Chylomicron-remnant clearance in homozygote and heterozygote Watanabe-heritable-hyperlipidaemic rabbits is defective

Lack of evidence for an independent chylomicron-remnant receptor

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Lymph chylomicrons radiolabelled in triacylglycerol and cholesteryl ester were injected into control and Watanabe heritable-hyperlipidaemic (WHHL) rabbits. Clearance of chylomicrons was slower in heterozygote and homozygote WHHL rabbits. Slower remnant clearance in WHHL rabbits was confirmed by monitoring the clearance from plasma of preformed chylomicron remnants. Use of chylomicron-like lipid emulsions injected into control and WHHL rabbits also confirmed the defect in remnant clearance in heterozygote WHHL and homozygote WHHL groups. Clearance from plasma of emulsion triolein was delayed in both WHHL groups compared with controls, owing to slower remnant clearance. The clearance from plasma of radioiodinated rabbit low-density lipoproteins (LDL) in heterozygote WHHL rabbits was the same as control rabbits. Defective chylomicron-remnant removal but normal LDL clearance in the heterozygote WHHL corresponded to elevated concentrations of plasma triacylglycerol and normal concentrations of plasma cholesterol. Receptor versus non-receptor clearances of chylomicron remnants were studied by comparing the clearance of emulsions with and without unesterified cholesterol respectively. Unlike control rabbits, there were no significant differences in the clearances of the two emulsion types in either the homozygote or heterozygote WHHL rabbits, indicating that the apolipoprotein-B₁₀₀/E receptor is the primary route for clearance of chylomicron remnants from plasma.

INTRODUCTION

The clearance of chylomicron remnants from plasma has been postulated to occur via a receptor-mediated process that is distinct from the removal of low-density lipoproteins (LDL) by the apolipoprotein-B₁₀₀/E receptor (LDL receptor). Present understanding is based mainly on a report by Kita *et al.* [1], who found that the disappearance of injected radiolabelled chylomicrons from the plasma fraction of density less than 1.006 g/ml was similar in homozygote Watanabe heritable-hyperlipidaemic (WHHL) rabbits (which lack functional LDL receptors) and normal rabbits.

The removal of chylomicron remnants is by a receptor-mediated process which recognizes apolipoprotein E as the ligand [2]. In contrast, apolipoprotein B₁₀₀ is responsible for the binding and uptake of LDL by the apolipoprotein-B₁₀₀/E receptor. Despite a number of studies *in vivo* and *in vitro*, a putative second receptor that binds chylomicron-remnant apolipoprotein E and mediates cellular uptake has not been confidently identified. In contrast, there is evidence to suggest that chylomicron remnants may be removed via the apolipoprotein-B₁₀₀/E receptor. Human fibroblasts take up chylomicron remnants via the apolipoprotein-B₁₀₀/E receptor [3]. The binding of chylomicron remnants to human HepG2 cells, rat liver cells in primary cultures and mouse macrophages was inhibited by 80–90% by specific anti-(apolipoprotein-B₁₀₀/E receptor) antibodies or LDL [4–6]. Chylomicron remnants, LDL and antibodies (apolipoprotein-B₁₀₀/E receptor) bind to the same proteins of rat liver membrane preparations [7] and mouse macrophages [6]. Furthermore, chylomicron-remnant binding to homozygote WHHL rabbit liver membranes was found to be markedly deficient [1].

Utilizing lipid emulsions which are similar in size and lipid composition to lymph chylomicrons, we recently showed that the

removal of chylomicron-like emulsion particles from the plasma of homozygote WHHL rabbits was delayed compared with normal rabbits [8]. Chylomicron-like emulsions were also cleared at a slower rate in cholesterol-fed rabbits [8], where apolipoprotein-B₁₀₀/E receptor activity is decreased [9].

The heterozygous form of familial hypercholesterolaemia is the most prevalent genetic lipid disorder of man [10]. WHHL rabbits are a model for this disease. Our data suggest that defective clearance of chylomicron remnants should be expected in heterozygous familial hypercholesterolaemia.

MATERIALS AND METHODS

Animals

Heterozygote WHHL rabbits were bred from homozygote bucks and British half-lop does. Rabbits were kept in individual cages and allowed free access to standard chow and water. Rabbits were used between 3 and 11 months of age, and body weight was 2.28–4.80 kg.

Chylomicron-like lipid emulsion

Lipid emulsions resembling chylomicrons in composition and size were prepared as previously described [11]. Mixtures of purified lipids were sonicated in Hepes buffer. Emulsion particles of the required composition and size (100–150 nm) were isolated by sequential gradient ultracentrifugation. Emulsion-particle size was determined with an automated particle sizer (Brookhaven Instruments Corp., New York, NY; U.S.A.) and confirmed by electron microscopy.

Emulsion particles used to monitor receptor-independent removal were modified by omitting unesterified cholesterol [12].

Radiolabelling and isolation of rat chylomicrons

Lymph chylomicrons were endogenously radiolabelled by

Abbreviations used: LDL, low-density lipoproteins; WHHL, Watanabe heritable hyperlipidaemic; FCR, fractional clearance rate.

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infusing whole milk containing 25 μCi of [^{14}C]palmitic acid and 30 μCi of [^3H]cholesterol into the duodenum of rats via a gastric cannula [13]. Lymph chylomicrons were collected via a cannula placed in the mesenteric lymph duct into tubes containing EDTA (5 mM). Lymph was removed from cells by centrifugation at 2000 g for 10 min. Chylomicrons were isolated by density-gradient ultracentrifugation [14]. Chylomicron diameter measured by electron microscopy had a mean of 110–120 nm.

Chylomicron-remnant formation

Chylomicron remnants were prepared as described previously [15]. Approx. 10 mg of chylomicron triacylglycerol was injected into rats which had their liver functionally excluded. Rats were bled 30 min after chylomicron injection, and remnants were isolated by density-gradient ultracentrifugation of the plasma [14].

LDL isolation and radiolabelling

Rabbit blood was collected by cardiac puncture from normal rabbits anaesthetized with ether. Plasma was separated by low-speed centrifugation and the density adjusted to 1.020 g/ml by addition of solid KBr. Plasma was centrifuged at 105000 g_{av} for 24 h at 4 °C in a Beckman SW28 rotor. The bottom two-thirds of each tube was collected and the density adjusted to 1.063 g/ml. The infranatant was overlaid with Tris buffer (pH 7) of density 1.063 g/ml. LDL was isolated by ultracentrifugation at 105000 g_{av} for 22 h in a Beckman SW28 rotor. The LDL was

washed by overlaying with at least 9 vol. of Tris buffer (pH 7) of equivalent density and centrifuging at 205000 g_{av} for 22 h in a Beckman SW41 rotor. The purity of the LDL fraction was confirmed by agarose-gel electrophoresis.

LDL was dialysed with multiple changes of 0.15 M-NaCl (pH 7.4) followed by 0.1 M-sodium borate at pH 8.4. Up to 3 mg of LDL in borate buffer (pH 8.4) was radiolabelled with 1 mCi of ^{125}I directly in Iodo-Gen-coated tubes (Pierce Chemical Co., Rockford, IL, U.S.A.). Over 97% of free ^{125}I was removed by passage through a Sephadex G-25 (fine grade) column, followed by extensive dialysis against 0.15 M-NaCl. Radiolabelled LDL was characterized on the basis of established procedures described by others [16,17]. Less than 8% of ^{125}I was associated with LDL lipid.

Emulsion, chylomicron and chylomicron-remnant kinetic studies

Chylomicron-like emulsions, native chylomicrons, chylomicron remnants and LDL were injected into normal control rabbits, homozygote WHHL rabbits and heterozygote WHHL rabbits. Injection of tracer was into a lateral ear vein of conscious rabbits. The amount of lipid injected was less than 2.5 mg in a maximum volume of 800 μl . Blood samples were subsequently taken from the opposite ear vein at regular time intervals between 3 and 45 min for chylomicron studies and up to 55.5 h for LDL turnovers. Coagulation of blood was prevented by the addition of 20 units of heparin. Plasma was separated immediately from blood cells by centrifugation (2000 g , 15 min) at 4 °C. For the

Table 1. Plasma lipid concentrations in normal control rabbits and heterozygote WHHL and homozygote WHHL rabbits

Blood was drawn from a lateral ear vein of conscious rabbits. Plasma was separated by low-speed centrifugation and plasma lipids were analysed. Data are means $\pm$ S.E.M.; numbers of samples analysed are shown in parentheses. In some cases individual rabbits were sampled several times over a 10-month period. Abbreviation: HDL, high-density lipoproteins. * Indicates significance at a probability less than 0.1%.

Rabbits	Plasma triacylglycerol (mg/dl)	Plasma total cholesterol (mg/dl)	Plasma unesterified cholesterol (mg/dl)	Plasma esterified cholesterol (mg/dl)	HDL triacylglycerol (mg/dl)	HDL total cholesterol (mg/dl)
Control	74 $\pm$ 5.9 (45)	67 $\pm$ 8.5 (46)	21 $\pm$ 2.5 (18)	48 $\pm$ 6.8 (18)	21 $\pm$ 1.1 (27)	26 $\pm$ 1.7 (31)
Heterozygote WHHL	119 $\pm$ 6.9* (80)	60 $\pm$ 4.1 (76)	19 $\pm$ 1.6 (41)	51 $\pm$ 4.1 (41)	19 $\pm$ 1.0 (53)	22 $\pm$ 1.2 (62)
Homozygote WHHL	571 $\pm$ 34* (13)	593 $\pm$ 28.0* (13)	196 $\pm$ 16.7* (6)	629 $\pm$ 67.9* (6)	7 $\pm$ 0.8* (10)	7 $\pm$ 1.1* (11)

Fig. 1. Clearance of chylomicron-like-emulsion triolein and cholesteryl oleate from plasma of control rabbits and heterozygote WHHL and homozygote WHHL rabbits

Chylomicron-like lipid emulsion containing radiolabelled triolein and radiolabelled cholesteryl oleate was prepared as described in the Materials and methods section. Two different radioisotopes were used for the two lipids. The emulsion was injected into an ear vein of conscious rabbits. Radioactivity in blood plasma sampled at regular time intervals from another ear vein was measured. The amount of radiolabelled triolein and cholesteryl oleate remaining in plasma is shown as a percentage of the dose injected. Plasma triolein and cholesteryl ester radioactivity is shown for control rabbits (○), heterozygote WHHL rabbits (●) and homozygote WHHL rabbits (□). Data are means for 8 normal, 10 heterozygote WHHL and 4 homozygote WHHL rabbits. Bars represent S.E.M.

chylomicron experiments, radioactivity in 400 μ l samples of plasma was determined by liquid-scintillation counting in a toluene-based fluor in dual-label mode with auto quench correction (Beckman LS3800 liquid-scintillation counter). For LDL clearance rates, 500 μ l of plasma was counted directly for radioactivity in a Packard Auto Gamma scintillation spectrometer (model 5320).

Clearance of emulsion or chylomicron lipid was mono-exponential over 3–15 min. The fractional clearance rate (FCR) was calculated for this time period by least-squares regression analysis. LDL clearance rates were calculated by using the two-pool model described by Matthews [18]. Total rabbit plasma volume was assumed to be 3.3 % of rabbit body weight.

Lipid analysis

Plasma triacylglycerol, free cholesterol, cholesteryl ester and HDL free and esterified cholesterol were determined with Boehringer test kits (nos. 877557, 237574, 310328 and 543004 respectively).

Statistical analysis

The concentrations of plasma lipids and the rates of lipoprotein clearance were compared between normal and WHHL rabbits by one-way analysis of variance. Significance was accepted for probabilities less than 5 %.

RESULTS

The plasma lipid concentrations for normal rabbits and heterozygote and homozygote WHHL rabbits are given in Table 1. The homozygote WHHL rabbits had higher concentrations of triacylglycerol, free cholesterol and cholesteryl ester compared with normal control rabbits and heterozygote WHHL rabbits. Triacylglycerol and total cholesterol were increased in similar proportion, both being about 8-fold more than in the control group. In contrast, in the heterozygote WHHL rabbits plasma triacylglycerol was significantly increased, but the free and esterified cholesterol concentrations were similar to those of the control group. The homozygote WHHL rabbits had significantly less HDL cholesterol and HDL triacylglycerol than did either the control group or the heterozygote WHHL rabbits.

The clearance of chylomicron-like emulsion from the plasma of normal and WHHL rabbits is shown in Fig. 1. Removal of triolein was always faster than cholesteryl oleate. For both the heterozygote and homozygote WHHL rabbits there was significantly

more cholesteryl oleate remaining in the plasma compartment compared with normal rabbits each time blood was taken. Similarly, at all times beyond 3 min more triolein radio-label remained in plasma of the WHHL rabbits. The FCRs of emulsion triolein and cholesteryl oleate calculated for the 3–15 min time period are shown in Table 2. In heterozygote and homozygote WHHL rabbits the rates of emulsion triolein removal were only 55 % and 31 % of those of control rabbits. Emulsion remnant removal calculated as the decline in plasma cholesteryl oleate was significantly slower in both types of WHHL rabbits, being approx. 60 % as fast in the heterozygote and 34 % as fast in the homozygote WHHL rabbits compared with controls.

Clearance from rabbit plasma of rat chylomicrons endogenously radiolabelled with triacylglycerol and cholesteryl ester is shown in Fig. 2. More radioactivity remained in plasma for both lipids in the homozygote and heterozygote WHHL rabbits compared with the control group. By 3 min after injection, about 20 % of the triacylglycerol component remained in the plasma of control rabbits, about 40 % in the heterozygote and about 80 % in the homozygote WHHL rabbits. Similarly, in the control rabbits 75 % of cholesteryl ester was removed 3 min after

Table 2. Clearance rates from plasma of chylomicron-like-emulsion triolein and cholesteryl oleate in normal control rabbits and heterozygote WHHL and homozygote WHHL rabbits

Rabbits	Clearance rate (fractions/min)	
	Cholesteryl oleate	Triolein
Control (8)	0.088 $\pm$ 0.010	0.163 $\pm$ 0.031
Heterozygous WHHL (10)	0.055 $\pm$ 0.006**	0.092 $\pm$ 0.012*
Homozygous WHHL (4)	0.025 $\pm$ 0.007**	0.073 $\pm$ 0.006

Chylomicron-like lipid particles containing radiolabelled triolein and radiolabelled cholesteryl oleate were prepared as described in the Materials and methods section. The emulsion was injected into ear veins of conscious rabbits. Fractional clearances of the two lipids were calculated from the decline in plasma radioactivity of the two radioisotopes over the first 15 min. The decline in plasma cholesteryl oleate is considered to represent particle removal. The decline in plasma triolein is the sum of two processes, lipolysis by endothelial lipases and removal of the particle. Data are means $\pm$ S.E.M. for numbers of animal in parentheses: * and ** reflect significance at a probability less than 1 % and 5 % respectively.

Fig. 2. Clearance of rat lymph chylomicrons from plasma of control rabbits and heterozygote WHHL and homozygote WHHL rabbits

Rat lymph chylomicrons were endogenously radiolabelled in triacylglycerol and cholesteryl ester with a different radioisotope for each lipid as described in the Materials and methods section. The lymph chylomicrons were injected into an ear vein of conscious rabbits. Blood was sampled at regular intervals from another ear vein, and the amount of radioactivity in plasma was measured. The amount of radiolabelled triacylglycerol and cholesteryl ester remaining in plasma is shown as a percentage of the dose injected. Plasma triacylglycerol and cholesteryl ester radioactivity is shown for control rabbits (○), heterozygote WHHL rabbits (●) and homozygote WHHL rabbits (□). Data are means for 5 normal rabbits, 5 heterozygote WHHL rabbits and 4 homozygote WHHL rabbits. Bars represent S.E.M.

Table 3. Clearance of lymph chylomicron lipids in normal control rabbits and heterozygote WHHL and homozygote WHHL rabbits

Rat lymph chylomicrons were endogenously radiolabelled in triacylglycerol and cholesteryl ester. Lymph chylomicrons were isolated by ultracentrifugation, and then a bolus injection of 2.5 mg chylomicron triacylglycerol was injected into ear veins of conscious rabbits. Fractional clearances of the two lipids were calculated from the decline in plasma radioactivity of the two radioisotopes with time. Data are means $\pm$ S.E.M. for the numbers of animals in parentheses: * and ** reflect significance at a probability less than 1% and 5% respectively.

Rabbit	Clearance rate (fractions/min)	
	Cholesteryl ester	Triacylglycerol
Control (5)	0.071 ± 0.006	0.352 ± 0.050
Heterozygous WHHL (5)	$0.039 \pm 0.008^*$	$0.137 \pm 0.014^{**}$
Homozygous WHHL (4)	$0.011 \pm 0.004^*$	$0.055 \pm 0.027^*$

Fig. 3. Chylomicron-remnant clearance in control rabbits and in homozygote WHHL rabbits

Chylomicron remnants were made by injecting rat lymph chylomicrons into hepatectomized rats. The lymph chylomicrons had been endogenously radiolabelled for triacylglycerol and cholesteryl ester by using a different radioisotope for each. Chylomicron remnants isolated by ultracentrifugation were injected into an ear vein of conscious rabbits. Blood samples were taken from another ear vein at regular time intervals. The amount of cholesteryl ester radioactivity (●, ■) and triacylglycerol (○, □) remaining in plasma is shown for control rabbits (●, ○) and homozygote WHHL rabbits (■, □) and is expressed as a percentage of the dose injected.

chylomicron injection, whereas only 45% and 20% of cholesteryl ester was removed in heterozygote and homozygote WHHL rabbits respectively. The FCRs for lymph chylomicron triacylglycerol and cholesteryl ester are given in Table 3. By analogy with the chylomicron-like emulsions, both triacylglycerol and cholesteryl ester were cleared at progressively slower rates in the heterozygote and homozygote WHHL rabbits compared with controls.

To clarify that slow chylomicron-remnant removal in WHHL rabbits was not due to slow formation of remnants *in vivo* (i.e. slow lipolysis of chylomicron triacylglycerol), we monitored the clearance of preformed remnants (Fig. 3). Normal rabbits cleared preformed chylomicron remnants much faster than did the homozygote WHHL rabbits. The FCR of cholesteryl ester was $0.088 \pm 0.010 \text{ min}^{-1}$ and $0.016 \pm 0.007 \text{ min}^{-1}$ for control and homozygote WHHL rabbits respectively ($P < 0.001$). In normal rabbits, the rate of triacylglycerol clearance was the same as particle (cholesteryl ester) clearance, i.e. $0.084 \pm 0.008 \text{ min}^{-1}$

Fig. 4. LDL clearance in normal rabbits and heterozygote WHHL and homozygote WHHL rabbits

Rabbit LDL was isolated from plasma of normal rabbits. The LDL was radiolabelled with ^{125}I as described in the Materials and methods section and then injected into conscious normal, heterozygote WHHL and homozygote WHHL rabbits. LDL clearance from plasma is shown as the amount of radioactivity remaining in plasma of control rabbits (○), heterozygote WHHL (●) and homozygote WHHL (□) rabbits. Radioactivity in plasma is given as a percentage of the dose injected. The data are means for 5 normal rabbits, 6 heterozygote WHHL rabbits or 1 homozygote WHHL rabbit. Bars represent S.E.M.

and $0.087 \pm 0.010 \text{ min}^{-1}$ respectively. In homozygote rabbits clearance of triacylglycerol was faster than of cholesteryl ester ($0.034 \pm 0.008 \text{ min}^{-1}$). It may be that, because the remnants are removed less readily in the WHHL rabbits, further lipolysis of particle triacylglycerol is able to occur.

The clearance of LDL in rabbits is presented in Fig. 4. The pattern of removal was bi-exponential. In homozygote WHHL rabbits, there was no rapid removal phase, and the amount of radiolabelled LDL remaining in plasma with time was much higher than in control rabbits. The FCR in the homozygote WHHL rabbit was 0.032 h^{-1} . In contrast, clearance rates of radiolabelled LDL from plasma of heterozygote WHHL rabbits and controls were similar and much faster, $0.185 \pm 0.033 \text{ h}^{-1}$ and $0.199 \pm 0.037 \text{ h}^{-1}$ respectively (means $\pm$ S.E.M.).

The clearances of chylomicron-like emulsions with or without cholesterol were compared in the same rabbit on consecutive days. Clearance patterns of the two emulsion types in normal and WHHL rabbits are shown in Fig. 5. Triacylglycerol removal was similar for the two emulsions in normal and WHHL rabbits (Table 4). Clearance of emulsion containing cholesterol in normal rabbits was rapid and exponential in nature. In emulsion lacking cholesterol, removal was very slow, being less than 3% as rapid as for normal emulsion. The clearances from plasma of cholesterol-free remnants were similar for the control rabbits and the two WHHL groups (Fig. 5). The difference between total rates of clearance (emulsion containing unesterified cholesterol) and receptor-independent removal (emulsion lacking unesterified cholesterol) was substantial in the control rabbits, but not different in the heterozygote and homozygote WHHL rabbits.

DISCUSSION

The clearance from plasma of chylomicron-like emulsion remnants is impaired in homozygote WHHL rabbits [8], presumably owing to the lack of functioning apolipoprotein-B₁₀₀/E receptors. In humans, an absolute deficiency of the LDL receptor (homozygote familial hypercholesterolaemia) is rare, and so perhaps of more importance is how heterozygotes of familial

Fig. 5. Clearance from plasma of chylomicron-like emulsion and lipid emulsion lacking unesterified cholesterol in control rabbits (a) and heterozygote WHHL (b) and homozygote WHHL (c) rabbits

Chylomicron-like emulsion containing radiolabelled cholesteryl oleate was prepared as described in the Materials and methods section. Removal of this emulsion in conscious control rabbits and heterozygote WHHL and homozygote WHHL rabbits was compared on consecutive days with that of a second emulsion which lacked unesterified cholesterol. The amount of radiolabelled cholesterol oleate remaining in plasma is shown as a percentage of the dose injected for emulsion containing unesterified cholesterol (○) and emulsion lacking unesterified cholesterol (●) in each group of rabbits. Data are means for two rabbits per treatment, and bars represent the range.

Table 4. Clearance of chylomicron-like emulsion and cholesterol-free emulsion in normal control rabbits and heterozygote WHHL and homozygote WHHL rabbits

Chylomicron-like lipid particles were prepared as described in the Materials and methods section. In a second emulsion type unesterified cholesterol was omitted from the preparation. Both cholesterol-containing and cholesterol-free emulsions contained radiolabelled triolein and radiolabelled cholesteryl oleate, a different radioisotope being used for each lipid. The two emulsions were injected on consecutive days into a lateral ear vein of conscious rabbits. Clearance rates for emulsion triolein and cholesteryl oleate were calculated from the amount of radioactivity remaining in plasma with time for the two radioisotopes respectively. Cholesteryl oleate reflects receptor-mediated uptake. Triolein removal is by lipolysis and particle uptake. Data are means $\pm$ range for two rabbits per treatment. The differences between columns (1) and (3) and columns (2) and (4) are shown for purposes of discussion.

Rabbits	Clearance rate (fractions/min) of chylomicron-like emulsion				Difference in removal	
	Containing unesterified cholesterol		Lacking unesterified cholesterol			
	Cholesteryl oleate (1)	Triolein (2)	Cholesteryl oleate (3)	Triolein (4)	(1)–(3)	(2)–(4)
Control	0.082 ± 0.024	0.182 ± 0.025	0.002 ± 0.006	0.166 ± 0.029	0.080	0.016
Heterozygous WHHL	0.032 ± 0.003	0.087 ± 0.001	0.017 ± 0.002	0.088 ± 0.003	0.015	–0.001
Homozygous WHHL	0.010 ± 0.005	0.080 ± 0.010	0.002 ± 0.001	0.079 ± 0.009	0.008	0.001

hypercholesterolaemia metabolize remnants. We have utilized the progeny of WHHL rabbits crossed with normal rabbits (heterozygote WHHL) to study the metabolism of chylomicrons.

Heterozygote WHHL rabbits might be expected to possess about 50% of normal apolipoprotein-B₁₀₀/E receptor activity. Consistent with this, the removal of chylomicron-like emulsions and of rat lymph chylomicrons was intermediate between clearances in control and homozygote WHHL rabbits (Fig. 1). Slower remnant clearance did not appear to be due to slow formation of remnants, because the clearance in WHHL rabbits of lymph chylomicrons was identical with that of preformed remnants (Fig. 2 versus Fig. 3). Therefore slower emulsion and chylomicron triacylglycerol clearance reflects impaired particle uptake and not decreased lipolysis by the endothelial lipases.

Our emulsion and chylomicron clearance data contrast with those reported by Kita *et al.* [1], who presented the removal of rat lymph chylomicrons in homozygote WHHL rabbits as the percentage of radioactivity remaining in the plasma fraction with density less than 1.006 g/ml. The procedures described in their study included ultracentrifuging at a temperature (12 °C) insufficient to melt crystallized chylomicron-remnant lipids [19]; therefore it is possible that a considerable proportion of their remnants had a density greater than 1.006 g/ml. Kita *et al.* [1] stated that the amount of radioactivity remaining in the plasma infranatant was about 40% (of the dose injected) for the

WHHL-rabbit plasma, and only 15% for the control rabbits. The amount of radioactivity in the 1.006 g/ml fraction was similar for the control rabbits and WHHL rabbits, meaning that the total plasma radioactivity (1.006 g/ml fraction plus infranatant) would have been less for their control group than for their WHHL group. This would agree with our previous observations [8] and the present study, supporting defective chylomicron remnant removal in WHHL rabbits.

In an earlier study [8], we suggested that competition by VLDL remnants and/or LDL for the apolipoprotein-B₁₀₀/E receptor may account for slower clearance of chylomicron remnants in WHHL rabbits. However, competition now seems unlikely to be the only cause, because clearance was delayed in heterozygote WHHL rabbits which had normal clearance rates of plasma LDL.

The efficient clearance of LDL, but slow clearance of chylomicron remnants, in the heterozygote WHHL rabbits was reflected in the plasma lipid concentrations. Heterozygote WHHL rabbits were normocholesterolaemic but hypertriacylglycerolaemic (Table 1). It is not clear why a partial deficiency of apolipoprotein-B₁₀₀/E receptor activity leads to impaired clearance of chylomicron remnants but not of LDL.

Chylomicron remnants are large in comparison with LDL and, unlike LDL, are unable to cross the capillary endothelium [20]. Therefore, chylomicron remnants are not exposed to the

majority of cellular apolipoprotein-B₁₀₀/E receptors in non-hepatic tissues. To accentuate further this difference in removal between the two lipoprotein types, the apolipoprotein-B₁₀₀/E receptor binds lipoprotein via apolipoprotein E with high affinity but low capacity, requiring a calculated four receptor sites per remnant particle [21]. In contrast, LDL binds to the apolipoprotein-B₁₀₀/E receptor via apolipoprotein B₁₀₀ with high affinity and high capacity, with only one binding site required per particle. Therefore chylomicron-remnant removal may be compromised at lower cellular apolipoprotein-B₁₀₀/E receptor densities.

We attempted to identify a receptor-removal process which was distinct from the apolipoprotein-B₁₀₀/E receptor system, by comparing the clearances of chylomicron-like emulsions and cholesterol-free particles. Chylomicron-like emulsion particles which lack unesterified cholesterol are lipolysed normally, but are not removed by high-affinity remnant-removal mechanisms [12]. If a second receptor pathway for chylomicron remnants existed, a difference in clearance would be found between normal emulsions and those lacking cholesterol when injected into homozygote WHHL rabbits. However, rates of clearance of the two emulsion types were the same in the homozygote and heterozygote WHHL rabbits (Fig. 5). Our findings in the heterozygote and homozygote rabbits can be explained by the absence of receptor-mediated removal of chylomicron remnants. Our findings do not support the existence of a second high-affinity removal mechanism for chylomicron remnants.

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