Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 665–669, January 1989
Medical Sciences

Increased clearance of plasma cholesterol after injection of apolipoprotein E into Watanabe heritable hyperlipidemic rabbits

(low density lipoprotein receptor)

NOBUHIRO YAMADA*†, HITOSHI SHIMANO*, HIROSHI MOKUNO*, SHUN ISHIBASHI*, TAKANARI GOTOHDA*, MASANOBU KAWAKAMI*, YOSHIO WATANABE‡, YASUO AKANUMA*, TOSHIO MURASE*, AND FUMIMARO TAKAKU*

*Third Department of Internal Medicine, University of Tokyo, Hongo, Tokyo, Japan; and ‡Institute for Experimental Animals, Kobe University, School of Medicine, Kobe 650, Japan

Communicated by Richard J. Havel, October 14, 1988

ABSTRACT **Apolipoprotein E (apoE) is known to play an important role in lipoprotein metabolism. We have studied the effect of apoE on the metabolism of plasma cholesterol by injecting apoE intravenously into rabbits deficient in low density lipoprotein receptors [Watanabe heritable hyperlipidemic (WHHL) rabbits]. Approximately 30 mg of apoE was injected per rabbit; a total of five WHHL rabbits were used. One hour later, plasma cholesterol levels fell 8.3% (from 488 ± 192 to 446 ± 174 mg/dl). After 3 hr, cholesterol levels had fallen by 19% (to 392 ± 152 mg/dl). The reduced levels were maintained for at least 8 hr after injection of apoE. Cholesterol in very low density lipoproteins (VLDLs) and intermediate density lipoproteins fell rapidly during the first 2 hr after injection, followed by a reduction in the low density lipoprotein cholesterol level. Changes in apolipoprotein B levels in each lipoprotein fraction were very similar to those of cholesterol. Plasma apoE levels 3 min after injection were elevated 3-fold to 22.8 ± 6.3 mg/dl and returned to initial levels 8 hr after injection. The rate of removal of intravenously injected ^{125}I-labeled VLDL that had been incubated with apoE was 3-fold higher than that of unmodified VLDL. From these results, we conclude that the injected apoE is incorporated into VLDLs and that VLDL particles carrying more apoE are removed from the blood more rapidly, resulting in reduced formation of low density lipoprotein and lowered cholesterol levels.**

Low density lipoprotein (LDL) cholesterol is mainly removed from blood plasma by hepatic LDL receptors that interact with two specific ligands on lipoproteins, apolipoprotein B-100 (apoB-100) and apolipoprotein E (apoE) (1–3). Lipoproteins rich in apoE, such as large very low density lipoprotein (VLDL) and β-VLDL, have a much higher affinity for LDL receptors than LDL, which contains only apoB-100 (4, 5). In addition, lipoproteins containing apoE can bind to receptors on the liver cell membranes that are responsible for the uptake of dietary cholesterol carried in chylomicron remnants (6, 7). This chylomicron-remnant receptor is thought to be distinct from the LDL receptor because chylomicron remnants are normally removed from the blood in human familial hypercholesterolemia (8) and in Watanabe heritable hyperlipidemic (WHHL) rabbits deficient in LDL receptors (9).

Recent research has demonstrated two lipoprotein subclasses containing apoB-100: B,E particles containing apoE and -B and B particles lacking apoE (10–12). The presence of apoE has been shown to have a profound influence on the removal of apoB-100 in VLDL particles from the blood of normal rabbits and their conversion to lipoproteins of higher density [intermediate density lipoprotein (IDL) and LDL] (10). In WHHL rabbits, the rate of removal of VLDL-B,E particles from the blood was 4-fold higher than that of VLDL-B particles (11). These results suggest that in WHHL rabbits a small number of LDL receptors expressed on the surface of hepatocytes or chylomicron-remnant receptors participate in the removal of lipoproteins containing several molecules of apoE by interacting with apoE.

The WHHL rabbit is an excellent animal model to test the effect of agents that could alter the interaction of lipoproteins with lipoprotein receptors. In the current study, we injected massive amounts of apoE into WHHL rabbits to enrich lipoproteins in the receptor-active protein apoE. We speculated that such lipoproteins would be removed much more efficiently through either the chylomicron-remnant receptor or the mutant LDL receptor resulting in the decreased level of plasma cholesterol.

MATERIALS AND METHODS

Animals. Male WHHL rabbits weighing 2.9–3.4 kg were fed a low-fat laboratory chow ad libitum and housed individually during the experiments.

ApoE. ApoE was purified from plasma of rabbits fed a diet containing 1% cholesterol (by weight) for more than 1 month. Plasma from blood containing 0.1% EDTA and 0.02% sodium azide was ultracentrifuged at a density of 1.03 g/ml for 20 hr at 16°C in a Beckman 50.2 rotor at 45,000 rpm; the supernatant lipoproteins were recentrifuged under the same condition to obtain β-VLDL rich in apoE (13). All steps of purification were carried out at 4°C (14, 15). After delipidation of β-VLDL by extraction with 200 vol of ethanol/ether [1:1 (vol/vol)] four times and evaporating the solvent under N_2, apolipoproteins were dissolved in 2 mM sodium phosphate (pH 7.4) containing 6 M urea with gentle stirring for 5 min, dialyzed against 2 mM sodium phosphate containing 50 mM NaCl and 1 M urea (pH 7.4; buffer A), and applied to a column (2 × 16 cm) of heparin-Sepharose 4B (Pharmacia, Uppsala) preequilibrated with 2 mM sodium phosphate (pH 7.4) containing 50 mM NaCl (buffer B). The column was first washed with 100 ml of buffer A, then with 1200 ml of buffer B, and finally with 2 mM sodium phosphate (pH 7.4) containing 500 mM NaCl and 6 M urea to elute apoE. The purity of apoE was evaluated by $NaDodSO_4$/polyacrylamide gel electrophoresis (16) and by immunoprecipitation of radioiodinated apoE with specific antibody prepared against rabbit apoE, kindly provided by R. J. Havel (University of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; WHHL, Watanabe heritable hyperlipidemic; apoE and apoB-100, apolipoproteins E and B-100, respectively.

†To whom reprint requests should be addressed.

California, San Francisco). In both cases, purity was greater than 95%. After dialysis against water and lyophilization, purified apoE was stored at −80°C. Before use, apoE was dissolved in 6 M guanidine hydrochloride (pH 7.4) at a concentration of ≈10 mg/ml and dialyzed extensively against 150 mM NaCl. We prepared ≈200 mg of apoE for the current study, which was pooled and used as a single lot.

***In Vivo* Studies.** ApoE in 150 mM NaCl was passed through a 0.45-μm filter and injected into rabbits through a marginal ear vein at 8.5 mg/ml per kg (body weight). As a control, 0.15 M NaCl [1 ml/kg (body weight)] was injected into the same rabbits 2 days before apoE injection. In both cases, 1 ml of blood was taken 3 min and 1, 2, 4, 8, and 24 hr after injection. Plasma samples obtained from five WHHL rabbits at each time were pooled and subjected to either density gradient ultracentrifugation (11, 17) or gel filtration chromatography (18). After density gradient ultracentrifugation, plasma lipoproteins were separated into 11 fractions with the following densities: 1.0040, 1.0052, 1.0086, 1.0135, 1.0182, 1.0238, 1.0278, 1.0348, 1.0402, 1.0478, and 1.050 g/ml. The first and second fractions were designated VLDL; the third, fourth, and one-half of the fifth fractions were designated IDL; and one-half of the fifth and the sixth to tenth fractions were designated LDL. For gel filtration chromatography, 1 ml of fresh pooled plasma was applied to a column of Bio-Gel A-5m (2 × 90 cm) (Bio-Rad) (18). Fractions were eluted with 0.15 M NaCl containing 0.1% EDTA and 0.02% sodium azide (pH 7.4) at a flow rate of 0.15 ml/min. The concentrations of triacylglycerols and cholesterol in plasma and lipoprotein fractions were determined by enzymatic assay (19, 20), apoE was determined by radioimmunoassay (14, 21), and apoB was determined by isopropanol precipitation as described (10, 22).

VLDL and lipoproteins with a density of 1.006–1.063, designated I+LDL, were isolated from WHHL rabbits and 1 mg of protein was iodinated by the iodine monochloride method (23) and incubated with 1 mg of lyophilized apoE at 4°C for 1–2 hr. The lipoproteins were then applied to a column (1 × 30 cm) of Sephacryl S-300 (superfine; Pharmacia, Uppsala) to remove unbound apoE. The VLDLs or I+LDLs incubated with apoE were designated VLDL–E or I+LDL–E, respectively. ^{125}I-labeled VLDL or ^{125}I-labeled I+LDL was passed through a 0.45-μm filter and injected intravenously into rabbits. Blood was withdrawn from an opposite ear vein. The next day, either ^{131}I-labeled VLDL–E or I+LDL–E was injected similarly. Radioiodinated apoB in samples of plasma obtained after injection was determined by isopropanol precipitation as described (10, 22). All data except those given in figures are presented as mean ± SD.

RESULTS

Plasma cholesterol, triacylglycerol, and apoE concentrations in WHHL rabbits before injection of apoE are shown in Table 1. After apoE injection, initial plasma cholesterol levels (488 ± 192 mg/dl) fell linearly by 19% to 393 ± 152 mg/dl by 4 hr; this level was maintained for the next 20 hr (Fig. 1). In one rabbit, there was a remarkable reduction from 350 to 244 mg/dl. The smallest reduction in plasma cholesterol level during the first 4 hr was from 386 to 334 mg/dl. After injection of saline, the initial level of plasma cholesterol (465 ± 173 mg/dl) remained almost constant in the first 4 hr and then fell gradually to 423 ± 144 mg/dl, as shown in Fig. 1. Analysis of fractions from a density gradient after ultracentrifugation (Fig. 2) showed that VLDL cholesterol fell 18% from 76 to 62 mg/dl during the first 4 hr and then increased 21% to 92 mg/dl as compared with the initial level. IDL cholesterol also

Table 1. Concentrations of plasma cholesterol, triacylglycerol, and apoE in WHHL rabbits

Rabbit	Cholesterol, mg/dl	Triacylglycerol, mg/dl	ApoE, mg/dl
1	658	434	10.8
2	314	526	10.6
3	350	340	3.4
4	730	145	5.2
5	386	185	5.8
Mean ± SD	488 ± 192	326 ± 162	7.2 ± 3.4

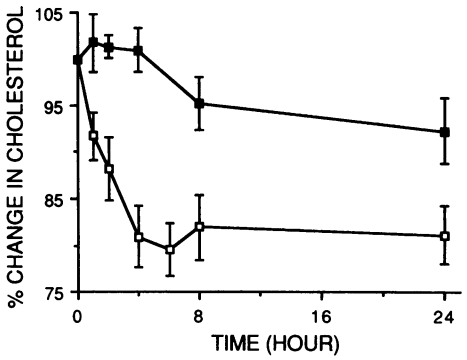

FIG. 1. Percentage change of plasma cholesterol concentrations after injection of apoE [8.5 mg/kg (body weight)] into five WHHL rabbits (□). As a control, 0.15 M NaCl was injected 2 days before injection of apoE into the same rabbits (■). (Bars = 1 SE.)

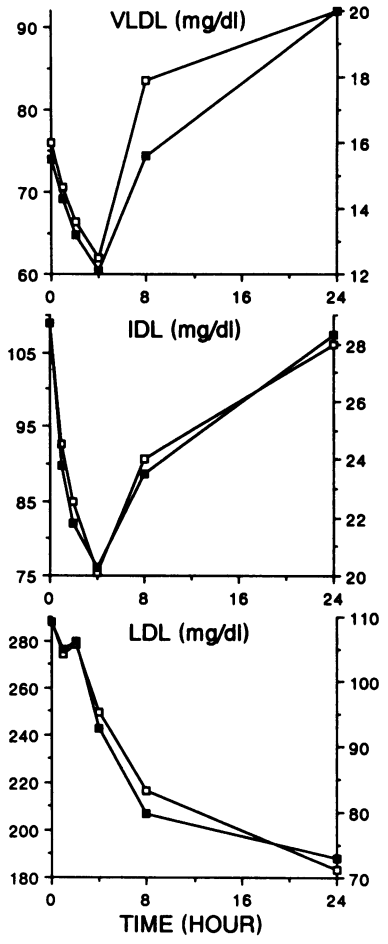

FIG. 2. Change of cholesterol (left axis, □) and apoB (right axis, ■) concentrations in VLDL (*Top*), IDL (*Middle*), and LDL (*Bottom*) fractions after injection of apoE [8.5 mg/kg (body weight)]. Lipoproteins were isolated from pooled plasma of five WHHL rabbits by density gradient ultracentrifugation. Values are given as mg/dl of plasma.

decreased 30% from 109 to 76 mg/dl during the first 4 hr and then returned to the initial level, whereas LDL cholesterol fell only 3.8% from 289 to 278 mg/dl during the first 2 hr and then fell more rapidly by 25% to 216 mg/dl during the next 6 hr. As shown in Fig. 2, changes in apoB concentration in lipoprotein fractions were very similar to those in cholesterol. Changes in plasma triacylglycerol concentrations were neither consistent nor significant; in four WHHL rabbits the levels fell 2.3%, 24%, 17%, and 16%, and in one rabbit the level increased 9.7% during the first 4 hr.

The mean concentration of apoE in five WHHL rabbits before injection was 7.2 ± 3.4 mg/dl. Three minutes after injection, the level increased to 22.8 mg/dl (Fig. 3*A*). The recovery of injected apoE in plasma 3 min after injection was 68.1% of injected amount, based upon the formula: plasma volume = 0.57 × body weight (in kg) × (100 − hematocrit). The low recovery could reflect rapid removal of degraded or aggregated apoE, which is known to aggregate readily in aqueous solution. The half-life of injected apoE was ≈1.5 hr. This observation is consistent with the rate of removal of the labeled apoE injected with 30 mg of unlabeled apoE (Fig. 3*A*). The distribution of apoE among lipoprotein classes was analyzed by gel permeation chromatography. As shown in Fig. 4, large VLDL was separated from small VLDL, IDL, LDL, and high density lipoprotein (HDL). Before injection of apoE, 7.6% of apoE was found in the large VLDL fraction and 20%, 37%, and 36% were found in small VLDL, IDL plus LDL, and HDL fractions, respectively. Increased amounts of apoE were found in all lipoprotein fractions 3 min after injection of apoE. Three minutes after injection, the fold

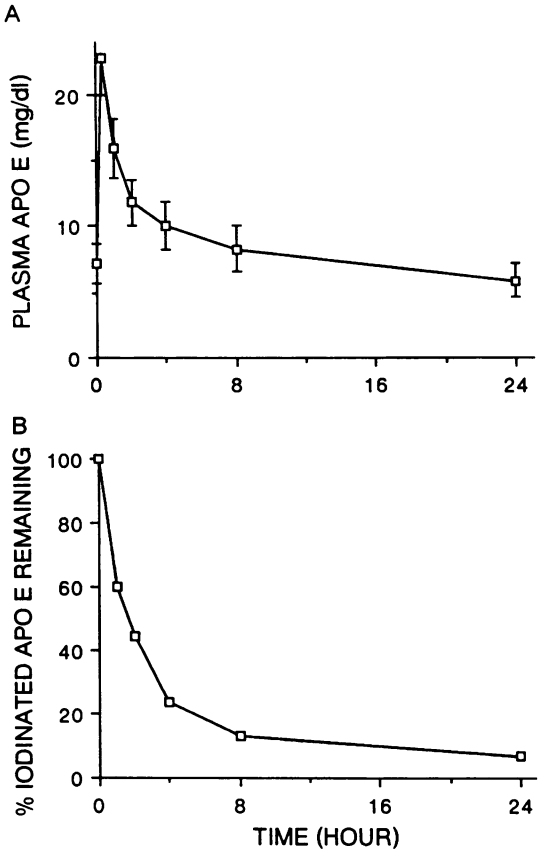

FIG. 3. (*A*) Change of plasma apoE concentrations after injection of apoE [8.5 mg/kg (body weight)] into five WHHL rabbits. Plasma apoE concentrations were determined by radioimmunoassay. (Bars = 1 SE.) (*B*) The removal from blood plasma of ^{125}I-labeled apoE injected with unlabeled apoE into two WHHL rabbits. ApoE was radioiodinated by the chloramine-T method described (14).

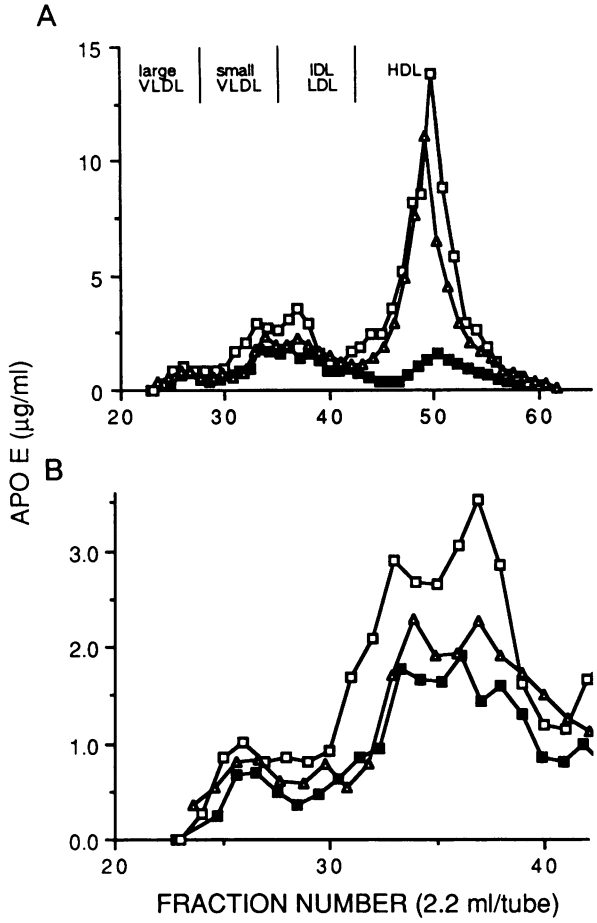

FIG. 4. (*A*) Gel filtration chromatography on Bio-Gel A-5m (2 × 90 cm column). Plasma (1 ml) pooled from five WHHL rabbits before (■) or 3 min (□) or 1 hr (△) after injection of apoE was applied to the column. Each fraction of the eluate contained 2.2 ml. (*B*) Fractions 23–42 shown in *A*. ApoE values are given as μg/ml of column eluate. To determine the distribution of each lipoprotein fraction, large VLDL, small VLDL, IDL, LDL, and HDL fractions were isolated from WHHL rabbits and applied to the column.

increases were 1.8 in large VLDL, 2.0 in small VLDL, 1.8 in IDL + LDL, and 5.5 in HDL fractions. One hour after injection, the content of apoE fell in all lipoprotein fractions but was still higher than before injection. The similar results were obtained in an experiment in which labeled apoE was injected with unlabeled apoE. Labeled apoE in lipoproteins containing apoB-100 decreased gradually during the first 8 hr after injection.

We found that the content of apoE in VLDL–E and I+LDL–E was 2- to 3-fold greater than that in original VLDL and I+LDL, as determined by radioimmunoassay and visualized in $NaDodSO_4$/polyacrylamide gel electrophoretograms. Clearance from plasma of radioiodinated VLDL–E and VLDL is shown in Fig. 5*A*. VLDL–E was removed from the blood plasma much faster than VLDL during the first 2 hr after injection; later the rates for VLDL–E and VLDL were very similar, whereas the rate of removal of I+LDL–E did not differ from that of I+LDL (Fig. 5*B*). The rate of removal of VLDL–E was 2.8-fold higher than that of VLDL during the first hr and 1.7-fold higher during the second hr.

DISCUSSION

Lipoproteins containing apoE are known to have a high affinity to lipoprotein receptors (3–7). ApoE can be incorporated into lipoproteins when incubated with lipoproteins or

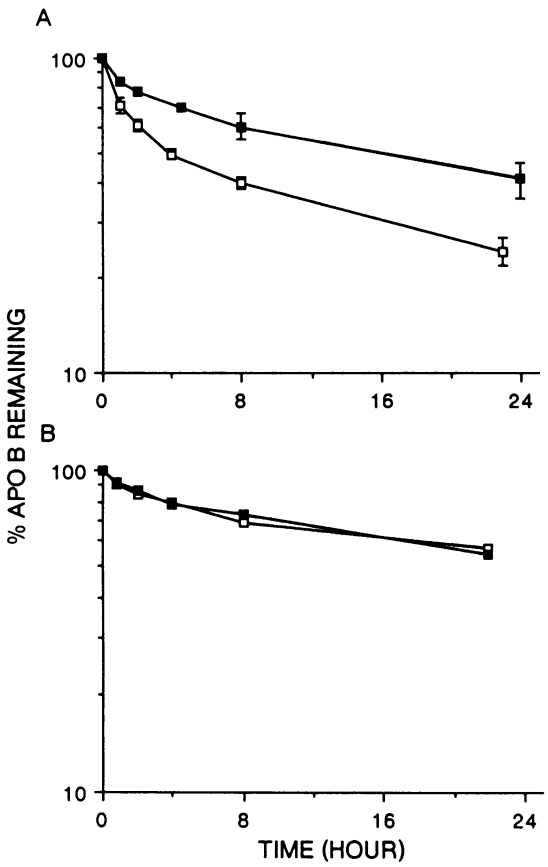

FIG. 5. Removal of ^{125}I-labeled apoB in plasma from blood plasma after injection of ^{125}I-labeled VLDL incubated with apoE (VLDL–E, □) and ^{125}I-labeled VLDL (■) into three WHHL rabbits (*A*) and injection of ^{125}I-labeled I+LDL ($1.006 < d < 1.063$) incubated with apoE (I+LDL–E, □) and ^{125}I-labeled I+LDL (■) into two WHHL rabbits (*B*). [Bars = 1 SE (where not shown, SE values fall within data point).]

injected *in vivo* (22, 24–26). In this report, we have shown that injection of a large amount of apoE into rabbits deficient in LDL receptors reduces plasma cholesterol by 20% between 4 and 8 hr after injection. In the early phase, the reduction was mainly in VLDL and IDL; later LDL cholesterol fell substantially. Lowered plasma cholesterol levels were maintained up to 24 hr after injection. The reduction of plasma cholesterol in the early phase is mainly explained by the removal of lipoproteins containing apoB because changes of both apoB and cholesterol in lipoprotein fractions were very similar. After injection of saline, plasma cholesterol levels were constant for the first 4 hr and then fell between 4 and 8 hr. It is, therefore, difficult to determine whether the decrease in plasma cholesterol in the late phase of the experiment was produced by apoE.

We determined the injected dose of apoE from preliminary *in vitro* experiments. By measuring the binding affinity of VLDL incubated with various amounts of apoE for LDL receptors on human fibroblast, we found that one-half to an equal amount of apoE to VLDL protein is required to obtain a maximal effect (H.M. and N.Y., unpublished data). Because the protein concentration of VLDL in WHHL rabbits is ≈30 mg/dl (11, 27), we decided to inject ≈30 mg of apoE.

As shown in Fig. 4, injection of apoE doubled the content of apoE in lipoproteins containing apoB-100. Before injection, apoB concentrations in VLDL and I+LDL isolated by density gradient ultracentrifugation were 15.5 and 137.9 mg/dl, respectively, and the corresponding values for apoE determined in fractions separated by gel filtration chromatography were 2.0 and 2.7 mg/dl, respectively. Based on these values, the average numbers of apoE molecule were estimated as 1.9 for VLDL and 0.29 for I+LDL from the molar ratio of apoE to apoB (based on molecular weights for apoB-100 of 512,000 and for apoE of 34,000). Immediately after injection of apoE, the average number of apoE molecules in VLDL and I+LDL particles was about 4 and 0.6, respectively, since the content of apoE was doubled. Previous studies, in which lipoproteins containing apoE were separated by immunosorption, demonstrated that 90% of both VLDL and IDL and 50% of LDL from WHHL rabbits are B,E particles. As in normal rabbits B,E particles in VLDL of WHHL rabbits were removed from the blood plasma more efficiently than B particles that lacked apoE. It has been proposed that multivalent binding of VLDL particles containing several apoE molecules promotes receptor-mediated endocytosis of lipoproteins containing apoB-100 (10, 11). The current study provides further evidence for this hypothesis by showing that incorporation of apoE into VLDL can influence the clearance of lipoproteins containing apoB-100.

In further support of this hypothesis, we found that the rate of removal of VLDL incubated with apoE (VLDL–E) was 3-fold higher than that of VLDL, whereas there was no difference between I+LDL–E and I+LDL (Fig. 5). This observation suggests that the number of apoE molecules on VLDL particles is critical for VLDL clearance. In contrast to VLDL, addition of apoE to IDL and LDL did not affect their turnover. A single molecule of apoE in IDL and LDL does not affect their turnover (10, 11). Rapid turnover of VLDL–E would be expected to reduce the formation of LDL from VLDL, resulting in the observed decrease in LDL cholesterol level.

There are two possible pathways to remove VLDL with additional apoE; the chylomicron-remnant receptor pathway is presumably intact in WHHL rabbits and the LDL receptor pathway. WHHL rabbits have a mutation of the LDL receptor gene and synthesize LDL receptors in which 4 amino acids are deleted from one of the binding domains (28). The abnormal LDL receptors are transported at a low rate from the endoplasmic reticulum to the Golgi apparatus, but a small number may reach the cell surface and could remove VLDL containing several apoE molecules. In the LDL-receptor-deficient state, we suggest that either chylomicron-remnant receptors or a small number of mutant LDL receptors can function to remove lipoproteins containing incorporated apoE.

Although lowering plasma cholesterol by injecting apoE may in principle reduce the rate of atherogenesis in WHHL rabbits, results of some studies suggest that VLDL containing several apoE molecules including β-VLDL promote cholesterol accumulation in macrophages (29, 30). Further research, including studies of the effects of sustained administration of apoE, is needed to determine the fate of cholesterol leaving the blood after injection of apoE, whether excreted as bile acids, secreted as VLDL, or incorporated into foam cells. ApoE has been produced biosynthetically by a bioengineering technique (31), and its availability should make it possible to determine whether apoE can be used to reduce the rate of atherogenesis in WHHL rabbits or other suitable animal models of accelerated atherogenesis.

We thank Dr. R. J. Havel for his helpful suggestions and discussion and Dr. N. Mori and T. H. Yoon for their collaboration in preparing apoE. This work was supported by a grant from Sandoz Foundation for Gerontological Research.

1. Brown, M. S. & Goldstein, J. L. (1986) *Science* **232**, 34–47.
2. Hofmann, S. L., Russell, D. W., Brown, M. S., Goldstein, J. L. & Hammer, R. E. (1988) *Science* **239**, 1277–1281.
3. Innerarity, T. L., Pitas, R. E. & Mahley, R. W. (1980) *Biochemistry* **19**, 4359–4365.

4. Windler, E., Kovanen, P. T., Chao, Y. S., Brown, M. S., Havel, R. J. & Goldstein, J. L. (1980) *J. Biol. Chem.* **255,** 10464–10471.
5. Gianturco, S. H., Bradley, W. A., Gotto, A. M., Jr., Morisett, J. M. & Peavy, D. L. (1982) *J. Clin. Invest.* **70,** 168–178.
6. Mahley, R. W., Hui, D. Y., Innerarity, T. L. & Weisgraber, K. H. (1981) *J. Clin. Invest.* **68,** 1197–1206.
7. Hui, D. Y., Innerarity, T. L. & Mahley, R. W. (1981) *J. Biol. Chem.* **256,** 5646–5655.
8. Havel, R. J., Goldstein, J. L. & Brown, M. S. (1980) in *Metabolic Control and Disease*, eds. Bondy, P. K. & Rosenburg, L. E. (Saunders, Philadelphia), 8th Ed., pp. 393–494.
9. Kita, T., Goldstein, J. L., Brown, M. S., Watanabe, Y., Hornick, C. A. & Havel, R. J. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 3623–3627.
10. Yamada, N., Shames, D. M., Stoudmire, J. B. & Havel, R. J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3479–3483.
11. Yamada, N., Shames, D. M. & Havel, R. J. (1987) *J. Clin. Invest.* **80,** 507–515.
12. Fielding, P. E. & Fielding, C. J. (1986) *J. Biol. Chem.* **261,** 5233–5236.
13. Havel, R. J., Eder, H. & Bragdon, J. (1955) *J. Clin. Invest.* **34,** 1345–1353.
14. Yamada, N., Murase, T., Akanuma, Y., Itakura, H. & Kosaka, K. (1982) *Horm. Metab. Res.* **14,** 303–306.
15. Shelburne, F. A. & Quarfordt, S. H. (1977) *J. Clin. Invest.* **60,** 944–950.
16. Weber, K. & Osborne, M. (1969) *J. Biol. Chem.* **244,** 4406–4412.
17. Shen, M. M. S., Krauss, R. M., Lindgren, F. J. & Forte, T. M. (1981) *J. Lipid Res.* **22,** 236–244.
18. van't Hooft, F. & Havel, R. J. (1981) *J. Biol. Chem.* **256,** 3963–3968.
19. Bucolo, G. & David, H. (1973) *Clin. Chem.* **19,** 476–482.
20. Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W. & Fu, P. C. (1974) *Clin. Chem.* **20,** 470–475.
21. Fainaru, M., Havel, R. J. & Imaizumi, K. (1977) *Biochim. Biophys. Acta* **490,** 144–155.
22. Yamada, N. & Havel, R. J. (1986) *J. Lipid Res.* **27,** 910–912.
23. McFarlane, A. S. (1958) *Nature (London)* **182,** 53–54.
24. Windler, E., Chao, Y. S. & Havel, R. J. (1980) *J. Biol. Chem.* **255,** 8303–8307.
25. Innerarity, T. L., Arnold, K. S., Weisgraber, K. H. & Mahley, R. W. (1986) *Arteriosclerosis* **6,** 114–122.
26. Imaizumi, K., Fainaru, M. & Havel, R. J. (1978) *J. Lipid Res.* **19,** 712–722.
27. Havel, R. J., Kita, T., Kotite, L., Kane, J. P., Hamilton, R. L., Goldstein, J. L. & Brown, M. S. (1982) *Arteriosclerosis* **2,** 467–474.
28. Yamamoto, T., Bishop, R. W., Brown, M. S., Goldstein, J. L. & Russell, D. W. (1986) *Science* **232,** 1230–1237.
29. Bates, S. R., Coughlin, B. A., Mazzone, T., Borensztajn, J. & Getz, G. S. (1987) *J. Lipid Res.* **28,** 787–797.
30. Soltys, P. A., Gump, H., Hennessy, L., Mazzone, T., Carey, K. D., McGill, H. C., Getz, G. S. & Bates, S. R. (1988) *J. Lipid Res.* **29,** 191–201.
31. Vogel, T., Weisgraber, K. H., Zeevi, M. I., Ben-Artzi, H., Levanon, A. Z., Rall, S. C., Innerarity, T. L., Hui, D. Y., Taylor, J. M., Kanner, D., Yavin, Z., Amit, B., Aviv, H., Gorecki, M. & Mahley, R. W. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8696–8700.