figure 4B).

**VLDL Synthesis Rate In Vivo**

To elucidate the possible mechanisms responsible for the hyperlipidemia exhibited in Tg rabbits, we measured the in vivo rates of VLDL secretion in fasting animals using Triton WR-1339 to block hydrolysis of TG-rich lipoproteins by lipoprotein lipase (LPL). The basal lines of plasma TG and VLDL-TG (d <1.006 g/mL) were quantified and compared between non-Tg and Tg rabbits. Data are expressed as mean±SEM (n=6 for each group). Plasma postheparin LPL and HL activity were analyzed. Data are expressed as mean±SEM (n=10 for non-Tg and n=11 for Tg). **P<0.01 vs non-Tg rabbits.

**Analysis of Plasma Enzymes**

To examine whether the expression of apoAII had any influence on lipoprotein-related enzymes and thereby modulate VLDL and HDL metabolism, thus causing subsequent hyperlipidemia, we measured postheparin LPL and hepatic lipase (HL) activity, and lecithin:cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP) activity. We found that postheparin LPL was significantly lower in Tg rabbits than that in non-Tg rabbits (Figure 4B). HL activity of Tg rabbits was also lower than that of non-Tg rabbits, although the difference was not statistically significant. However, compared to non-Tg rabbits, the LCAT and CETP levels of Tg rabbits were not significantly changed: LCAT activity: 56±6 nmol/mL/h/37°C in Tg versus 47±4 nmol/mL/h/37°C in non-Tg (n=4 for each group); CETP activity: 12.0±1.0 in Tg versus 13.4±0.9 nmol/mL/h in non-Tg (n=11 for each group).

**Discussion**

To the best of our knowledge, this is the first study to investigate human apoAII using Tg rabbits, an animal without an endogenous apoAII gene.20 This unique apoAII Tg rabbit model allowed us to examine several physiological functions of apoAII in vivo.

First, the expression of human apoAII in Tg rabbits led to increased plasma levels of TG, TC, and PL in both fasting and postprandial states. Analysis of lipoprotein density fractions revealed a marked increase in VLDL and IDL along with increased chylomicron remnants in the postprandial state, which are responsible for the hyperlipidemia exhibited in Tg rabbits. Several possible mechanisms are related to the increased VLDL and IDL levels in Tg rabbits; these include increased hepatic synthesis, impaired LPL-mediated hydrolysis of TG-rich lipoproteins by LPL. The basal lines of plasma TG and VLDL-TG (d <1.006 g/mL) were quantified and compared between non-Tg and Tg rabbits. Data are expressed as mean±SEM (n=6 for each group). Plasma postheparin LPL and HL activity were analyzed. Data are expressed as mean±SEM (n=10 for non-Tg and n=11 for Tg). **P<0.01 vs non-Tg rabbits.

![Figure 4. Analysis of lipoproteins by capillary isotachophoresis (cITP). A, Representative electropherograms of cITP lipoprotein subfractions from non-Tg and apoAII-Tg rabbits. Three peaks can be identified in HDL subfractions representing fast (f)-, intermediate (i)-, and slow (s)-migrating HDL. In non-HDL fractions, there are 4 peaks (from left to right) representing fast (f)-TRL (chylomicron remnant fraction), slow (s)-TRL (VLDL/IDL), and fast (f)- and slow(s)- LDL. B, Lipoprotein subfractions in plasma from non-Tg rabbits and apoAII-Tg rabbits were determined and expressed as peak areas relative to that of an internal marker. Data are expressed as mean±SEM (n=9 for non-Tg and n=12 for Tg). RFU indicates relative fluorescence units. **P<0.01 vs non-Tg rabbits.](http://atvb.ahajournals.org/)

![Figure 5. Post-Triton VLDL production rate in fasting non-Tg and Tg rabbits (A) and postheparin lipase activity (B). Blood was drawn at 0 minutes (before Triton WR-1339) and 2 hours, 4 hours, and 6 hours after Triton WR-1339 injection. Plasma TG and VLDL-TG (d <1.006 g/mL) were quantified and compared between non-Tg and Tg rabbits. Data are expressed as mean±SEM (n=6 for each group). Plasma postheparin LPL and HL activity were analyzed. Data are expressed as mean±SEM (n=10 for non-Tg and n=11 for Tg). **P<0.01 vs non-Tg rabbits.](http://atvb.ahajournals.org/)
lasis, and delayed catabolism in the liver. It seems unlikely that the expression of apoAII per se enhances hepatic VLDL production because Triton WR-1339 injection revealed that the rate of hepatic VLDL synthesis of Tg rabbits was similar to that of non-Tg rabbits, and that there was no increased lipid accumulation in the hepatocytes (fatty liver, a hallmark of enhanced VLDL synthesis) of Tg rabbits. Moreover, we found that Tg rabbits had significantly lower postheparin LPL activity, suggesting that hyperlipidemia induced by apoAII expression may be caused by the reduced LPL activity. It is likely that a small amount of apoAII present in VLDL particles may replace apoCII, thereby interfering with apoCII as an LPL activator; alternatively, apoAII may directly inhibit LPL activity. It has been reported that the presence of apoAII in VLDL makes it a poor substrate for LPL hydrolysis. As such, reduced LPL activity in Tg rabbits may also help explain why Tg rabbits showed increased postprandial chylomicron remnants accompanied by increased apoB48. In support of this notion, our preliminary study showed that Tg rabbits are more susceptible to cholesterol diet-induced hyperlipidemia than non-Tg rabbits. (Inoue T, Koike T, Fan J, unpublished data, 2009).

Second, the expression of apoAII resulted in several characteristic changes in HDL levels of Tg rabbits: (1) a marked reduction in plasma HDL-C; (2) a reduction in fast- or α-migrating HDL and an increase in slow- or pre-β-migrating HDL particles; and (3) a reduction in apoAI content in HDL particles compared with that in non-Tg rabbits, suggesting that apoAII plays multiple roles in HDL metabolism. Whether a reduced level of fast-migrating HDLs and an increased level of slow migrating HDLs in Tg rabbits were attributable to reduced production or enhanced catabolism is unknown. Because the intestinal and hepatic expression of apoAI mRNA of Tg rabbits was similar to that of non-Tg rabbits (hepatic expression: 0.56±0.16 arbitrary unit in All-Tg versus 1.00±0.03 in non-Tg and intestinal expression: 383±34 in All-Tg versus 376±13 in non-Tg), it is not likely that apoAII transgene per se inhibited apoAI synthesis. We speculate that apoAII/apoAI HDLs may be catabolized faster than those of apoAII-only HDLs, although this hypothesis remains to be verified as the urine excretion of apoAI was not changed in Tg rabbits compared to non-Tg rabbits (supplemental Figure II). In addition, apoAII has long been considered to participate in HDL remodeling and particle size modulation through interaction with plasma enzymes. For example, apoAII may act as an activator, inhibitor, or cofactor for LCAT, CETP, HL, and LPL, although this needs to be conclusively proven. It has been proposed that apoAII may displace apoAII from the surface of HDL, thereby reducing the capability of the particles to act as a substrate for LCAT. In the current study, we did not observe any significant reduction in LCAT and CETP activity in Tg rabbits compared to non-Tg rabbits; therefore, it is still unclear whether apoAII has any direct effect on the activity of these enzymes.

A possible mechanism for the reduction in HDL levels in Tg rabbits may be the decreased availability of VLDL surface components caused by an inhibition of LPL activity. Although previous studies suggested that apoAII may be a mediator for HL, relatively low postheparin HL activity in Tg rabbits compared with non-Tg rabbits (though not statistically different) may suggest that apoAII is an inhibitor rather than an activator of HL. However, these results regarding HL activity in rabbits should be interpreted carefully because rabbits are considered to have a lower HL activity than other species.

Taken together, apoAII Tg rabbits exhibited prominent hyperlipidemia and decreased levels of plasma HDL, and thus they can become a new model for the study of human familial combined hyperlipidemia (FCHL). Previous studies have demonstrated that increased levels of plasma apoAII are biochemically and genetically associated with human FCHL. Furthermore, phenotypic features of apoAII Tg rabbits also indicate that apoAII Tg rabbits may be potentially proatherogenic. To address these possibilities, we are now investigating whether apoAII Tg rabbits are susceptible to cholesterol-induced atherosclerosis.

In conclusion, we successfully established Tg rabbits expressing human apoAII. ApoAII Tg rabbits exhibited apparent hyperlipidemia and lower HDL levels, suggesting that apoAII plays an important role in the metabolism of both VLDL and HDL. Although the precise mechanisms remain to be elucidated, Tg rabbits may also provide a unique means to investigate apoAII functions in lipid metabolism and the relationship of apoAII with FCHL and atherosclerosis.

Sources of Funding
This work was supported in part by grants-in-aid for scientific research from the Ministry of Education, Culture, Sports, and Technology, Japan (19790026, 71790514, 19390009, and 21659078), the Takeda Science Foundation, Ono Medical Research Foundation, Naito Foundation for the Promotion of Science, Uehara Memorial Foundation, Japan Heart Foundation, and a research grant from AstraZeneca, a research grant for cardiovascular disease from the Ministry of Health, Labor, and Welfare of Japan.

Disclosures
None.

References


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Arterioscler Thromb Vasc Biol. 2009;29:2047-2053; originally published online September 24, 2009;
doi: 10.1161/ATVBAHA.109.190264
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

An erratum has been published regarding this article. Please see the attached page for:
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In the article “Expression of Human ApoAII in Transgenic Rabbits Leads to Dyslipidemia: A New Model for Combined Hyperlipidemia” by Koike et al., which appeared in the December 2009 issue of the journal (Arterioscler Thromb Vasc Biol. 2009;29:2047–2053; DOI: 10.1161/ATVBAHA.109.190264), the term “pre-beta HDL” was incorrectly expressed as “beta HDL” in two locations:

1. Page 2050, left column, lines 5 and 6: The correct version should read, “Almost all HDLs in Tg rabbits migrated to the pre-β position.”
2. Page 2052, left column, first full paragraph, lines 1 through 8: The correct version should read, “Second, the expression of apoAII resulted in several characteristic changes in HDL levels of Tg rabbits: (1) a marked reduction in plasma HDL-C; (2) a reduction in fast- or α-migrating HDL and an increase in slow- or pre-β-migrating HDL particles; and (3) a reduction in apoAI content in HDL particles compared with that in non-Tg rabbits, suggesting that apoAII plays multiple roles in HDL metabolism.”

The online version of the article has been corrected.

The authors regret the error.

DOI: 10.1161/ATV.0b013e3181df7a89
S-Fig.l.
S-Fig.II.
Supplement figure legends

Supplement figure I.  Electron microscope analysis of lipoproteins
Lipoprotein density fractions were examined by negative-stain electron microscopy, and representative micrographs are shown. The distribution of the sizes of lipoproteins is illustrated at the bottom panel. The average particle diameter (± SEM) and the total number of particles counted are listed above the graphs.

Supplement figure II.  Analysis of apoAI in urine by immunoblotting analysis
Rabbit 24-h urine was collected and concentrated 10x using a protein concentrator (Centricon plus-20, Millipore Corp., Bedford, MA). Kidney protein was extracted. Urine (30µl) and kidney protein (10µg) were electrophoresed by 10% SDS-PAGE followed by immunoblotting using human apoAI polyclonal Ab (AbD Serotec, Oxford, UK). Plasma HDL fraction (density, 1.10-1.21 g/ml) was isolated by sequential density ultracentrifugation and used as a positive control.
Supplemental data for materials and methods

Analysis of plasma apolipoprotein AII and lipids
The plasma lipids and lipoprotein profiles of F1 transgenic (Tg) rabbits were compared with non-Tg littermates at the age of 3-4 months. Blood was obtained from rabbits that had been either fasted for 16 hours (fasting state) or been re-fed for 6 hours (postprandial state), and EDTA-plasma was collected after centrifugation at 4°C for 20 min. The presence of human apolipoprotein (apo) AII proteins in plasma was determined by 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE), followed by immunoblotting with monoclonal antibody (mAb) against human apoAII (Intracel, Frederick, MD). Plasma concentrations of human apoAII in Tg rabbits were quantified by immunonephelometry (ApoAII auto-N, Daiichi, Sekisui Medical Co, Tokyo, Japan). Total cholesterol (TC), triglycerides (TG), high-density lipoproteins (HDL)-cholesterol (HDL-C), phospholipids (PL), free fatty acids, glucose, and insulin were measured using Wako assay kits (Wako Pure Chemical Industries Ltd, Osaka, Japan).

Analysis of plasma lipoproteins
Plasma lipoproteins were isolated by small-volume sequential ultracentrifugation with a Beckman TLA100.2 rotor as described previously\(^1\). Isolated individual density fractions were resolved by electrophoresis in 1% agarose universal gels (Helena Laboratories, Saitama, Japan). Then, the gels were stained with Fat Red 7B. Cholesterol and TG contents in each density fraction were measured
using Wako assay kits. The sizes of the lipoproteins within five lipoprotein density fractions (d<1.006, d=1.006~1.02, 1.02~1.04, 1.04~1.06, and 1.06~1.08 g/ml) were analyzed by negative-staining electron microscopy. Plasma apoB100 and apoB48 contents in very-low-density lipoproteins (VLDL) fractions (d<1.006 g/ml) obtained from both fasting and postprandial rabbits were evaluated by SDS-PAGE immunoblotting using polyclonal apoB Ab.

The lipoprotein profiles were also analyzed using capillary isotachophoresis (cITP) technique using a Beckman P/ACE MDQ system (Beckman-Coulter Inc., Tokyo, Japan) as described previously. cITP can separate lipoproteins into three subfractions according to lipoprotein electric charge: HDL subfractions (consisting of fast-, intermediate- and slow-migrating HDLs), two TG-rich lipoprotein (TRL) subfractions, and two low-density lipoproteins (LDL) subfractions. Levels of cITP lipoprotein subfractions were quantified and expressed as the peak area relative to that of an internal marker (5-carboxy-fluorescein).

**Measurement of VLDL synthesis**

The VLDL secretion rate was determined using Triton WR-1339 injection to inhibit VLDL clearance from the plasma as described previously. Briefly, rabbits were fasted overnight and then intravenously injected with 20% Tyloxapol (Sigma) solution (200 mg/ml in 0.9% NaCl) at a dose of 400 mg/kg BW. Blood was collected at 0, 2, 4, and 6 hours later after the Triton WR-1339 injection. Plasma TG and VLDL-TG (density fractions, d<1.006 g/ml, isolated by ultracentrifugation) were measured as described above.
Analysis of plasma enzymes

Hepatic lipase (HL) and lipoprotein lipase (LPL) activities were measured by a recently reported method\(^8,9\). In brief, HL and LPL proteins were prepared from the postheparin plasma of fasting rabbits using heparin-Sepharose column chromatography. Lipase activity was measured by examining the increase in absorbance at 546 nm (sub; 660 nm) due to the production of quinonediimine dye using a previously described assay procedure\(^10\). Plasma lecithin:cholesterol acyltransferase (LCAT) activity in fasting rabbits was analyzed by a dipalmitoyl lecithin substrate method as performed by SRL Inc. (Tokyo, Japan).

Plasma cholesteryl ester transfer protein (CETP) activity was measured using a commercial assay kit (BioVision, Mountain View, CA) as described previously\(^11\). This method employs a donor molecule containing a fluorescent self-quenched neutral lipid that is transferred to an acceptor molecule in the presence of CETP. CETP-mediated transfer of the fluorescent neutral lipid to the acceptor molecule results in an increase in fluorescence (excitation: 465 nm; emission: 535 nm).

1. Fan J, McCormick SP, Krauss RM, Taylor S, Quan R, Taylor JM, Young SG. Overexpression of human apolipoprotein B-100 in transgenic rabbits results in increased levels of LDL and decreased levels of HDL. *Arterioscler Thromb Vasc Biol.* 1995;15:1889-1899.


