

Partial ileal bypass reduces the production rate of low density lipoproteins in Watanabe heritable hyperlipidemic rabbits

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Abstract Partial ileal bypass surgery in homozygous Watanabe heritable hyperlipidemic (WHHL) rabbits resulted in a decrease of low density lipoproteins (LDL)-cholesterol from 14.2 ± 2.4 to 7.0 ± 1.2 mmol/l. To investigate the effect of partial ileal bypass on receptor-mediated and receptor-independent LDL catabolism, turnover studies were performed of radiolabeled native LDL and chemically modified LDL (methyl-LDL) in WHHL rabbits after partial ileal bypass, in WHHL control rabbits, and in New Zealand White ("normal") rabbits. The plasma LDL pool in WHHL control rabbits was increased 10-fold. The receptor-mediated LDL clearance was essentially zero in WHHL rabbits, both in controls and after ileal bypass surgery; the fractional catabolic rates for total LDL were equal in both WHHL groups and were also similar to that for methyl-LDL in the normal rabbits. Seventy percent of the total LDL clearance in the normal rabbits occurred via the LDL receptor pathway. In the animals with a partial ileal bypass, the plasma LDL-protein pool was appreciably lower than in WHHL controls (41.6 ± 5.7 vs 73.4 ± 9.9 mg/kg, $P < 0.02$). The absolute catabolic rate was almost 50% lower in the PIB group (21.4 ± 2.0 vs 40.0 ± 7.5 mg · kg⁻¹ · day⁻¹, $P < 0.02$). These results indicate that the decrease of LDL after partial ileal bypass surgery in WHHL rabbits is the result of a reduced production rate of LDL.—Stalenhoef, A. F. H., J. L. M. van Niekerk, P. N. M. Demacker, and A. van 't Laar. Partial ileal bypass reduces the production rate of low density lipoproteins in Watanabe heritable hyperlipidemic rabbits. *J. Lipid Res.* 1984. 25: 1350–1357.

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Homozygous Watanabe heritable hyperlipidemic (WHHL) rabbits are characterized by grossly elevated levels of plasma lipoproteins (1, 2). They exhibit a nearly complete genetic deficiency of low density lipoprotein (LDL) receptors in liver and other tissues (3, 4), consequently, the rate of removal of LDL from the circulation is markedly decreased (5). In addition, the hepatic uptake of very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL) is impaired, leading to an increased conversion of VLDL into LDL

(6). The defect in the LDL receptor gene, leading to a massive increase in plasma LDL levels and premature atherosclerosis in homozygous WHHL rabbits, is analogous to the defect in familial hypercholesterolemia; this animal is therefore a unique model for its human counterpart (7).

Recently, the effect of partial ileal bypass (PIB) on plasma lipoproteins and atherosclerotic plaque formation in WHHL rabbits was studied (8). In animals operated at the age of 3 months, a sustained decrease in plasma cholesterol of 52% (range 29–67%) was obtained, while a sham operation had no effect. Plaque formation, measured 30 weeks after operation, was significantly reduced (8). Plasma cholesterol in these animals was also lowered by administration of cholestyramine (mean decrease 38%) (9). To explain the decrease in plasma cholesterol, it was suggested that WHHL rabbits should be considered as a model for the receptor-defective mutant of familial hypercholesterolemia (10), in which a certain number of LDL receptors are still present. Interruption of the enterohepatic circulation would then result in an increase of LDL receptors and consequent decrease of plasma cholesterol. To study the underlying mechanism in more detail, LDL turnover studies were performed in WHHL rabbits after PIB and in control rabbits. Receptor-mediated and receptor-independent LDL catabolism was measured. The results indicate that LDL catabolism is unaffected after PIB, while LDL production rate decreases by about 50%.

Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; WHHL, Watanabe heritable hyperlipidemic; NZW, New Zealand White; apo, apolipoprotein; FCR, fractional catabolic rate; ACR, absolute catabolic rate; EDTA, sodium salt ethylenediamine tetraacetic acid; PIB, partial ileal bypass, TCA, trichloroacetic acid.

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MATERIALS AND METHODS

Rabbits

Homozygous WHHL rabbits were raised in Nijmegen by crossing and backcrossing between New Zealand White (NZW) and WHHL rabbits (a generous gift from Dr. Y. Watanabe). At the age of 9 months, the rabbits underwent either a sham operation or a partial ileal bypass (PIB). During the sham operation a laparotomy was performed and the total length of the small bowel was measured. The PIB operation was performed essentially as described by Buchwald (11). The distal third of the small bowel was bypassed by a functional end-to-side anastomosis between ileum and colon ascendens immediately above the ileocecal valve. All animals received antibiotics perioperatively. They were fed ad libitum with a chow diet (Lk04, Hope Farms, Woerden, the Netherlands).

Lipoproteins, radiolabeling, reductive methylation

Blood was taken from the ear arteries and mixed with EDTA, 1 mg/ml. Lipoproteins were isolated by density gradient ultracentrifugation in swinging bucket rotors in a IEC-B60 ultracentrifuge (Damon/IEC, Needham Heights, MA) as described previously (12). The lipoproteins separated this way are present as sharp bands in the following density ranges: VLDL + IDL, $d < 1.025$ g/ml; LDL, $1.025 < d < 1.063$ g/ml; and HDL, 1.063–1.185 g/ml. The LDL isolated this way contained negligible amounts of serum proteins (12). The interassay coefficient of variation for the determination of LDL cholesterol of pooled serum stored frozen was 3.7% ($n = 18$). For the determination of the apoLDL concentration, LDL was isolated by sequential ultracentrifugation in rotor 468 (168,000 g) between densities d 1.019 and 1.063 g/ml and recentrifuged once at density d 1.063 g/ml. After bleeding WHHL rabbits (plasma cholesterol 7–9 mmol/l), LDL for iodination was prepared by ultracentrifugation between densities of 1.019 and 1.050 g/ml in the presence of 0.01% EDTA, 0.04% azide, and 5 μ g/ml gentamycin, pH 7.4. LDL was then recentrifuged once at d 1.050 g/ml and dialyzed overnight at 4°C against 0.15 M NaCl, 0.01% EDTA, and 5 μ g/ml gentamycin, pH 7.4. The LDL isolated this way was then diluted with saline to 6 mg of protein/ml and portions were iodinated with either ^{125}I or ^{131}I by a modification (13) of the method of McFarlane (14). Unreacted iodine was removed by chromatography on Sephadex G-25 M (columns PD-10, Pharmacia Fine Chemicals, Uppsala, Sweden). The ^{125}I -labeled LDL ("native" LDL) was then dialyzed overnight at 4°C against 0.15 M NaCl, 0.01% EDTA, pH 7.4. The ^{131}I -labeled LDL was chemically modified by re-

ductive methylation as described by Weisgraber, Innerarity, and Mahley (15) with a 60-min reaction sequence. The reaction mixture was chromatographed on Sephadex G-25 and the methyl-LDL was dialyzed overnight at 4°C against 0.15 M NaCl, 0.01% EDTA, pH 7.4. More than 80% of lysine residues was methylated as estimated with a colorimetric assay based on the reaction of 2,4,6-trinitrobenzene-1-sulfonic acid with primary amines (16). Chemical modification of the lysine residues of LDL has been shown to abolish the binding ability to specific LDL receptors without effect on nonspecific processes (3, 15).

The efficiency of labeling was 35–50%. Lipid labeling was 6–8%, and more than 98% of the radioactivity was precipitated by 10% TCA. Specific activities were between 350–600 cpm/ng protein. LDL was prepared, labeled, and modified this way on two different occasions and used in two experiments.

LDL turnover

After mixing ^{125}I -labeled native LDL and ^{131}I -labeled methyl-LDL with rabbit plasma and sterilization through a Millipore filter (0.44 μm), portions of this mixture were injected in two experiments into a marginal ear vein of 6 and 7 animals, respectively; the amount of radioactivity was 36–47 μCi for ^{125}I and 40–43 μCi for ^{131}I . Blood samples drawn 3 min after injection were used to calculate the plasma volume by measuring the isotope dilution. Two-ml blood samples were then taken at intervals (into 2 mg EDTA) for 72 hr. Total plasma and a 10% TCA-precipitate were assayed for ^{125}I and ^{131}I in a Philips PW4800 automatic gamma counter. The plasma disappearance curves of TCA-insoluble radioactive iodine were analyzed by the method of Matthews (17), which assumes a simple two-compartment model where plasma apoLDL equilibrates with an extravascular compartment and where all irreversible loss of apoLDL occurs from the plasma compartment. The fractional catabolic rate (FCR) was obtained (18) and the absolute catabolic rate (ACR) was calculated by multiplying the FCR (pools/day) by the apoLDL pool size. The apoLDL pool size was calculated by multiplying apoLDL concentration by plasma volume, corrected for body weight.

Other analyses

Total cholesterol was measured in plasma and lipoprotein fractions by the CHOD-PAP method (no 237574, Boehringer Mannheim GmbH, F.R.G.) (19). Triglycerides were estimated with a semi-automated colorimetric method (20). Phospholipids and free cholesterol were determined by enzymic kits (nos 691844 and 310328, respectively, of Boehringer Mannheim GmbH, F.R.G.). Protein was estimated according to Lowry et al. (21).

TABLE 1. Weight and plasma lipid and lipoprotein concentrations in WHHL control rabbits (four sham, two not operated) and in WHHL rabbits after a partial ileal bypass (PIB); for comparison, results in normal NZW rabbits are given^a

Rabbit	Weight kg	Total Plasma			VLDL + IDL			LDL			HDL		
		Cholesterol	Triglycerides	Cholesterol	Triglycerides	Cholesterol	Triglycerides	Cholesterol	Triglycerides	Cholesterol	Triglycerides	Cholesterol	Triglycerides
WHHL Controls (n = 6)	2.64 ± 0.11	12.48 ± 2.38	3.57 ± 1.11	3.61 ± 0.99	1.43 ± 0.44	8.13 ± 1.56	1.78 ± 0.40	0.17 ± 0.02	0.10 ± 0.02				
PIB (n = 4)	2.42 ± 0.19	6.99 ± 1.27	5.58 ± 4.54	2.80 ± 0.71	2.85 ± 2.73	3.44 ± 1.72	1.66 ± 0.31	0.17 ± 0.01	0.14 ± 0.04				
P values ^b	NS	<0.02	NS	NS	NS	<0.02	NS	NS	NS			NS	
NZW (n = 3)	2.99 ± 0.35	1.56 ± 0.63	0.60 ± 0.24	0.15 ± 0.09	0.18 ± 0.07	0.64 ± 0.35	0.17 ± 0.12	0.59 ± 0.25	0.13 ± 0.02				

^a Values are means ± SD.

^b P values refer to statistical comparisons between the WHHL control and PIB groups; NS, not significant.

Statistical analyses were performed with the nonparametric test for unpaired data according to Wilcoxon to compare WHHL-PIB and control rabbits. P-values less than 5% were considered to be significant. All results are expressed as mean ± 1 standard deviation.

RESULTS

Weight, plasma lipids and lipoproteins in the PIB-operated animals and controls

The PIB-operated animals lost weight, but were stable 5 weeks after the operation at about 90% of their pre-operation weight (2.68 ± 0.09 and 2.42 ± 0.19 kg, respectively). A mean decrease of 50% in total plasma cholesterol was seen in these animals (14.2 ± 2.4 to 7.0 ± 1.2 mmol/l, respectively). The sham-operated animals hardly lost weight and were stabilized after 5 weeks at about 110% of their pre-operation level (2.37 ± 0.19 and 2.63 ± 0.14 kg, respectively). Their plasma cholesterol levels did not change after operation (11.5 ± 1.9 and 11.8 ± 1.4 mmol/l, respectively).

In the group of control WHHL rabbits, two unoperated animals with comparable weight and plasma lipid levels were included next to the four sham-operated animals. Plasma lipoprotein concentrations of this WHHL control group and PIB rabbits are summarized in **Table 1**. Mean body weight in these groups did not differ significantly. Plasma cholesterol and LDL cholesterol were significantly lower in the PIB group with mean values of 56% and 42% of those of the WHHL controls, respectively. In the PIB group the concentration of VLDL + IDL-cholesterol was lower and that of triglycerides higher, but these differences were not significant. There was no effect of PIB on the HDL-lipid levels. Results in three NZW rabbits of the same age (9 months) clearly confirm the differences in plasma lipoprotein concentrations with the WHHL rabbits (**Table 1**).

The content of protein, phospholipids, and cholesterol of LDL (in density gradient between $1.025 < d < 1.063$ g/ml) was similar in the different groups (**Table 2**). In the PIB group LDL was enriched in triglycerides.

Effect of PIB on the receptor-independent and receptor-mediated LDL catabolism

The effect of PIB on the receptor-independent and receptor-mediated LDL catabolism was studied by measuring the turnover of ¹²⁵I-labeled native LDL and ¹³¹I-labeled methyl-LDL simultaneously in all animals (**Fig. 1** and **Table 3**). In the operated animals, this was done 5 weeks after the operation. The plasma apoLDL pool was significantly lower in the WHHL-PIB group com-

TABLE 2. Composition of LDL (% mass) of WHHL control rabbits (four sham, two not operated), in WHHL rabbits after a partial ileal bypass (PIB), and in normal NZW rabbits^a

Component	WHHL-Controls (n = 6)	WHHL-PIB (n = 4)	NZW (n = 3)
Cholesteryl esters	36.6 ± 5.3	25.5 ± 11.4	32.2 ± 4.8
Triglycerides	14.8 ± 2.5	25.6 ± 8.0 ^b	16.9 ± 7.1
Cholesterol	7.8 ± 0.6	6.5 ± 1.7	7.3 ± 1.2
Phospholipids	19.0 ± 2.0	21.4 ± 5.1	20.4 ± 1.5
Protein	21.9 ± 1.2	21.2 ± 0.5	23.3 ± 1.1

^a Results are mean ± SD.

^b $P < 0.02$ compared with WHHL-controls.

pared to the WHHL controls. The mean FCR for ¹²⁵I-LDL, which reflects the total clearance for LDL by both receptor-mediated and receptor-independent processes, was markedly decreased in both WHHL groups. The mean FCR for ¹³¹I-methyl-LDL, as a measure for receptor-independent removal of LDL, was essentially the same as that for ¹²⁵I-LDL, indicating that there was no receptor-mediated clearance of LDL. This was the case in all WHHL rabbits, either after a sham operation or not operated at all, and in the PIB group. In contrast, the mean receptor-mediated clearance of LDL in the NZW rabbits was 1.53 ± 0.74 pools/day (70% of total LDL removal).

Since these studies were performed during steady-state conditions as appeared from daily determinations of plasma lipids and apoLDL concentrations, the absolute catabolic rate (ACR) of LDL equals the production rate of LDL. In the WHHL-PIB group the ACR was signif-

icantly lower than in the WHHL controls ($P < 0.02$), indicating a mean decrease in LDL synthesis of almost 50% (Table 3). In the WHHL-PIB group the mean LDL production rate was still higher than in the NZW rabbits.

DISCUSSION

Interruption of the enterohepatic circulation of bile acids has been shown to be effective in lowering plasma LDL levels in heterozygous familial hypercholesterolemia. The average decrease in serum cholesterol in the reported series ranges from 33 to 40% and in LDL cholesterol from 36 to 46% (22). Due to the fecal loss the synthesis of bile acids is increased 3- to 10-fold and the conversion of cholesterol to bile acids is promoted (23). Along with an enhanced cholesterol synthesis, the number of LDL receptors is increased after PIB, as

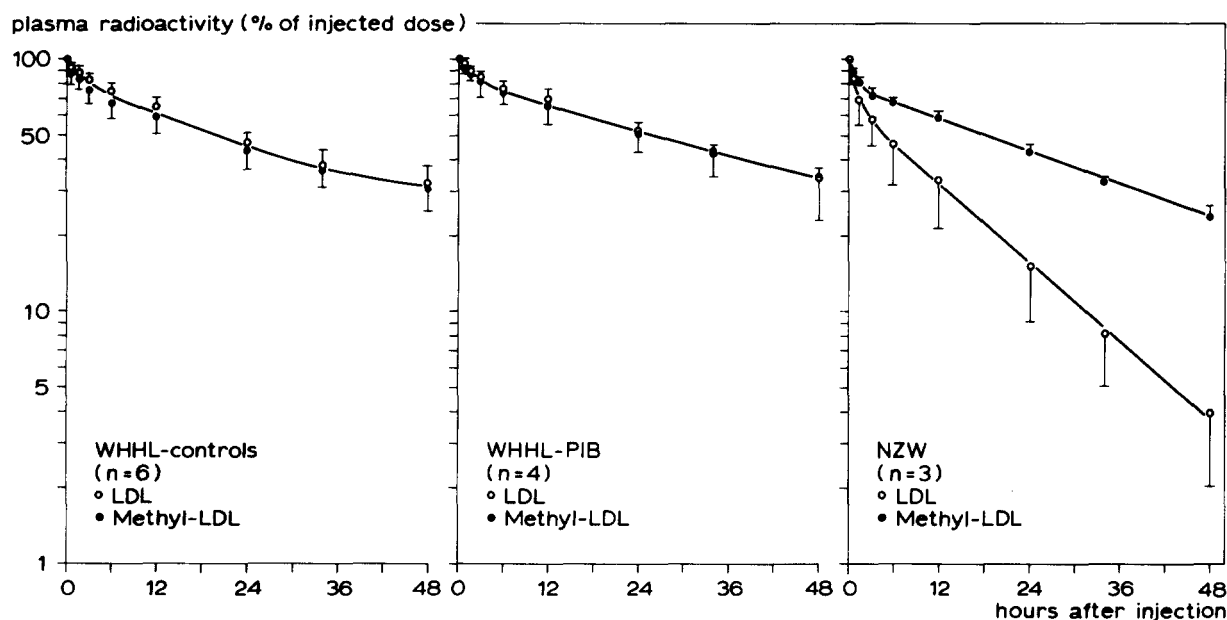


Fig. 1. Disappearance of total plasma TCA-insoluble radioactivity after intravenous injection of ¹²⁵I-labeled native LDL and ¹³¹I-labeled methyl-LDL in WHHL control rabbits, WHHL rabbits 5 weeks after partial ileal bypass surgery, and in New Zealand White rabbits. Values are mean ± SD.

TABLE 3. Kinetic data for apoLDL and apo-methyl-LDL turnover in WHHL control rabbits, after partial ileal bypass (PIB), and in normal NZW rabbits

Rabbits	#	Sex	Plasma Volume ml	ApoLDL Pool mg/kg	FCR (pools per day)			ACR of ApoLDL (mg · kg ⁻¹ · day ⁻¹)		
					Total	Receptor-Independent	Receptor-Dependent	Total	Receptor-Independent	Receptor-Dependent
WHHL										
Sham operated										
	1	M	79	66.7	0.52	0.44	0.08	34.7	29.3	5.4
	2	M	78	64.3	0.65	0.76	(-0.11)	41.8	48.9	(-7.1)
	3	F	89	89.6	0.55	0.61	(-0.06)	49.3	54.7	(-5.4)
	4	F	82	73.9	0.44	0.45	(-0.01)	32.5	33.3	(-0.8)
Not operated										
	1	F	84	80.1	0.60	0.53	0.07	48.1	42.5	5.6
	2	F	90	65.9	0.51	0.61	(-0.10)	33.6	40.2	(-6.6)
Mean ± SD (n = 6)			84 ± 5	73.4 ± 9.9	0.55 ± 0.07	0.57 ± 0.12	(-0.02 ± 0.08)	40.0 ± 7.5	41.5 ± 9.5	(-1.5 ± 5.8)
PIB										
	1	F	80	43.1	0.45	0.49	(-0.04)	19.4	21.1	(-1.7)
	2	M	85	49.0	0.46	0.60	(-0.14)	22.5	29.4	(-6.9)
	3	F	77	36.4	0.55	0.40	0.15	20.0	14.6	5.4
	4	F	80	38.0	0.62	0.68	(-0.06)	23.6	25.8	(-2.2)
Mean ± SD (n = 4)			81 ± 3	41.6 ± 5.7	0.52 ± 0.08	0.54 ± 0.12	(-0.02 ± 0.12)	21.4 ± 2.0	22.7 ± 6.4	(-1.4 ± 5.1)
P value			NS	<0.02	NS	NS	NS	<0.02	<0.02	NS
NZW										
	1	M	106	3.3	3.04	0.65	2.39	10.0	2.1	7.9
	2	M	125	8.9	1.85	0.76	1.09	16.5	6.8	9.7
	3	M	91	10.4	1.66	0.55	1.11	17.3	5.7	11.5
Mean ± SD (n = 3)			107 ± 17	7.5 ± 3.7	2.18 ± 0.75	0.65 ± 0.11	1.53 ± 0.74	14.6 ± 4.0	4.9 ± 2.5	9.7 ± 1.8

P values refer to statistical comparisons between WHHL control and PIB groups; NS, not significant; FCR, fractional catabolic rate; ACR, absolute catabolic rate.

measured by turnover studies with native and chemically modified LDL (24).

In contrast to its effect in heterozygous patients, PIB has no effect or only a moderate effect in homozygous familial hypercholesterolemia (22). The variable response to therapy in these patients has been attributed to differences in biochemical defects (25); although familial hypercholesterolemia appears to be determined by a single gene coding for the LDL receptor, several different types of mutations have been characterized, of which three involved production of normal sized proteins (26). In receptor-negative subjects (less than 2% of the normal number of LDL receptors in the cell-surface) there may be no response to therapy, whereas in receptor-defective subjects (2–30% of the normal number of LDL receptors) an increase in this number may be induced. In the WHHL rabbits a clearcut, sustained decrease in serum cholesterol was seen after PIB (8) and from this observation, it was suggested that this mutant rabbit resembles one of the receptor-defective mutant phenotypes of familial hypercholesterolemia, in which it is possible to induce LDL receptors. In normal rabbits it has been shown that bile acid-binding resins promote the receptor-mediated catabolism and hepatic uptake of LDL (27). On the other hand, fasting rabbits for 9 days results in a down-regulation of the hepatic LDL receptor and concomitant increase in plasma LDL levels (18); in these rabbits there is an accumulation of cholesteryl esters in the liver (18), due to a reduced conversion of cholesterol to bile acids. In the rabbit, these studies also underline the pivotal role of the hepatic LDL receptor in the regulation of the plasma LDL concentration (28) and the influence of changes in bile acid excretion.

In agreement with previous studies (5), the receptor-mediated LDL catabolism was 70% of the total LDL removal in the normal rabbits, whereas there was no receptor-mediated LDL catabolism in our WHHL rabbits. The markedly delayed clearance of LDL, in combination with a 2.5-fold increase in LDL synthesis rate, accounts for the 10-fold increase in LDL levels. Our LDL turnover data in WHHL controls are identical to those reported by Bilheimer, Watanabe, and Kita, using the same method (5). After PIB, LDL concentrations decreased 50%, but the receptor-mediated clearance of LDL was still absent. It must therefore be concluded that the LDL receptors are not induced in WHHL rabbits by bile acid drainage. Since the FCR was unchanged after PIB, the ACR of LDL decreased nearly 50% (Table 3), so that a decreased synthesis of LDL must be regarded as the cause for the reduction in LDL.

The question arises whether the decreased synthesis of LDL is due to a relative malnutrition after PIB in this study. Earlier studies in 3-month-old rabbits showed

a normal gain in weight after PIB without significant differences in body weight at 30 weeks after the operation, compared with sham-operated rabbits; still there was a sustained decrease in plasma cholesterol (8). Furthermore, treatment with cholestyramine decreased plasma cholesterol to a similar extent as PIB, without any influence on body weight (9). Therefore, the observed results in the present study can be assumed to result from bile acid drainage rather than malnutrition.

The composition of LDL was determined in order to study a possible selective uptake of cholesteryl esters from LDL by the liver to meet the high requirements for bile acid synthesis. LDL was relatively enriched in triglycerides after PIB, but the decrease in cholesteryl esters or cholesteryl ester/protein ratio was not significant (Table 2), which makes a selective uptake less likely. The changes in the composition of LDL may be the result of a decrease in the size of the LDL pool accompanied by an increase in triglyceride levels in lipoproteins with lower density. Exchange of cholesteryl esters for triglycerides between VLDL and LDL may then yield the observed changes at steady state.

The decrease in LDL synthesis after PIB may be due to an altered metabolism of VLDL. In normal rabbits, only a small fraction of VLDL is converted into LDL (6); Kita et al. (6) reported a delayed clearance of VLDL in WHHL rabbits and a markedly increased conversion of VLDL apoB to LDL. Hornick et al. (29) provided evidence from WHHL liver perfusion studies that there is no increased secretion of apoB or de novo secretion of LDL, so that the increased synthesis of LDL in these rabbits appears to result solely from an increased conversion of VLDL to LDL. On the other hand, an independent LDL production has been reported from kinetic studies in vivo in normal rabbits (30) and in familial hypercholesterolemia (31). If there is independent LDL synthesis in WHHL rabbits also, this could be reduced after PIB to meet the requirements for cholesterol in the liver to compensate for the large loss of bile acids in the feces. VLDL may be used for this purpose also. Despite the absence of LDL receptors in WHHL rabbits, more than half of LDL is degraded in the liver of these animals (about 90% in parenchymal cells), just as in normal rabbits (32). The cholesteryl ester content was found to be increased in WHHL livers and the rate of cholesterol synthesis in the liver was suppressed (33). Apparently, receptor-independent transport mechanisms are able to deliver sufficient amounts of cholesterol to the liver in these animals.

In conclusion, a marked reduction in plasma LDL concentration was found in WHHL rabbits after partial ileal bypass, caused by a reduced production of LDL. Additional VLDL turnover studies may help to explain this reduction in LDL synthesis. ■■

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