Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 6096–6100, October 1983
Medical Sciences

Secretion of lipoproteins from the liver of normal and Watanabe heritable hyperlipidemic rabbits

(animal model for familial hypercholesterolemia/deficient low density lipoprotein receptor)

CONRAD A. HORNICK*, TORU KITA†‡, ROBERT L. HAMILTON*, JOHN P. KANE*, AND RICHARD J. HAVEL*§

*Cardiovascular Research Institute and Departments of Medicine and Anatomy, University of California School of Medicine, San Francisco, California 94143; and †Departments of Molecular Genetics and Internal Medicine, University of Texas Health Science Center at Dallas, Dallas, Texas 75235

Contributed by Richard J. Havel, June 27, 1983

ABSTRACT **We compared the rate of accumulation of lipoproteins in perfusates of isolated livers from normal New Zealand White rabbits and Watanabe heritable hyperlipidemic (WHHL) rabbits, in which a gene mutation has produced a virtually complete deficiency of low density lipoprotein (LDL) receptors. The rate of accumulation of apolipoprotein B-100 did not differ in perfusates of livers from normal and mutant animals and little or no apolipoprotein B-48 was detected. In both groups, virtually all apolipoprotein B accumulated in very low density lipoprotein (VLDL). Experiments in which [^{3}H]lysine was added to the perfusates showed that the apolipoprotein B that accumulated in VLDL was newly synthesized by the liver whereas the small amount of apolipoprotein B found in lipoproteins of higher density appeared to be washed out of extravascular spaces during perfusion. Perfusate VLDL from both groups contained more triglycerides and less cholesteryl esters than their counterparts from blood plasma. As compared with perfusate VLDL from normal livers, those from livers of WHHL rabbits were enriched in cholesteryl esters. Experiments in which Triton WR-1339 was injected into the blood of intact rabbits confirmed the observations with perfused livers. Previous studies have shown that the extent to which VLDL is converted to LDL is increased several-fold in WHHL rabbits. Taken together with our present results, which fail to provide evidence for increased secretion of apolipoprotein B or *de novo* secretion of lipoproteins other than VLDL that contain apolipoprotein B, it can be concluded that overproduction of LDL in rabbits lacking LDL receptors is solely the result of altered metabolism of VLDL.**

High levels of low density lipoprotein (LDL) caused by deficiency of specific LDL receptors and accelerated atherosclerotic disease are cardinal features of homozygous familial hypercholesterolemia (FH) in humans (1). These same features characterize the Watanabe heritable hyperlipidemic (WHHL) rabbit, which provides an instructive animal model of the human disease (2–5).

In human homozygous FH and in WHHL homozygotes, hypercholesterolemia is caused in part by impaired catabolism of LDL, a direct result of the receptor deficiency, but, in addition, the rate of transport of apolipoprotein (apo) B, the protein component of LDL, is increased 2- to 5-fold (6, 7). Apo B of LDL is normally produced during the catabolism of very low density lipoprotein (VLDL), which is secreted from the liver (8). In human homozygotes, the rate of production of VLDL apo B is reportedly normal and much lower than that of LDL (9). Thus, it has been concluded that in these individuals apo B of LDL is produced independently of VLDL. Recent studies of WHHL rabbits have shown that the concentration of apo B is increased in VLDL and intermediate density lipoprotein (IDL), which represents partially catabolized VLDL, as well as LDL (5). In normal rabbits, most of the apo B of hepatogenous VLDL appears to be taken up by the liver and only a small fraction is converted, by way of IDL, to LDL (10, 11). In contrast, the rate of hepatic uptake of apo B of VLDL is reduced in WHHL rabbits, and more apo B accumulates in IDL, which subsequently is converted to LDL (11). The increased rate of production of LDL evidently could result from impaired hepatic uptake of partially catabolized VLDL, because of the deficiency of hepatic LDL receptors. However, the rate at which the liver secretes apo B and the form in which it is secreted in WHHL rabbits have not been determined, so that the pathogenesis of the increased transport of LDL in this animal is uncertain. We now provide evidence that neither increased secretion of apo B in VLDL nor independent secretion of apo B in LDL contribute to the increased production of LDL in WHHL rabbits.

MATERIALS AND METHODS

Animals. Male New Zealand White (NZW) rabbits were purchased from Nitabell (Hayward, CA) or from Hickory Hill Rabbitry (Flint, TX). Male homozygous WHHL rabbits were raised in Dallas (11). Animals were fed Purina rabbit laboratory chow and were used at 2–3 months of age (body weight, 1–2 kg) for liver perfusion experiments or at 3–4 months (2–2.5 kg) for *in vivo* experiments.

Liver Perfusions. The perfusion apparatus and silastic membrane oxygenator were modified from those described by Hamilton *et al.* (12) to accommodate the larger rabbit livers. The recirculating perfusate consisted of 90 ml of a suspension of washed rabbit erythrocytes (25% hematocrit) in Krebs–Henseleit buffer (pH 7.4) with 0.5 mg of glucose per ml. Livers from anesthetized rabbits (13) were perfused at 1.0–1.5 ml/g per min. Viability of the organ was evaluated by its appearance, rate of flow of bile, and by perfusate pH, P_{CO_2}, and P_{O_2}. After 30 min, the recirculation was interrupted, 30 ml of fresh perfusate was passed through the liver, and recirculation was immediately reinstituted with 90 ml of fresh perfusate. To prevent vasospasm, 5 mg of papaverine·HCl (Eli Lilly) was added to the perfusate at intervals as needed. Livers in which more than small areas of cyanosis appeared on the surface were discarded. To measure synthesis of apo, [4,5-^{3}H]lysine (Amersham) was added to perfusates every 30 min.

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: apo, apolipoprotein; FH, familial hypercholesterolemia; IDL, intermediate density lipoprotein(s); HDL, high density lipoprotein(s); LDL, low density lipoprotein(s); VLDL, very low density lipoprotein(s); NZW, New Zealand White; WHHL, Watanabe heritable hyperlipidemic; ρ, density.

‡ Present address: Dept. of Internal Medicine, Kyoto Univ., Kyoto, Japan.
§ To whom reprint requests should be addressed.

Intravenous Injection of Triton WR-1339. NZW and WHHL rabbits, matched for weight, were anesthetized, and a catheter was inserted into the internal jugular vein (7); the animals were allowed to recover for 48–72 hr. Triton WR-1339 (Ruger, Irvington, NJ) at 200 mg/ml in 0.15 M NaCl was injected into an ear vein at a dose of 400 mg/kg of body weight. Blood samples of 3 ml were obtained at intervals from the indwelling catheter.

Analyses. At the end of each perfusion experiment, lipoproteins were isolated from the perfusate plasma by sequential ultracentrifugation (14). For measurement of lipoprotein composition (5), lipoprotein fractions were recentrifuged at their upper density limits. Albumin and apo E were estimated in whole perfusate by radial immunodiffusion (15) and radioimmunoassay (16), respectively. Proteins of isolated perfusate lipoproteins were separated on 0.1% $NaDodSO_4$/3% polyacrylamide gels and visualized by staining with Coomassie brilliant blue (17). Gels were sliced into 2-mm sections, dissolved in 30% H_2O_2, and assayed for ^3H (18). Agarose gel electrophoresis (19) and electron microscopic evaluation (20) of lipoproteins were carried out as described.

RESULTS

Body and liver weights of normal NZW and WHHL rabbits used in perfusion experiments were closely similar, as was the concentration of major lipid classes in liver (Table 1). Serum triglyceride and cholesterol levels of WHHL rabbits were grossly elevated.

P_{O_2} and P_{CO_2} in the venous inflow ranged from 350 to 450 mm Hg and 23 to 25 mm Hg, respectively. Corresponding values in the venous outflow were 70–100 and 30–40 mm Hg and did not change systematically during the perfusion. Hourly rates of bile flow for both normal NZW and WHHL perfusions were approximately 0.04 ml/g of liver. Inflow pH fell gradually from 7.4 to 7.1. Triglycerides accumulated at a linear rate in perfusates of normal NZW and WHHL rabbits (Fig. 1). The mean rate of accumulation was slightly higher (25%) in WHHL perfusates, as estimated from regression analysis. The mean rate of accumulation of cholesterol was moderately higher (62%) in WHHL perfusates but that of cholesteryl esters was about 4-fold higher than that of normal animals. Albumin and apo E also accumulated at linear rates (Fig. 1). For albumin, the rate was 34% lower in WHHL perfusates, whereas that of apo E was 57% higher than that of normals. Hourly rates of secretion of cholesterol and phospholipids into bile were virtually identical in the two groups at about 1.8 and 6.0 μg/g of liver, respectively.

VLDL was the major class of lipoproteins that accumulated after 3 hr in perfusates of both normal and WHHL livers, accounting for 75–96% of the lipids and 71–76% of the total protein recovered (Table 2). VLDL in both groups had slow pre-β mobility in agarose gel electrophoretograms. The mass of cholesteryl esters recovered in VLDL was about 3-fold higher

Table 1. Characteristics of normal NZW and WHHL rabbits used in perfusion studies

	Normal NZW	WHHL
Weight, g		
Body	1,550 ± 223	1,396 ± 340
Liver	51 ± 8	51 ± 24
Serum lipids, mg/dl		
Triglycerides	118 ± 49	736 ± 315*
Total cholesterol	87 ± 33	741 ± 263*
Liver lipids, mg/g		
Esterified cholesterol	0.28 ± 0.24	0.13 ± 0.11
Triglycerides	11.7 ± 4.8	16.0 ± 8.1
Free cholesterol	3.16 ± 0.45	2.76 ± 0.57
Phospholipids	33.5 ± 3.8	34.2 ± 8.1

Values are mean ± SD. For NZW animals, $n = 4$; for WHHL animals, $n = 7$.

*Significantly different from normal NZW ($P < 0.05$, Student's t test, unpaired).

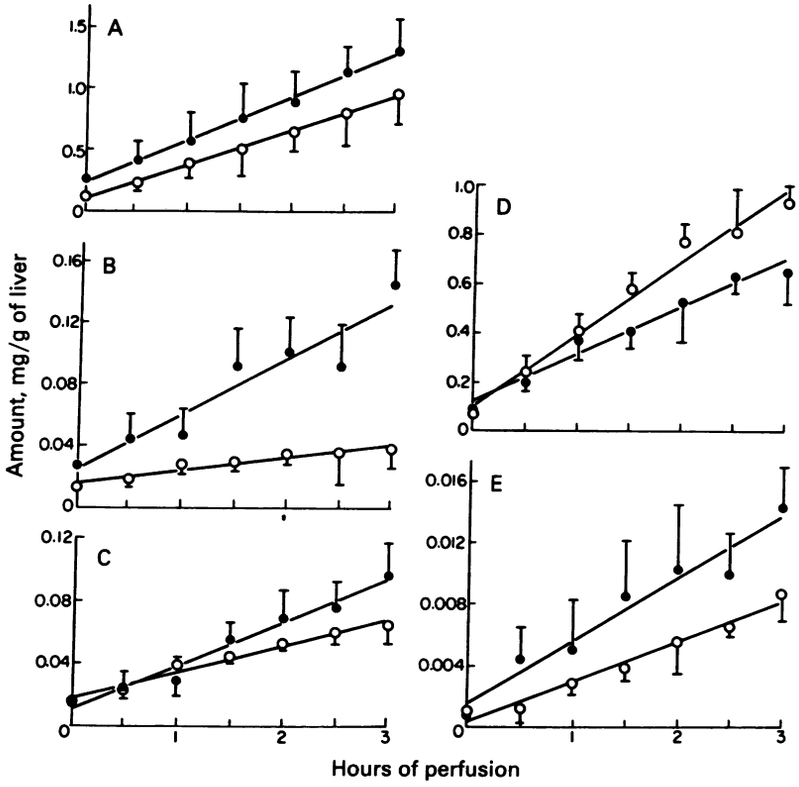

FIG. 1. Accumulation of lipids and proteins in perfusates during perfusion of isolated rabbit livers for 3 hr. Two-milliliter samples of whole perfusate were collected every 30 min, centrifuged at 750 × g for 30 min to remove erythrocytes, and assayed. Values shown, corrected for sampling losses, are mean ± 1 SD for four normal NZW livers (○) or four WHHL livers (●). The slopes of the regression lines were determined by the method of least squares and compared by analysis of covariance. These slopes were (WHHL vs. normal NZW): *A*, triglycerides, 0.354 vs. 0.280 ($P < 0.001$); *B*, cholesteryl esters, 0.0362 vs. 0.00864 ($P < 0.001$); *C*, free cholesterol, 0.0285 vs. 0.0176 ($P < 0.001$); *D*, albumin, 0.193 vs. 0.291 ($P < 0.01$); *E*, apo E, 0.00406 vs. 0.00258 ($P < 0.001$).

Table 2. Yield of lipoprotein lipids and protein from perfused livers of normal NZW and WHHL rabbits

		Amount recovered after 3 hr of perfusion, mg					
	Rabbits	Triglycerides	Cholesteryl esters	Cholesterol	Phospholipids	Protein	apo B
VLDL ($\rho < 1.010$ g/ml)	Normal NZW	13.78 ± 6.14 (96.3)	0.46 ± 0.16 (73.9)	0.79 ± 0.28 (83.7)	2.53 ± 1.37 (81.9)	1.31 ± 0.45 (70.5)	0.81 ± 0.25 (72.5)
	WHHL	12.86 ± 2.25 (94.3)	1.30 ± 0.27* (87.6)	0.98 ± 0.35 (90.4)	1.50 ± 0.29 (80.6)	1.16 ± 0.23 (75.8)	0.64 ± 0.17 (79.0)
IDL ($1.010 < \rho < 1.019$ g/ml)	Normal NZW	0.25 ± 1.10 (1.7)	0.05 ± 0.06 (7.7)	0.02 ± 0.01 (2.2)	0.08 ± 0.05 (2.6)	0.05 ± 0.03 (2.9)	0.03 ± 0.02 (3.2)
	WHHL	0.37 ± 0.19 (2.3)	0.06 ± 0.02 (3.5)	0.03 ± 0.02 (1.2)	0.10 ± 0.04 (5.5)	0.05 ± 0.02 (3.1)	0.02 ± 0.01 (2.4)
LDL ($1.019 < \rho < 1.063$ g/ml)	Normal NZW	0.13 ± 0.08 (6.9)	0.07 ± 0.06 (11.7)	0.05 ± 0.04 (5.8)	0.15 ± 0.12 (5.5)	0.08 ± 0.06 (4.3)	0.07 ± 0.04 (6.2)
	WHHL	0.26 ± 0.12 (1.9)	0.08 ± 0.03 (5.8)	0.04 ± 0.02 (3.7)	0.12 ± 0.08 (6.9)	0.14 ± 0.11 (7.4)	0.10 ± 0.07 (10.1)
HDL ($1.063 < \rho < 1.21$ g/ml)	Normal NZW	0.16 ± 0.04 (1.1)	0.05 ± 0.03 (6.7)	0.08 ± 0.03 (8.4)	0.27 ± 0.07 (9.5)	0.41 ± 0.18 (22.3)	
	WHHL	0.24 ± 0.13 (1.5)	0.05 ± 0.03 (3.0)	0.04 ± 0.02 (3.5)	0.14 ± 0.03 (7.1)	0.24 ± 0.08 (13.8)	

Values are adjusted to a liver weight of 50 g and are mean ± SD for four rabbits in each group. Values in parentheses are percentage of amount recovered for each component.
*Significantly different from normal NZW ($P < 0.05$).

in WHHL perfusates than in normal animals, whereas that of other measured components did not differ significantly (Table 2). Mean recovery of triglycerides in IDL, LDL, and HDL was consistently, but not significantly, higher in WHHL perfusates. In additional experiments, the rate of accumulation of triglycerides and cholesteryl esters was measured in VLDL and lipoproteins of higher density (>1.010 g/ml) at intervals of 30 min. Whereas these lipids accumulated at a linear rate in VLDL, their concentration in residual lipoproteins remained almost constant between 30 and 180 min of perfusion (data not shown). The recovery of apo B in VLDL, IDL, and LDL did not differ significantly; however, in VLDL, the mean value in WHHL perfusates, like that of phospholipid, was moderately lower than that found in normal animals (Table 2). As judged from the intensity of stained bands in polyacrylamide gels, essentially all of the apo B in perfusate VLDL, IDL, and LDL was B-100 (Fig. 2). No B-48 was detected in these lipoproteins in perfusates of normal NZW or WHHL livers.

VLDL was composed mainly of triglycerides in perfusates from both groups of animals (Table 3). VLDL from WHHL perfusates contained much more cholesteryl ester than that of normals. The composition of perfusate IDL was similar in the two groups. LDL and HDL of WHHL perfusates contained somewhat more of the two nonpolar lipids (triglycerides and cholesteryl esters) but none of these differences was statistically significant.

Negatively stained perfusate VLDL were somewhat larger than serum VLDL (5). Ninety percent of particle diameters ranged from 250 to 1,050 Å. Perfusate IDL (200–450 Å) and LDL (150–300 Å) resembled their counterparts in blood plasma. Most perfusate HDL was found as particles ≈100 Å in diameter, but discoidal forms (42 × 240 Å) were common, occasionally forming rouleaux. No differences were observed between these lipoproteins from normal NZW and WHHL perfusates.

Proteins of VLDL were highly labeled after 3 hr of perfusion and to approximately the same extent in normal NZW and WHHL perfusates (Table 4). One-third to one-half of the ^{3}H was in apo B-100. By contrast, little labeling of apo B was found in perfusate LDL.

After injection of Triton WR-1339 into normal NZW and WHHL rabbits, the plasma triglyceride concentration rose at a constant rate during the next 6.5 hr (Fig. 3). The rates of increase in the two groups of animals were indistinguishable. In normal NZW and WHHL rabbits alike, virtually all of the increase in triglycerides was found in VLDL. Mean values of VLDL triglycerides 4 hr after injection of Triton were 640 ± 51 mg/dl for normal rabbits ($n = 3$) and 749 ± 54 mg/dl for WHHL rabbits ($n = 4$). When L-[^{35}S]methionine was injected intravenously 1 hr after injection of Triton, the accumulation of ^{35}S in apo B of VLDL during the next 3 hr did not differ significantly in normal and WHHL rabbits (data not shown).

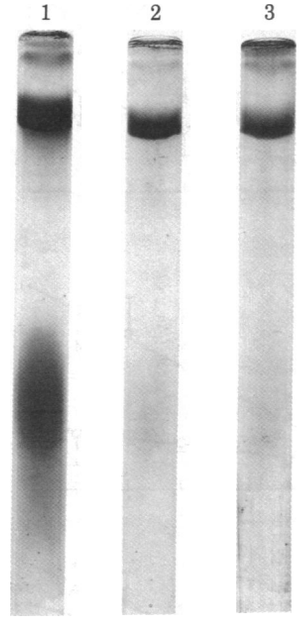

FIG. 2. $NaDodSO_4$/polyacrylamide gel electrophoretograms of perfusate lipoproteins from a normal rabbit, stained with Coomassie brilliant blue. Lanes: 1, VLDL; 2, IDL; 3, LDL. The prominent band near the top of each gel has the same mobility as that of apo B-100 from human or rabbit LDL.

Table 3. Composition of perfusate lipoproteins in normal NZW and WHHL rabbits

	Amount, % total							
	VLDL		IDL		LDL		HDL	
	Normal NZW	WHHL	Normal NZW	WHHL	Normal NZW	WHHL	Normal NZW	WHHL
Cholesteryl esters	2.6 ± 1.9	11.8 ± 1.3*	10.9 ± 3.2	15.5 ± 3.9	14.9 ± 3.4	21.1 ± 4.2	4.4 ± 2.6	11.7 ± 5.5
Triglycerides	73.0 ± 6.3	68.5 ± 1.4	53.8 ± 10.7	54.0 ± 8.8	25.4 ± 12.2	38.1 ± 5.5	16.1 ± 6.2	30.1 ± 10.9
Free cholesterol	4.2 ± 0.5	5.5 ± 1.3	4.6 ± 1.6	4.8 ± 1.9	10.8 ± 2.8	6.0 ± 1.2	7.9 ± 3.9	6.2 ± 2.9
Phospholipids	12.8 ± 4.2	8.0 ± 0.5	18.5 ± 8.3	17.7 ± 5.8	32.0 ± 7.7	18.5 ± 5.9	27.9 ± 2.4	20.0 ± 6.3
Protein	7.3 ± 1.6	6.2 ± 0.4	12.3 ± 5.2	8.0 ± 1.3	16.8 ± 0.5	16.2 ± 3.4	43.7 ± 8.9	32.0 ± 3.0

*Significantly different from control NZW ($P < 0.05$).

DISCUSSION

Two key observations have emerged from these studies of lipoprotein secretion from livers of normal and WHHL rabbits, both of which have been confirmed by additional observations in intact animals. First, lipoproteins containing apo B-100 were secreted essentially as VLDL [density (ρ) < 1.010 g/ml], independent of the presence of functioning hepatic LDL receptors. This observation for normal animals is consistent with previous observations in rats (21), guinea pigs (22), and monkeys (23). The similar observation in WHHL rabbits provides strong evidence that independent secretion of LDL (i.e., LDL not formed from VLDL) does not contribute to the increased rate of production of LDL observed in these animals (7). On the basis of experience in other species (22, 24), efforts were made to wash out LDL from the space of Disse before starting measurements of lipoprotein accumulation in perfusates. These efforts evidently were largely successful, even in WHHL rabbits in whom plasma levels of VLDL, IDL, and LDL are very high (5). The small amount of IDL and LDL found in perfusates after 3 hr of recirculating perfusion accumulated mainly during the first 30 min and probably represented plasma lipoproteins washed out of the space of Disse. Little apo B of the LDL was labeled by [^{3}H]lysine, in contrast to that of VLDL, which accumulated progressively. Furthermore, in intact rabbits given Triton WR-1339, plasma triglycerides accumulated exclusively in VLDL, consistent with the observations in perfused livers.

Second, our observations suggest that livers of WHHL rabbits do not secrete more VLDL particles than those of control animals. The amount of apo B that accumulated in VLDL of WHHL perfusates was actually somewhat less than that of controls. More apo B might be expected to accumulate in WHHL perfusates than in controls even if the rate of secretion of VLDL particles were not increased, because less of the nascent lipoprotein would be taken up by receptor-mediated endocytosis during perfusion. Therefore, the lack of a significant difference between control and WHHL rabbits provides strong evidence that an increased rate of VLDL particle secretion does not contribute to the increased rate of production of LDL particles in WHHL rabbits. These observations in perfused livers are consistent with the observations in intact rabbits injected with Triton WR-1339. The rates of accumulation of VLDL triglyceride were virtually the same in control and WHHL rabbits and no triglycerides accumulated in lipoproteins of higher density. Taken together, these observations support the conclusion that increased LDL production in WHHL rabbits is caused by an increase in the fraction of VLDL converted to LDL (11).

The perfusate VLDL of WHHL rabbits contained a much larger amount of cholesteryl esters than that of control animals. Similar observations have been made for perfusate VLDL of livers from rats in which LDL receptors are induced by treatment of 17α-ethinylestradiol (25). In both cases, the rate of hepatic uptake of cholesterol-rich lipoproteins is thought to be increased. This could lead to increased secretion of cholesteryl esters, synthesized by acyl-CoA:cholesterol *O*-acyltransferase, from the liver. In samples taken at the end of perfusion, we found no accumulation of cholesteryl esters in the liver. However, Dietschy *et al.* (26) have observed that the concentration of cholesteryl esters is moderately increased in unperfused livers of WHHL rabbits. The increased rate of accumulation of apo E in perfusates of WHHL livers resembles that found in perfused livers of cholesterol-fed rats (27) and guinea pigs (22), which also secrete cholesterol-enriched VLDL. However, apo E may have accumulated to a greater extent in perfusates of WHHL livers because of reduced uptake. The possibility that the cholesteryl esters of VLDL were derived from the action of lecithin-cholesterol acyltransferase secreted into the perfusate cannot be excluded, but this seems unlikely because other lipoproteins were not enriched with cholesteryl esters to the extent observed for VLDL. VLDL in blood plasma of WHHL rabbits contain considerably more cholesteryl esters than that of perfusate VLDL (5). Most of these esters in plasma VLDL are probably produced by lecithin-cholesterol acyltransferase. The same seems to be true for plasma VLDL of control NZW rabbits (5).

Our observations provide additional evidence that the rabbit is a suitable model for studying the regulation of lipoprotein

Table 4. Incorporation of [^{3}H]lysine into perfusate apolipoproteins of VLDL and LDL

		Total ^{3}H recovered, cpm	^{3}H in apo B-100 band, cpm	Total protein loaded, μg	Total apo B loaded, μg	Specific activity of apo B-100, cpm/μg
Normal NZW	VLDL	7,313	2,783	80	54	52
	VLDL	6,022	3,014	80	46	66
	LDL	1,329	200	23	20	10
	LDL	307	37	14	10	4
WHHL	VLDL	6,035	1,831	69	40	46
	VLDL	3,251	1,152	86	40	29
	LDL	918	76	54	40	2

Values are for VLDL and LDL isolated from individual perfusates after 3 hr of perfusion; 100 μCi of [^{3}H]lysine was added to the perfusate every 30 min.

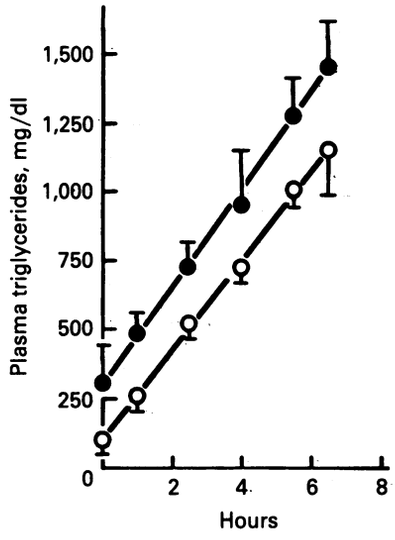

FIG. 3. Accumulation of serum triglycerides after injection of Triton WR-1339 into normal NZW (○) and WHHL (●) rabbits. Animals were starved for 16 hr before Triton WR-1339 (400 mg/kg) was injected intravenously. At the indicated times after injection, blood was obtained and unfractionated serum was used for measurement of triglyceride content. Each value represents mean ± SD for four (NZW) or three (WHHL) animals.

metabolism, as related to human health and disease. As in humans, rabbit VLDL, IDL, and LDL contain almost entirely apo B-100 and little if any B-48 (5). As shown here, the rabbit liver secretes little if any apo B-48. Furthermore, like the human, the rabbit has an active mechanism for transferring core lipids among lipoproteins (28). Finally, the rabbit converts an appreciable fraction of apo B-100 of VLDL to LDL and the extent of conversion is subject to regulation (11). In all these respects the rabbit seems to be a more suitable model for the study of human lipoprotein metabolism than the rat (29). In humans homozygous for deficiency of functional LDL receptors, LDL is thought to be secreted independently of VLDL (9). Further studies in WHHL rabbits may clarify this apparent discrepancy.

We thank Drs. Michael Brown and Joseph Goldstein for useful discussions. This research was supported by grants from the National Institutes of Health (HL-14237 and HL-20948) and the Moss Heart Foundation.

1. Goldstein, J. L. & Brown, M. S. (1979) *Annu. Rev. Genet.* **13**, 259–289.
2. Tanzawa, K., Shimada, Y., Kuroda, M., Tsujita, Y., Arai, M. & Watanabe, Y. (1980) *FEBS Lett.* **18**, 81–84.
3. Kita, T., Brown, M. S., Watanabe, Y. & Goldstein, J. L. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2268–2272.
4. Buja, L. M., Kita, T., Goldstein, J. L., Watanabe, Y. & Brown, M. S. (1983) *Arteriosclerosis* **3**, 87–101.
5. Havel, R. J., Kita, T., Kotite, L., Kane, J. P., Hamilton, R. L., Goldstein, J. L. & Brown, M. S. (1982) *Arteriosclerosis* **2**, 467–474.
6. Simons, A. K., Reichl, D. & Myant, N. B. (1975) *Atherosclerosis* **21**, 283–298.
7. Bilheimer, D. W., Watanabe, Y. & Kita, T. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3305–3309.
8. Havel, R. J., Goldstein, J. L. & Brown, M. S. (1980) in *Metabolic Control and Disease*, eds. Bondy, P. K. & Rosenberg, L. E. (Saunders, Philadelphia), 8th Ed., pp. 393–494.
9. Soutar, A. K., Myant, N. B. & Thompson, G. R. (1977) *Atherosclerosis* **28**, 247–256.
10. Ghiselli, G. (1982) *Biochim. Biophys. Acta* **711**, 311–315.
11. Kita, T., Brown, M. S., Bilheimer, D. W. & Goldstein, J. L. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5693–5697.
12. Hamilton, R. L., Berry, M. N., Williams, M. C. & Severinghaus, E. B. (1974) *J. Lipid Res.* **15**, 182–186.
13. 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.
14. Havel, R. J., Eder, H. & Bragdon, J. (1955) *J. Clin. Invest.* **34**, 1345–1353.
15. Mancini, G., Carbonaro, A. D. & Herman, J. F. (1965) *Immunochemistry* **2**, 235.
16. Havel, R. J., Kotite, L., Vigne, J.-L., Kane, J. P., Tun, P., Phillips, N. & Chen, G. C. (1980) *J. Clin. Invest.* **66**, 1351–1362.
17. Kane, J. P., Hardman, D. A. & Paulus, H. E. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2465–2469.
18. Felker, T. E., Hamilton, R. L., Vigne, J.-L. & Havel, R. J. (1982) *Gastroenterology* **83**, 652–663.
19. Pagnan, A., Havel, R. J., Kane, J. P. & Kotite, L. (1977) *J. Lipid Res.* **18**, 613–622.
20. Hamilton, R. L., Havel, R. J., Kane, J. P., Sata, T. & Blaurock, A. (1971) *Science* **172**, 475–478.
21. Hamilton, R. L., Williams, M. C., Fielding, C. J. & Havel, R. J. (1976) *J. Clin. Invest.* **58**, 667–680.
22. Guo, L. S. S., Hamilton, R. L., Ostwald, R. & Havel, R. J. (1982) *J. Lipid Res.* **23**, 543–555.
23. Johnson, R. L., St. Clair, R. W. & Rudel, L. L. (1981) *Arteriosclerosis* **1**, 381a (abstr.).
24. Nakayer, N., Chung, B. H., Patsch, J. R. & Taunton, O. D. (1977) *J. Biol. Chem.* **254**, 7530–7533.
25. Windler, E. E. T., Kovanen, P. T., Chao, Y.-s., Brown, M. S., Havel, R. J. & Goldstein, J. L. (1980) *J. Biol. Chem.* **255**, 10464–10471.
26. Dietschy, J. M., Kita, T., Suckling, J. E., Goldstein, J. L. & Brown, M. S. (1983) *J. Lipid Res.* **24**, 469–480.
27. Noel, S.-P., Wong, L., Dolphin, P. J., Dory, L. & Rubenstein, D. (1979) *J. Clin. Invest.* **64**, 674–683.
28. Barter, P. J. & Lally, J. I. (1978) *Atherosclerosis* **31**, 355–364.
29. Faergeman, O., Sata, T., Kane, J. P. & Havel, R. J. (1975) *J. Clin. Invest.* **56**, 1396–1403.