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 \((d<1.006, d=1.006\sim1.02, 1.02\sim1.04, 1.04\sim1.06, \text{ and } 1.06\sim1.08 \, g/ml)\) were analyzed by negative-staining electron microscopy\(^2\). 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\(^1\).

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\(^3\). 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\(^4, 5\). 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\(^6, 7\). 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.


