ApoVLDL of the Watanabe Heritable Hyperlipidemic rabbit and the cholesterol-fed rabbit

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Abstract The Watanabe Heritable Hyperlipidemic rabbit (WHHL rabbit) and the cholesterol-fed rabbit have been reported to show elevations of very low density (VLDL), intermediate density (IDL), and low density lipoproteins (LDL), and a broad-beta band on agarose-gel electrophoresis. We have studied the lipid and lipoprotein composition of WHHL rabbits and cholesterol-fed rabbits using ultracentrifugal analysis and isoelectric focusing. The total cholesterol (TC)/triglyceride (TG) ratios of VLDL, IDL, and LDL in WHHL rabbits were slightly elevated, but almost normal compared with those of cholesterol-fed rabbits, whose TC/TG ratios were markedly elevated compared with normolipidemic rabbits. ApoVLDL of WHHL rabbits showed no compositional changes in apoE and apoC isoforms, and no marked changes in the apoE/apoC ratios. But apoVLDL of cholesterol-fed rabbits showed a significant decrease of apoC-III, a significant increase of apoC-V, and marked elevation of the apoE/apoC ratio. We conclude that serum lipoprotein compositions in the WHHL rabbit were normal, while the lipoprotein lipid and apolipoprotein composition in the WHHL rabbit are different from those in cholesterol-fed rabbits.—**Wakasugi, T., H. Mabuchi, Y. Sakai, T. Sakai, A. Yoshimura, A. Watanabe, J. Koizumi, S. Miyamoto, R. Takeda, and Y. Watanabe.** ApoVLDL of the Watanabe Heritable Hyperlipidemic rabbit and the cholesterol-fed rabbit. *J. Lipid Res.* 1984. **25:** 246–253.

Supplementary key words isoelectric focusing electrophoresis • apoE • apoC

The Watanabe Heritable Hyperlipidemic rabbit (WHHL rabbit) is the first inbred strain with spontaneous hyperlipidemia and develops atherosclerosis and xanthoma on the digital joints. The inheritance is an autosomal recessive trait. There are elevations of VLDL, IDL, and LDL levels, and a broad-beta band on agarose-gel electrophoresis (1, 2). Kondo and Watanabe (1) reported that the hyperlipidemia in the WHHL rabbit was analogous to type III or II hyperlipidemia in man. Recently, deficiences of the LDL-receptor on cultured fibroblasts (3, 4), cultured hepatocytes (5), and membrane of liver and adrenal gland (6) were demonstrated in the WHHL rabbit. Thus, the WHHL rabbit is thought to be an animal model for familial hypercholesterolemia (FH) in man (3, 4).

Cholesterol-fed rabbits also show elevations of VLDL, IDL, and LDL levels, and a broad-beta band on agarose-gel electrophoresis. They also develop atherosclerosis (7), as does the WHHL rabbit. The hepatic lipoprotein receptors of cholesterol-fed rabbits were reported to be saturated and suppressed (8), or undetectable (5). The abnormality of receptor activity was phenotypically analogous to that in the WHHL rabbit liver.

The resemblance of the WHHL rabbit's lipid profile to type III hyperlipidemia raised a possibility of some abnormalities in apolipoprotein E (apoE) isoforms as in primary dysbetalipoproteinemia in man (9). Therefore the purpose of this study was to compare the lipoprotein lipid and VLDL apolipoprotein compositions of normal, cholesterol-fed rabbits and WHHL rabbits, in order to determine if the similarities in lipoprotein electrophoretic patterns in the WHHL rabbit and in human dysbetalipoproteinemia are due to some abnormalities in apoVLDL composition, particularly apoE, in the WHHL rabbit.

METHODS

Animals and sample collections

Eight male Japanese white rabbits weighing 2.8–3.4 kg were fed, for 1 week, 100 g/day of normal standard laboratory chow (Type ORC-4; Oriental Yeast Co., Ltd., Japan). Twenty ml of blood was obtained after an overnight fast (normal rabbits). These rabbits were then fed 100 g/day of commercial chow containing 1% cholesterol (Oriental Yeast Co., Ltd., Japan). Ten ml of blood was obtained after 1 week (Chol-fed rabbit). Ten ml of blood

Abbreviations: VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; TC, total cholesterol; TG, triglyceride; PL, phospholipid; apoVLDL, apolipoproteins in VLDL; apoE, apolipoprotein E; apoC, apolipoprotein C; FH, familial hypercholesterolemia; WHHL, Watanabe Heritable Hyperlipidemic rabbit; IEF, isoelectric focusing.

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was also obtained from eight male WHHL rabbits fed 100 g/day of normal chow for 1 week.

Analysis of lipoprotein lipids

VLDL ($d < 1.006$ g/ml), IDL ($1.006 < d < 1.019$ g/ml), LDL ($1.019 < d < 1.063$ g/ml), and HDL ($d > 1.063$ g/ml) fractions were obtained sequentially by preparative ultracentrifugation of 1 or 2 ml of serum according to standard procedures (10).

Total cholesterol (TC) and phospholipid (PL) in serum and each lipoprotein fraction were determined enzymatically (11, 12). Triglycerides (TG) were determined by the method of Fletcher (13) using triolein as a standard. Protein concentration in VLDL was determined by the method of Lowry et al. with modification (14, 15).

Isoelectric focusing (IEF)

VLDL was isolated by ultracentrifugation from serum at d 1.006 g/ml (105,000 *g*, 10°C, 20 hr). VLDL was refloated under identical conditions.

The washed VLDL containing 250–300 μg of apoVLDL was delipidated two times with 5 vol of acetone–ethanol 1:1 (v/v) and diethylether according to Warnick et al. (16). The apoVLDL was solubilized in 200 μl of 8 mol/l urea containing Tris (hydroxymethyl) aminomethane-HCl (10 mmol/l, pH 8.6) and dithiothreitol (10 mmol/l) at 4°C for 4–6 hr (16).

Electrophoresis was performed essentially according to Warnick et al. (16). IEF gels, which contained 7.5% acrylamide, 0.2% N,N′-methylene bisacrylamide, 8 mol/l urea, and 2% ampholine, pH 4–6, were focused at a constant voltage of 150 V for 16 hr at 4°C. Proteins were fixed, stained (Coomassie brilliant blue), and destained (17). Relative proportions of the isoforms were determined by densitometry at 550 nm with a scanning densitometer. Isoelectric points were determined according to Warnick et al. (16).

Identification of protein bands and comparison with human apoVLDL

ApoVLDL from a normal rabbit was electrophoresed in a discontinuous buffer system on 10% polyacrylamide gels that contained 8 mol/l urea (15). One gel was stained to locate the apoE and apoC bands with their characteristic mobilities (7), and these regions were then sliced from the parallel unstained gels electrophoresed together. Human apoE and apoC bands were sliced from the electrophoresed gels of normal human apoVLDL. These slices were used in the next two analyses.

SDS electrophoresis. SDS electrophoresis was performed as follows according to Neville (18). The upper reservoir buffer contained 0.04 mol/l boric acid, 0.041 mol/l Tris (hydroxymethyl) aminomethane (pH 8.64), and 0.1% SDS. The upper polyacrylamide gel (T = 3.2%, C = 6.25%) contained buffer of 0.0267 mol/l H_2SO_4-0.054 mol/l Tris(hydroxymethyl)aminomethane, pH 6.1. The lower polyacrylamide gel (T = 9.4%, C = 0.9%) contained buffer of 0.0237 N HCl-1.3147 mol/l Tris(hydroxymethyl)aminomethane, pH 9.81. The lower reservoir buffer was the same as the lower gel buffer. Two of the slices containing rabbit apoE were incubated at 100°C for 30 min in upper-gel buffer containing 0.2% SDS, 0.2% β-mercaptoethanol and 0.005% bromophenol blue. These incubated gel slices were placed on top of an SDS gel and electrophoresed. Gels containing rabbit apoC, human apoE, or human apoC were also incubated and electrophoresed together.

Isoelectric focusing. Gel slices were placed on top of IEF gels and subjected to focusing as described in the previous section. Rabbit apoVLDL was also electrophoresed in order to identify the IEF bands.

Data were analyzed using Student's *t*-test and Wilcoxon's test.

RESULTS

Serum and lipoprotein lipids (Table 1)

The serum TC levels were significantly elevated (517 ± 160, 891 ± 230 and 1657 ± 392 (mean ± SD) mg/dl) by feeding the 1% cholesterol chow diet for 1, 2, and 4 weeks, respectively, compared to the level in normal rabbits (58 ± 19 mg/dl). The serum TC levels in rabbits fed the 1% cholesterol chow diet for 1 week did not differ from WHHL rabbits, whose serum TC levels were significantly higher than normal rabbits. However, serum TC levels of rabbits fed the 1% cholesterol chow diet for 2 or 4 weeks were significantly higher than in WHHL rabbits. Therefore, we compared WHHL rabbits with normal rabbits and rabbits fed the 1% cholesterol chow diet for only 1 week (Chol-fed rabbits). The data from this study cannot be extrapolated to all cholesterol-fed animals regardless of their plasma cholesterol concentrations.

The TC levels of VLDL, IDL, and LDL fractions in Chol-fed and WHHL rabbits were significantly higher (>10-fold) than in normal rabbits. There were no significant differences between Chol-fed and WHHL rabbits in the TC levels. However, the HDL-TC levels in WHHL rabbits were significantly lower than in normal and Chol-fed rabbits.

In WHHL rabbits, serum and lipoprotein TG levels were significantly greater than in normal rabbits except for HDL-TG, which was significantly lower. In contrast to WHHL rabbits, lipoprotein TG levels in Chol-fed rabbits were similar to those in normal rabbits except for the significant decrease of HDL-TG level. The changes

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TABLE 1. Serum and lipoprotein lipids

	Serum	VLDL	IDL	LDL	HDL
Total cholesterol (mg/dl)					
Normal	58 ± 19	6 ± 3	5 ± 4	17 ± 12	24 ± 4
Chol-fed	517 ± 160[a]	120 ± 62[a]	97 ± 38[a]	228 ± 59[a]	22 ± 3[a]
WHHL	609 ± 224[a]	157 ± 105[a]	75 ± 25[a]	312 ± 188[a]	10 ± 4[a,c]
Triglyceride (mg/dl)					
Normal	63 ± 19	20 ± 7	6 ± 4	13 ± 8	14 ± 2
Chol-fed	33 ± 20[b]	12 ± 10	5 ± 2	10 ± 5	7 ± 4[a]
WHHL	509 ± 333[a,d]	228 ± 183[a,d]	48 ± 33[a,d]	166 ± 55[a,c]	7 ± 2[a]
Phospholipid (mg/dl)					
Normal	85 ± 14	9 ± 3	5 ± 3	14 ± 9	56 ± 7
Chol-fed	205 ± 66[a]	37 ± 19[a]	36 ± 15[a]	92 ± 25[a]	45 ± 8[b]
WHHL	414 ± 128[a,d]	98 ± 64[a]	48 ± 18[a]	203 ± 89[a,d]	38 ± 3[a,d]

Levels of lipids were determined in serum and ultracentrifugally separated lipoprotein fractions of eight normolipidemic rabbits (Normal), four rabbits fed a 1% cholesterol chow diet for 1 week (Chol-fed), and eight WHHL rabbits (mean ± SD).

[a] $P < 0.01$ versus normal rabbits.
[b] $P < 0.05$ versus normal rabbits.
[c] $P < 0.01$ versus Chol-fed rabbits.
[d] $P < 0.05$ versus Chol-fed rabbits.

of serum and lipoprotein PL levels in Chol-fed and WHHL rabbits were similar to those of TC levels, but the degree of change of PL levels from normal values was smaller than those of TC levels.

Lipid ratios in lipoprotein fractions (Table 2)

The TC/TG ratio in Chol-fed rabbits was markedly higher than in normal rabbits, 99-fold for VLDL, 38-fold for IDL, and 24-fold for LDL. The ratios in WHHL rabbits were significantly (twofold) higher than in normal rabbits. Although there were large variations of serum and lipoprotein TC levels among rabbits, there were no specific differences of the ratios between rabbits with high cholesterol concentration and those with low values.

The TC/PL ratios in these lipoprotein fractions of Chol-fed and WHHL rabbits were significantly increased, differences in ratios between normal and WHHL rabbits were smaller than those between normal and Chol-fed rabbits.

TC/TG and TC/PL ratios in HDL decreased in WHHL rabbits and increased in Chol-fed rabbits.

The ratio of VLDL-TC/serum TG, which is elevated in type III hyperlipoproteinemia in man (19), increased significantly in Chol-fed rabbits by 56-fold and in WHHL rabbits by only 3-fold.

There were marked differences in these ratios, especially TC/TG and VLDL-TC/serum TG, beteen Chol-fed and WHHL rabbits. The ratios in Chol-fed rabbits were more than 15 times higher than in WHHL rabbits.

TABLE 2. Lipid ratios in lipoprotein fractions

	VLDL	IDL	LDL	HDL
TC/TG ratio				
Normal	0.29 ± 0.11	0.83 ± 0.19	1.26 ± 0.32	1.76 ± 0.28
Chol-fed	28.8 ± 37.8[b]	31.7 ± 23.4[a]	30.4 ± 18.1[a]	4.16 ± 2.33[b]
WHHL	0.74 ± 0.23[a]	1.95 ± 0.98[a,c]	1.98 ± 1.20[c]	1.54 ± 0.66[d]
TC/PL ratio				
Normal	0.64 ± 0.27	1.15 ± 0.18	1.20 ± 0.20	0.42 ± 0.04
Chol-fed	3.49 ± 1.26[a]	2.79 ± 0.41[a]	2.51 ± 0.32[a]	0.49 ± 0.08
WHHL	1.58 ± 0.29[a,c]	1.60 ± 0.30[a,c]	1.49 ± 0.23[b,c]	0.27 ± 0.12[a,c]
VLDL-TC/serum TG ratio				
Normal 0.09 ± 0.02 : Chol-fed 5.04 ± 3.42[a] : WHHL 0.30 ± 0.11[a,c]				

TC/TG and TC/PL ratios in each lipoprotein fraction and VLDL-TC/serum TG were determined in eight normolipidemic rabbits (Normal), four rabbits fed a 1% cholesterol chow diet for 1 week (Chol-fed), and eight WHHL rabbits (mean ± SD).

[a] $P < 0.01$ versus normal rabbits.
[b] $P < 0.50$ versus normal rabbits.
[c] $P < 0.01$ versus Chol-fed rabbits.
[d] $P < 0.01$ versus Chol-fed rabbits.

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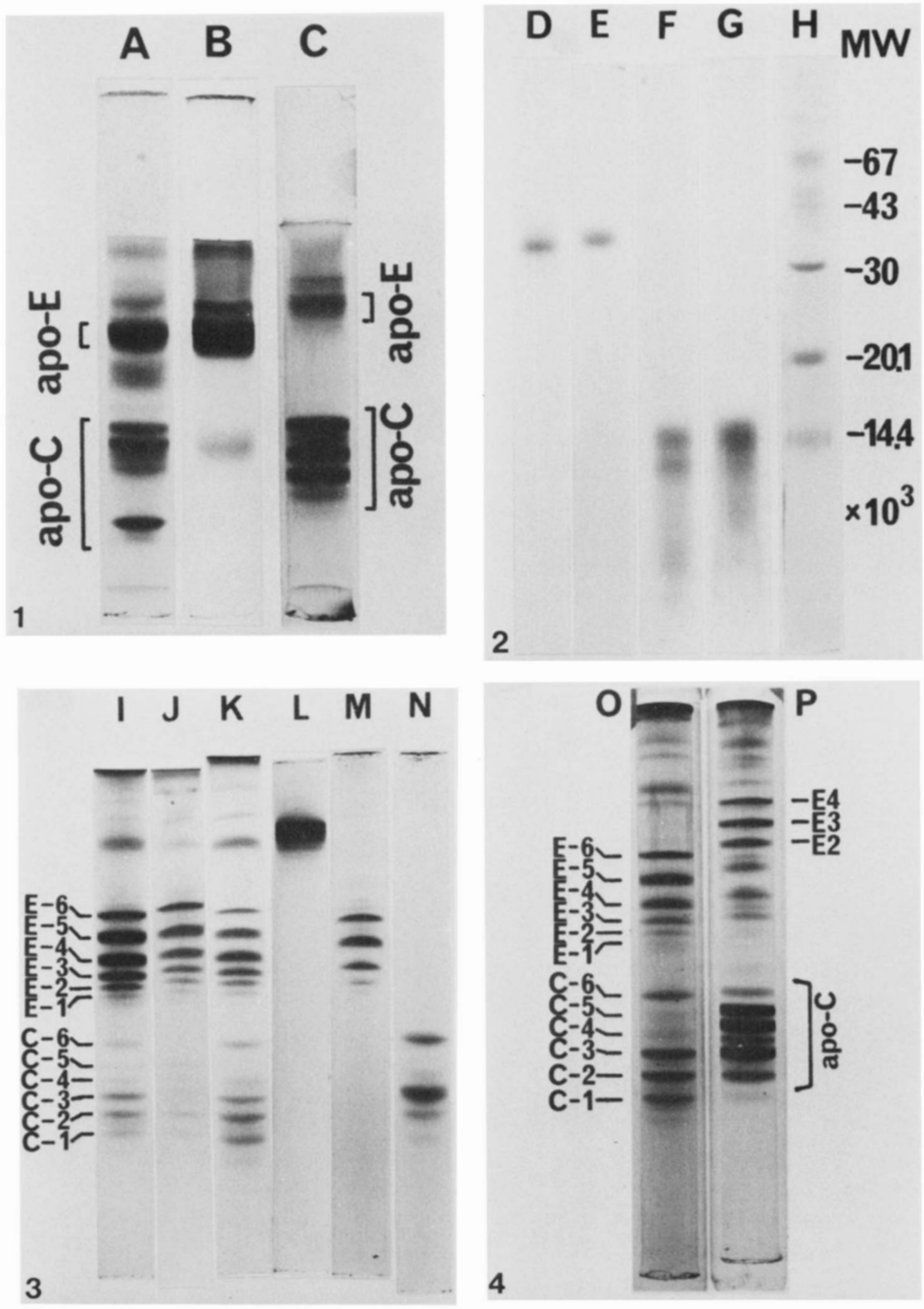

Fig. 1. Isoelectric focusing pattern of rabbit apoVLDL and identification of protein bands. 1) Polyacrylamide-gel disc electrophoresis in a discontinuous buffer system. A, Normal rabbit (TC 25 mg/dl); B, cholesterol-fed rabbit (TC 401 mg/dl); C, human VLDL. ApoE and apoC are indicated according to their characteristic mobilities (7). 2) SDS electrophoresis was performed according to Neville (18). The upper gel contained 3.2% polyacrylamide and H_2SO_4-Tris(hydroxymethyl)aminomethane buffer, pH 6.1. The lower gel contained 9.4% polyacrylamide and Tris(hydroxymethyl)aminomethane-HCl buffer, pH 9.81. Gel slices (from parallel gels electrophoresed as in Fig. 1-1) were incubated in buffer of the upper gel containing 0.2% SDS, 0.2% β-mercaptoethanol, and 0.005% bromophenol blue. The incubated gel slices were placed on top of SDS-gels and electrophoresed. Columns D, E, F, and G are the electrophoretic patterns of the protein designated in Fig. 1-1 as rabbit apoE, human apoE, rabbit apoC, and human apoC, respectively. Rabbit and human apoE had identical R_f values. Rabbit and human apoC also had the same R_f values. 3) IEF electrophoresis. Gel slices (from parallel gels electrophoresed as in Fig. 1-1) were placed on top of IEF-gels, and focused as outlined in Methods. Columns M and N are IEF patterns of proteins designated as apoE and apoC (rabbit) in Fig. 1-1, respectively. Columns I, J, and K are the IEF patterns of apoVLDL of a WHHL rabbit (TC 370 mg/dl), apoVLDL of a cholesterol-fed rabbit (TC 479 mg/dl), and apoVLDL of a normal rabbit, respectively. Column L is rabbit albumin. Gels K, M, and N were electrophoresed together; the others were run separately. 4) Comparison of IEF patterns of rabbit and human apoVLDL. Rabbit apoVLDL (column O) and human apoVLDL (column P) were electrophoresed together. There were no apoE isoforms of rabbit corresponding to human apoE-3 and apoE-4.

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IEF pattern of rabbit apoVLDL

ApoVLDL from normal rabbits were subjected to polyacrylamide gel electrophoresis by Kane's method (15). Protein bands, presumed to be apoE and apoC according to their characteristic mobilities (7) (**Fig. 1-1**), were sliced from the gels and subjected to SDS electrophoresis and IEF as described in Methods. Human apoE and presumed rabbit apoE had identical R_f values by SDS electrophoresis. Human apoC and the presumed rabbit apoC also had identical R_f values (Fig. 1-2).

The IEF patterns of VLDL apoE and apoC are shown in Fig. 1-3. Rabbit apoE was separated into at least six isoforms by IEF electrophoresis. The most basic isoform was designated E-6, and more acidic isoforms were E-5, E-4, E-3, E-2, and E-1, respectively. Apparent pI values at 4°C of the apoE isoforms in 8 mol/l urea ranged from approximately 5.4 to 5.7 (**Fig. 2**). These pI values were

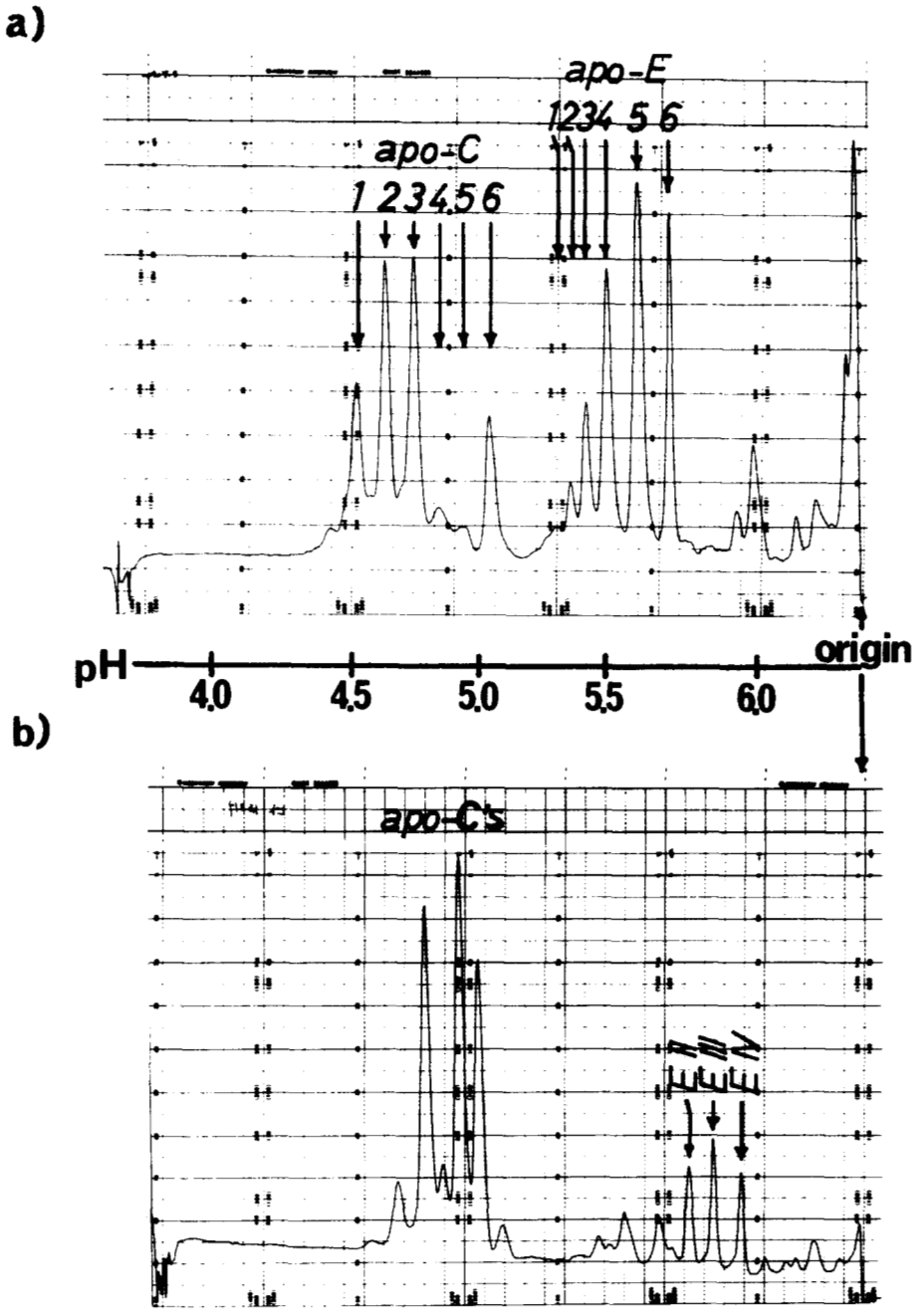

Fig. 2. Densitometric pattern of rabbit and human apoVLDL. IEF gels of rabbit and human apoVLDL were scanned at 550 nm. a) Normal rabbit apoVLDL; b) human apoVLDL. Apparent pI values (at 4°C) of rabbit apoproteins were as follows: apoE, 5.4–5.7; apoC, 4.4–5.2.

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slightly more acidic than those of human apoE. There were no clear apoE bands corresponding to human apoE-3 and apoE-4 (Fig. 1-4). ApoVLDL proteins more acidic than apoE were primarily C-apoproteins. Apparent pI values of these proteins ranged from approximately 4.4 to 5.2 (Fig. 2). The most basic of the C-apoproteins was designated C-6, and other more acidic isoforms were C-5, C-4, C-3, C-2, and C-1, respectively. For comparison, IEF patterns of apoVLDL from a Chol-fed rabbit and a WHHL rabbit, whose serum TC levels were 479 and 370 mg/dl, respectively, are shown in Fig. 1-3.

ApoVLDL composition of normal, Chol-fed, and WHHL rabbits (Table 3)

The relative proportions of apoE isoforms in WHHL rabbits did not differ from those of normal rabbits. However, E-4 increased and E-6 decreased significantly in Chol-fed rabbits. Since there were no changes of apoE isoforms in rabbits fed the 1% cholesterol chow diet for 2 or 4 weeks compared with those of normal rabbits (data not shown), the observed changes of E-4 and E-6 bands in Chol-fed rabbits might not be meaningful.

The relative proportions of apoC isoforms in Chol-fed and WHHL rabbits changed as follows. In Chol-fed rabbits, C-3 decreased and C-5 increased significantly compared with bands of normal rabbits. These changes were also demonstrated in rabbits fed the 1% cholesterol chow diet for 2 or 4 weeks (C-3, 25.9 ± 3.7 and 20.1 ± 5.1%, respectively; C-5, 8.7 ± 1.6 and 15.9 ± 4.4%, respectively). There were significant correlations between C-3 and VLDL-TC ($r = -0.52$, $P < 0.05$), and between C-5 and VLDL-TC ($r = 0.69$, $P < 0.01$). In WHHL rabbits, only the C-6 increased significantly compared with normal rabbits. Since there was no significant correlation between C-6 and lipoprotein lipids, the observed change of C-6 in WHHL rabbits might not be meaningful.

We summed the total area under all of the isoforms for the two proteins, and calculated the apoE/apoC ratio. This ratio was significantly ($P < 0.01$) increased in Chol-fed rabbits (18.6 ± 9.7), but did not change in WHHL rabbits (8.0 ± 6.8), when compared with normal rabbits (3.2 ± 2.1).

DISCUSSION

Japanese white rabbits fed a 1% cholesterol chow diet for 1 week and WHHL rabbits had equally elevated levels of cholesterol in serum, VLDL, IDL, and LDL. However, the HDL-TC level decreased significantly in WHHL rabbits. Serum and lipoprotein triglyceride (except HDL) levels were markedly increased in WHHL rabbits, but were unchanged or decreased in Chol-fed rabbits. These changes observed in WHHL rabbits and Chol-fed rabbits were identical to those previously reported (3, 7).

WHHL rabbits, Chol-fed rabbits, and patients with homozygous FH all have hypercholesterolemia (3, 7, 20), but there are marked differences in the serum TG levels between WHHL rabbits (hypertriglyceridemic) and patients with FH (almost normal TG levels (20)), and between WHHL rabbits and Chol-fed rabbits (normotriglyceridemic). In order to clarify these differences, we examined the lipoprotein lipid ratios of normal, Chol-fed, and WHHL rabbits.

Chol-fed rabbits had much higher TC/TG ratios in VLDL, IDL, and LDL than normal rabbits. In contrast

TABLE 3. The relative proportions of rabbit apoE and apoC isoforms

	ApoE					
	E-1	E-2	E-3	E-4	E-5	E-6
	%					
Normal	0.9 ± 1.1	3.5 ± 4.4	12.9 ± 8.7	17.2 ± 6.3	35.8 ± 6.0	29.7 ± 4.8
Chol-fed	1.7 ± 0.3	5.8 ± 1.1	12.8 ± 1.4	24.7 ± 0.7[b]	35.0 ± 2.2	20.1 ± 2.2[a]
WHHL	0.8 ± 0.5	2.5 ± 0.7	7.9 ± 2.1	19.5 ± 5.1	37.0 ± 2.8	32.3 ± 6.1
	ApoC					
	C-1	C-2	C-3	C-4	C-5	C-6
	%					
Normal	10.2 ± 5.2	36.8 ± 11.8	34.7 ± 9.3	7.1 ± 4.3	2.1 ± 1.1	11.3 ± 11.4
Chol-fed	16.6 ± 3.8	29.8 ± 4.7	21.5 ± 3.4[b]	11.2 ± 2.7	13.8 ± 4.1[a]	6.0 ± 2.9
WHHL	7.1 ± 3.5	28.7 ± 9.6	32.7 ± 11.1	3.5 ± 2.8	4.7 ± 3.3	23.4 ± 9.5[b]

VLDL from eight normolipidemic rabbits (Normal), four rabbits fed a 1% cholesterol chow diet for 1 week (Chol-fed), and eight WHHL rabbits was isolated and washed once by ultracentrifugation. The VLDL was delipidated and the apoproteins were subjected to IEF as described in Methods. The proteins were fixed, stained, and destained (17), and the gel was scanned at 550 nm (mean ± SD).

[a] $P < 0.01$ versus normolipidemic rabbits.

[b] $P < 0.05$ versus normolipidemic rabbits.

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to Chol-fed rabbits, the ratio in WHHL rabbits was slightly higher than in normal rabbits. There were also marked differences in the VLDL-TC/serum TG ratio between Chol-fed and WHHL rabbits. The ratio was 56-fold greater in Chol-fed rabbits and only 3-fold higher in WHHL rabbits than in normal rabbits. These data showed that lipoproteins of Chol-fed rabbits were markedly enriched with cholesterol. Lipoproteins of WHHL rabbits were slightly cholesterol-rich but the lipid ratios were essentially normal.

The lipid profile of Chol-fed rabbits might be analogous to that seen in human type III hyperlipoproteinemia, inasmuch as lipoproteins in Chol-fed rabbits were cholesterol-rich, and their VLDL showed β-mobility on agarose-gel electrophoresis (β-VLDL) (21). Patients with FH have been reported to show a slightly increased TC/TG ratio in VLDL (22) and LDL (23), as did WHHL rabbits in the present study. Therefore, the lipid compositions of WHHL rabbit lipoproteins are analogous to those of human FH, and different from those of type III hyperlipoproteinemia; WHHL rabbits had increased concentrations of lipoproteins with normal compositions. There remain differences between WHHL rabbits and patients with FH. WHHL rabbits have high levels of VLDL and IDL, while patients with homozygous FH do not.

Utermann, Jaeschke, and Menzel (9) reported a deficiency of VLDL apoE-3 in human primary type III hyperlipoproteinemia, in which there were elevated levels of VLDL and IDL (21). Our first interest in this study was whether or not the apoVLDL of the WHHL rabbit was abnormal, inasmuch as it was previously reported to resemble type III hyperlipoproteinemia (1) based on the broad-β band on agarose-gel electrophoresis and the elevation of VLDL and IDL levels.

Chol-fed rabbits showed marked and significant elevations in the apoE/apoC ratio of VLDL and several compositional changes of C-apoproteins. In contrast to Chol-fed rabbits, WHHL rabbit had no meaningful abnormalities in the apoE/apoC ratio or in the IEF patterns of these apoproteins, when compared with normal and Chol-fed rabbits. We conclude that the lipoproteins of WHHL rabbits were essentially normal in lipid and apolipoprotein composition compared with rabbits fed a 1% cholesterol chow diet for a week.

Kita et al. (6) reported that the hepatic LDL-receptors which bind β-VLDL and LDL were defective in WHHL rabbits and that EDTA-resistant β-VLDL binding sites were decreased. The receptor corresponding to the human apoE receptor (24) has not yet been demonstrated in rabbits. Even though the chylomicron remnant receptor (25) functions normally in the WHHL rabbit, it does not mediate normal clearance of plasma VLDL and LDL, because the clearance of VLDL and LDL has been shown to be delayed in WHHL rabbits (26). Therefore, VLDL and IDL probably accumulate because of the decreased functioning β-VLDL binding sites in WHHL rabbits.

Binding sites for β-VLDL and LDL in liver, which are probably identical to the LDL-receptor, were suppressed (8) or undetectable (5) in cholesterol-fed rabbits. The differences in the serum TG levels between Chol-fed rabbits and WHHL rabbits cannot be the result of the change in LDL-receptor activity alone, because the receptor decreased in both types of rabbits as discussed above. There may be some unknown mechanism other than the change in LDL-receptor activity for the difference between Chol-fed and WHHL rabbits. For example, the activity of lipoprotein lipase may increase enough to catabolize the increased amount of secreted VLDL in cholesterol-fed rabbits, inasmuch as there are changes in the proportions of apoC isoforms. In WHHL rabbits these changes may be insufficient to affect lipoprotein lipase activity. Accumulation of chylomicron remnants in plasma (27) in cholesterol-fed rabbits may account for the abnormalities of VLDL. The hepatic uptake of chylomicron remnants may be less active in cholesterol-fed rabbits than in normal rabbits, inasmuch as the clearance of remnants was delayed (27). The LDL-receptor was not detected because of its suppression in cholesterol-fed rabbits (8), whereas, it was not detected in WHHL rabbits because of a genetic deficiency. This difference of pathogenesis for LDL-receptor abnormality may account for the difference of serum TG levels. Havel et al. (28) reported on the concentration and composition of lipoproteins in blood plasma of the WHHL rabbits. They discussed the point that the differences of the lipoprotein abnormalities between WHHL rabbits and human homozygous FH were likely to reflect a difference in lipoprotein metabolism in the species.

In cholesterol-fed rabbits, there are several changes in lipid and apolipoprotein compositions which have not been reported in human FH. Lipoproteins of WHHL rabbits were almost normal in lipid and apolipoprotein compositions as in human FH. Therefore, the WHHL rabbit is a promising model of human FH, but the cholesterol-fed rabbit is not.

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