A study of the chylomicron metabolism in WHHL rabbits after fat loading

Discrepancy between results based on measurement of apoprotein B-48 or retinyl palmitate

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We studied the metabolism of chylomicrons in homozygous Watanabe heritable hyperlipidaemic (WHHL) rabbits and in cholesterol-fed or normally fed New Zealand White (NZW) rabbits by measuring the concentrations of apoprotein B-48 and of retinyl palmitate in their serum after feeding fat plus this vitamin according to two different protocols. Compared with NZW controls, retinyl palmitate accumulated in both hyperlipidaemic groups under study, not only in the $d < 1.019$ fraction but also in the low-density lipoprotein (LDL) fraction. A strong correlation was found between the retinyl palmitate concentration in either the $d < 1.019$ fraction or the LDL fraction of the WHHL rabbits and the concentrations of cholesterol and triacylglycerols in these fractions. This suggests that retinyl palmitate is exchanged rapidly between exogenous and endogenous lipoproteins. This is supported by the lack of a correlation between the retinyl palmitate concentrations and the intensity of the apoprotein B-48 band in the respective $d < 1.019$ fractions or LDL fractions; in most fractions, in which large amounts of retinyl palmitate were present, the intensity of the apoprotein B-48 band was not increased compared with the fasting concentrations. Assuming that retinyl palmitate is a marker for the transfer of exogenous lipids, the results of our experiments indicate that the removal of exogenous lipids is delayed by complexing to endogenously synthesized lipoproteins. However, the clearance of apoprotein B-48 is normal and thus independent of the LDL-receptor activity.

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 (apo) B-100/E receptor (LDL receptor). The present understanding is based mainly on results of Kita *et al.* [1], who found that the disappearance of injected radiolabelled chylomicrons from the plasma fraction of $d < 1.006$ was similar in homozygous Watanabe heritable hyperlipidaemic (WHHL) rabbits, which lack functional LDL receptors, and normal rabbits. By means of the retinyl palmitate labelling method, however, it was found that the clearance of lipoprotein particles of intestinal origin was delayed in WHHL homozygotes as well as in cholesterol-fed New Zealand White (NZW) rabbits [2]. In another study, lymph chylomicrons, radiolabelled in triacylglycerol and cholesterol ester, were cleared more slowly in homozygous WHHL rabbits [3]. This suggests that chylomicron remnants are, at least in part, removed via the apo B-100/E receptor. This is in line with the deficient binding of chylomicron remnants to liver membranes of WHHL homozygotes [1]. To clear this apparent discrepancy in the literature concerning chylomicron metabolism in WHHL rabbits, we studied the appearance and disappearance of chylomicrons after feeding or loading fat by frequent determination of apo B-48 in the $d < 1.019$ fraction by SDS/PAGE. We also applied the retinyl palmitate labelling method, in which use is made of the specific incorporation of retinyl palmitate into chylomicrons in the intestinal mucosal cells. Retinyl palmitate is incorporated in the apolar core of chylomicrons and remains in the particle during transport in the lymphatic and vascular spaces [4]. In man, the suitability of this method has been demonstrated clearly, because

the exchange of retinyl palmitate to lipoproteins of higher density is less than 10% [5,6].

For comparison with the experiments described above, we also performed studies in NZW rabbits fed on normal chow or chow supplemented with 1% cholesterol.

MATERIALS AND METHODS

Animals

Homozygous WHHL rabbits were raised by crossing and back-crossing with NZW rabbits. They were maintained on a regular chow diet (containing 2.6% of energy from fat, including 1% from polyunsaturated fat; LK04 diet, Hope Farms, Woerden, The Netherlands). NZW rabbits fed either on normal chow or on chow containing 1% cholesterol (Hope Farms) for 1 week were also studied. Body weights of the rabbits ranged between 2350 and 2630 g. Rabbits were fed in the morning; the food was usually consumed completely within 5 h. Unless indicated otherwise, blood samples were obtained from an ear vein after an overnight fast, after approx. 19 h of fasting. Serum was isolated after standing for 1 h at room temperature. To prevent degradation of apoproteins, 0.9 mg of phenylmethanesulphonyl fluoride was added to 10 ml of serum.

Vitamin A feeding test

For this, five drops of Vitamin A (13.7 mg, 25000 I.U.; Arovit; Hoffman-La Roche) were mixed with 7.5 ml of cream and absorbed on to 25 g of rabbit chow. This mixture was quantitatively supplied to overnight-fasted rabbits (five NZW rabbits on normal chow, and five homozygous WHHL rabbits and three NZW rabbits on chow containing 1% cholesterol for 1 week). At 15:00 h food was consumed completely; the rabbits were then

Abbreviations used: LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; HDL, high-density lipoprotein; apo, apoprotein; WHHL, Watanabe heritable hyperlipidaemic; NZW, New Zealand White.

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fed with another 30 g of food not containing additional fat and vitamin A. Blood was obtained the next morning after fasting overnight. The rabbits were then fed as usual with 100 g of food, which was completely consumed within 5 h. After fasting overnight the rabbits were bled again, which delivered the day-2 sample (24 h later). In the same way the day-3 sample (48 h later) was obtained.

Intragastric fat-loading test

Intragastric fat loading was performed in five WHHL homozygotes and four NZW rabbits of similar body weight. At 2 days preceding the experiment, all animals were sedated and a silicone catheter was inserted into an ear vein; the length of the catheter was adjusted so that the tip was situated in the right atrium. A solution of 10 % (w/v) dextran in 0.15 M-NaCl was instilled into the catheter to prevent clotting during sampling of blood at the times indicated. On the day of the experiment, 15 ml of cream containing ten drops of vitamin A was given to each animal by an intragastric catheter. While the animals continued to fast, blood was sampled just before loading and after 4, 6, 9, 13, 18, 23 and 29 h.

For the isolation of mesenteric lymph, rabbits received an intragastric fat load as described above. Then 2 h later they were anaesthetized by intramuscular injection of Hypnorm (0.5 ml/kg) followed by atropine (0.25 mg/rabbit) and ventilation with N_2O/O_2 (2:1) through a trachea with a Loosco infant ventilator at 20 cycles/min (volume 2 litres, pressure 10 cmH₂O), with electrocardiographic and temperature monitoring. After the abdomen was opened, a polyethylene cannula filled with heparin solution (1000 units/ml) was inserted 1–2 mm into the main mesenteric lymph duct. The cannula was positioned so that a regular lymph flow occurred. Lymph was collected into a tube containing EDTA (1 mg/ml). The lymph isolated after cannulation had triacylglycerol concentrations between 1.7 and 4.1 mM and cholesterol concentrations between 0.8 and 1.2 mM. After collection, lymph was diluted 5-fold with a $d = 1.006$ solution containing 3 % (w/v) saccharose, followed by ultracentrifugation for 1 h at 14 000 rev./min (20 000 g) in a MSE swinging-bucket rotor (S3383) in a MSE Prespin 75 ultracentrifuge.

Lipoprotein solution

Lipoprotein fractions were isolated at 14 °C by sequential ultracentrifugation for 16–22 h at 168 000 g in an IEC B-60 ultracentrifuge in the fixed-angle rotor 468 [7]. Very-low-density lipoprotein (VLDL) was subfractionated by cumulative rate centrifugation as described previously [8]. In short, in a polycarbonate ultracentrifuge tube (3441-125; MSE), we dissolved 0.476 g of KBr in 3.4 ml of serum to raise the density to 1.10 g/ml. This solution was subsequently overlaid with 2.5 ml of $d = 1.065$, 2.5 ml of $d = 1.020$ and 2.9 ml of $d = 1.006$ solutions. Ultracentrifugation was carried out in a SW 40 Ti rotor at 20 °C in a Beckman L7-55 ultracentrifuge. Four consecutive runs were done, calculated to float VLDL particles of the following *S_f* classes to the top of the tube: *S_f* > 400; *S_f* 175–400; *S_f* 100–175; *S_f* 15–100 [8]. Each fraction was recovered by careful aspiration of the top 1 ml after each run, and the tube was then re-filled with 1 ml of $d = 1.006$ solution. After the fourth and final centrifugation, the LDL located in the middle of the tube was also isolated.

Analytical methods

Cholesterol and triacylglycerols in serum and in lipoprotein fractions were determined by enzymic methods using commercially available reagents (CHOD-PAP reagent from Boehringer, Mannheim, Germany, and Sera-PAK Triglycerides from Miles, Milano, Italy). Agarose-gel electrophoresis of lipoproteins

was performed as described previously [9]. Apoproteins B-48 and B-100 were analysed by discontinuous analytical PAGE (3.5/4.5% gels) [10]. The $d < 1.019$ fractions and the LDL fractions isolated from 2 ml of serum were always diluted to the same volume, 1.5 ml and 2.0 ml respectively. Because of the large differences in concentrations, the gels were loaded with 100 μ l and 40 μ l of the $d < 1.019$ fractions of NZW and WHHL rabbits respectively, and with 100 μ l and 20 μ l of the LDL fraction respectively. For the cholesterol-fed rabbits, the dilutions of the fractions and the amounts loaded on to the gels were similar to those for the WHHL rabbits. Retinyl palmitate concentrations were determined by h.p.l.c. (Si-60; Varian 8500) [11]. Samples to be analysed were kept in the dark and were frozen until assay.

For quantitative measurement, 20 μ l of retinal oxime (3.06 nmol) was added as an internal standard to 1 ml of each lipoprotein fraction. Extraction was performed with 2 $\times$ 4 ml of acetone/diethyl ether (1:1, v/v). The recovery of retinyl palmitate after extraction was 85–95 % ($n = 10$). The extracts were combined, dried under nitrogen, and redissolved in 15 μ l of dioxan. This solution was diluted with 100 μ l of hexane, and 40 μ l of this mixture was applied to the Si-60 column and eluted isocratically. Eluted peaks were detected at 328 nm. Peak heights were converted into absolute amounts by means of the internal standard.

RESULTS

Serum lipids and lipoproteins in the various groups of animals

Table 1 summarizes the results on serum lipid and lipoprotein concentrations of the three groups of rabbits in the vitamin A feeding test. The values of the rabbits in the intragastric fat-loading test were of similar magnitude.

Vitamin A feeding test

Basal fasting retinyl palmitate concentrations in the $d < 1.019$ fractions of WHHL and cholesterol-fed NZW rabbits were increased compared with normal fed NZW rabbits (Fig. 1). These differences were much more pronounced in the samples obtained 24 h and 48 h after the fat load. In the controls, basal fasting values were already attained at 24 h after fat feeding, whereas in both other groups fasting values of retinyl palmitate were still increased 48 h after fat feeding. Assuming that retinyl palmitate is a valid marker for chylomicrons, these data would indicate that homozygous WHHL and cholesterol-fed NZW rabbits have a delayed removal of chylomicrons and their remnants. Surprisingly, after fat feeding to WHHL rabbits most retinyl palmitate was found in the LDL fraction, especially in the fasting sample after 24 h. In the cholesterol-fed NZW rabbits 3 times more retinyl palmitate was present in the $d < 1.019$ fraction

Table 1. Serum lipids and lipoprotein concentrations in the rabbits studied

Values (mM) are means $\pm$ s.d.

Rabbits ... n ...	NZW 5	WHHL homozygotes 5	Cholesterol- fed NZW* 3
Serum cholesterol	3.51 $\pm$ 1.20	16.6 $\pm$ 2.8	14.9 $\pm$ 4.5
Serum triacylglycerols	0.52 $\pm$ 0.33	2.0 $\pm$ 1.0	0.36 $\pm$ 0.21
$d < 1.019$ cholesterol	0.37 $\pm$ 0.21	3.2 $\pm$ 2.5	8.11 $\pm$ 2.81
$d < 1.019$ triacylglycerols	0.21 $\pm$ 0.19	1.9 $\pm$ 2.2	0.23 $\pm$ 0.14
LDL-cholesterol	2.03 $\pm$ 1.24	12.8 $\pm$ 2.4	3.20 $\pm$ 0.41
LDL-triacylglycerols	0.27 $\pm$ 0.16	0.9 $\pm$ 0.3	0.06 $\pm$ 0.02
HDL-cholesterol	0.68 $\pm$ 0.12	0.10 $\pm$ 0.04	0.66 $\pm$ 0.10

* Fed 1 % cholesterol for 1 week.

Fig. 1. Vitamin A concentrations in lipoprotein fractions after fat feeding

After the rabbits had eaten cream and vitamin A, retinyl palmitate was determined in the $d < 1.019$ fraction (a) and in the LDL fraction (b) of NZW ($n = 5$), WHHL homozygote rabbits ($n = 5$) and NZW rabbits on 1% cholesterol (CHOL) ($n = 3$). Determinations were performed before and 1 or 2 days after the feeding test.

than in the LDL fraction. These findings suggest that in both groups of hyperlipidaemic rabbits there is an exchange of retinyl palmitate concentrations between chylomicrons and the VLDL, intermediate-density lipoprotein (IDL) and LDL. This is supported by the strong correlation of the concentration of retinyl palmitate in the VLDL + IDL fractions of the NZW rabbits on normal chow 1 day after fat feeding with the cholesterol or triacylglycerol content in these fractions ($r = 0.84$ and 0.88 respectively; $n = 5$; $0.05 < P < 0.1$). Retinyl palmitate concentrations in the $d < 1.019$ fractions of the WHHL homozygotes correlated strongly with the cholesterol and/or triacylglycerol concentrations in the $d < 1.019$ fractions ($r = 0.99$ and 0.92 respectively; $n = 5$; $P < 0.05$). Because of the small group, data from the cholesterol-fed NZW rabbits were not conclusive in this respect. Owing to the small inter-individual variation in LDL concentrations, cholesterol did not correlate with retinyl palmitate in these fractions of the normal fed NZW rabbits ($r = 0.05$); for the WHHL homozygotes this relationship was significant ($r = 0.87$; $P < 0.05$).

Intragastric fat-loading test

Retinyl palmitate concentrations in the $d < 1.019$ fraction rose steadily and similarly in both groups of rabbits in the first 9–13 h (Fig. 2). In the NZW rabbits a peak was obtained 13 h after loading; in the WHHL rabbits a peak was reached after approx. 23 h, but at a much higher level. The increase of retinyl palmitate in the LDL fractions in both groups of rabbits showed a trend similar to that of the respective $d < 1.019$ fraction. In the NZW rabbits a plateau was reached after 13 h, and in the WHHL rabbits between 23 and 29 h.

Study of intestinal lymph formation

If the small intestine is divided into six parts of equal length (segments of 33–39 cm, total length 198–234 cm; $n = 4$) most intestinal blood vessels are present in the third and fourth segments. After fat loading, these vessels become white-coloured. Maximal uptake of chylomicrons by these vessels took place

Fig. 2. Vitamin A concentrations in lipoprotein fractions after intragastric fat loading

After NZW ($n = 4$) and WHHL ($n = 5$) rabbits had been loaded with cream and vitamin A, blood samples were obtained at the indicated times, followed by determination of retinyl palmitate in the $d < 1.019$ fraction and in the LDL fraction.

Fig. 3. SDS/PAGE of high-molecular-mass apoproteins in various lipoprotein fractions and in intestinal lymph

From left to right: HDL fraction of a NZW rabbit; HDL fraction of a WHHL rabbit; intestinal lymph of a NZW rabbit after fat loading; $d < 1.019$ fraction of a WHHL rabbit; LDL fraction of a WHHL rabbit. Abbreviations: E, apo E; A-1, apo A-1; B-48, apo B-48; B-100, apo B-100; alb, albumin.

between 6 and 14 h after loading. It may thus be expected that the whole fat load is processed to chylomicrons within 16–20 h.

Determination of apo B-48 in intestinal lymph

The intestinal lymph isolated contained, despite the thorough washing procedure, large amounts of albumin. Fig. 3 shows that apo B-48 is the only high-molecular-mass apoprotein in intestinal lymph of rabbits. A band with similar migration is usually present in the $d < 1.019$ fraction of fasting WHHL rabbits (Fig.

Fig. 4. SDS/PAGE of high-molecular-mass apoproteins in various density fractions of rabbits to determine apo B-48 before and 24 h after consumption of fat

Top: $d < 1.019$ fraction of NZW rabbits ($n = 4$, left), of WHHL rabbits before fat loading ($n = 4$, middle), and of WHHL rabbits 24 h after fat loading ($n = 3$, right). Gels were loaded with 100 μ l and 40 μ l of the $d < 1.019$ fraction from NZW and WHHL rabbits respectively. Middle: LDL fraction of NZW rabbits ($n = 3$, left), of WHHL rabbits before fat loading ($n = 3$, middle), and of WHHL rabbits 24 h after fat loading ($n = 3$, right). Gels were loaded with 100 μ l and 20 μ l, respectively. Bottom: $d < 1.019$ fraction of NZW rabbits fed with 1% cholesterol before fat feeding ($n = 3$, left) or 24 h after fat feeding ($n = 2$, middle). At the right, LDL fractions of these rabbits 24 h after fat loading are shown. Abbreviations: H, the $d < 1.019$ fraction of a human pooled serum; E, apo E; A, apo A-1; B-48, apo B-48; B-100, apo B-100.

3). The corresponding LDL fraction used as reference contains two faint bands migrating faster than apo B-100, probably apo B-74 and apo B-26, rather than apo B-48. This is strengthened by the intensities of both minor bands: that of the presumed B-74 is 2–3-fold stronger than that of B-26, in agreement with their estimated molecular masses.

Measurement of apo B-48 after loading or feeding fat

During the intragastric fat-loading test the variation in the intensity of the presumed apo B-48 band in the $d < 1.019$ fraction of WHHL rabbits was minimal. There was no clear relationship with the increase in vitamin A in the $d < 1.019$ fraction. Compared with apo B-100, the intensity of the presumed apo B-48 band was always less than 5% (results not shown).

In the fat-feeding test a faint apo B-48 band was found in one of the four VLDL+IDL fractions analysed from fasted NZW rabbits (Fig. 4). Similar fractions from fasted WHHL rabbits contained a faint band at the region specific for apo B-48; some other minor bands were also present. The day after the vitamin A-enriched food was consumed, the VLDL+IDL fraction from

Fig. 5. Distribution of retinyl palmitate over various density classes in a NZW and a WHHL rabbit at 29 h after fat loading

The various Sf classes were isolated by flotation and density ultracentrifugation: A, Sf > 400; B, Sf 175–400; C, Sf 100–175; D, Sf 15–100; E, LDL fraction; F, HDL fraction ($d > 1.063$ fraction).

three of the four WHHL rabbits was negative for the presumed apo B-48 band (Fig. 4), despite the maximal values then found for retinyl palmitate in these rabbits.

Compared with NZW rabbits, the LDL fraction of the WHHL rabbits contained next to the apo B-100 band several other proteins with higher or lower molecular masses than apo B-100, probably apo B-200, apo B-100, apo B-74 and apo B-26; none of the bands had a migration similar to apo B-48 (Figs. 3 and 4). This pattern did not change in the fasted samples obtained 1 day after fat-feeding (Fig. 4).

In all the cholesterol-fed NZW rabbits, apo B-48 was present in the $d < 1.019$ fraction; the intensity of these apo B-48 bands did not change the day after the oral fat load (Fig. 4). Again, some minor bands were present between the presumed B-48 band and apo B-100. The LDL fractions contained, next to the strong apo B-100 band, a clear band, presumably apo B-74, and two minor bands, probably apo B-48 and apo B-26; none of them increased in intensity the day after the oral fat load.

Agarose-gel electrophoresis

The samples obtained during the intragastric fat-loading test were also analysed for chylomicrons by agarose-gel electrophoresis. In none of the samples was a clear band with zero mobility seen on agarose-gel electrophoresis. After storage of the samples at 4 °C for 24 h, no white turbid material had migrated to the meniscus. Both findings indicate that the samples are free from chylomicrons.

Flotation analysis

Serum from a NZW and a WHHL rabbit obtained at 29 h after fat-loading was fractionated into several flotation classes. In the Sf > 400 fractions from both rabbits no turbidity was present. In the serum of the WHHL rabbit, turbidity increased with decreasing Sf value and was maximal in the fraction with Sf values between 15 and 100. This was also the only fraction from the NZW serum that was slightly turbid. Surprisingly, the vitamin A content was related to the turbidity and lipid content of the VLDL subfractions and of LDL (Fig. 5).

DISCUSSION

In the present study we found that rabbits, being omnivorous and therefore consuming only small amounts of fat, have an active chylomicron metabolism. The chylomicron particles are secreted by the intestinal lymph vessels at a maximal rate 6–14 h after intragastric loading. Surprisingly, retinyl palmitate concentrations in the $d < 1.019$ fraction of the WHHL rabbits, a marker for chylomicrons, was maximal between 19 and 29 h after loading, pointing to a defect in removal of chylomicrons and their

remnants in these rabbits. This seems to be supported by the fact that retinyl palmitate concentrations in the $d < 1.019$ fractions of WHHL rabbits and of cholesterol-fed NZW rabbits were increased considerably as compared with NZW rabbits 1 or 2 days after feeding fat with this vitamin. However, the LDL fractions of the WHHL rabbits in both protocols and of the cholesterol-fed NZW rabbits 1 or 2 days after fat feeding also contained large amounts of retinyl palmitate. In the fat-feeding experiments it was shown that the increase in retinyl palmitate in the $d < 1.019$ fractions and in the LDL fractions showed a similar trend; no delay was observed in the increase in retinyl palmitate in the LDL fraction as compared with that in the $d < 1.019$ fraction. Such a delay might be expected if the chylomicrons during their processing to chylomicron remnants increase in density, so that they are isolated in the LDL fraction. However, there is no precedent for such a conversion, at least not in man [12]. The increased amounts of retinyl palmitate in the LDL fraction and in the VLDL+IDL fraction of WHHL rabbits must then be due to exchange of this vitamin between the chylomicrons and the endogenous lipoproteins during the conversion of chylomicrons into chylomicron remnants. That most of the retinyl palmitate in these fractions is associated with endogenous lipoproteins and not with chylomicrons or with their remnants can be deduced from the frequent absence of apoprotein B-48 in these fractions several hours after feeding or loading fat at time intervals when retinyl palmitate concentrations were elevated. In agreement with previous findings [13], the intensity of the apoprotein B-48 band was always less than 5% of that of the apo B-100 band. Apparently, apo B-48 is rapidly removed from the circulation of homozygous WHHL rabbits, whereas retinyl palmitate, the presumed marker of chylomicrons, accumulates in the plasma, owing to exchange. In this respect it is not surprising that the retinyl palmitate concentrations in the $d < 1.019$ fractions in the WHHL rabbits correlate significantly with their lipid contents. Also, a similar strong correlation in the LDL fraction of the WHHL rabbits supports the exchange hypothesis. Indeed, rabbits, and especially WHHL rabbits, have an elevated cholesterol ester transfer capacity [14,15]. On the basis of our findings and those of Zilversmit *et al.* [16], it is likely that other lipophilic products, such as cholesterol and triacylglycerols, are also exchanged. The very similar cholesterol/triacylglycerol composition data for the $d < 1.019$ fraction and the LDL fraction of WHHL rabbits support this. By this exchange, also observed by Kita *et al.* [1], the endogeneously produced lipoproteins carry large amounts of absorbed lipids, which alters their composition and which may also influence their interaction with the LDL receptor. Indeed, the binding of WHHL LDL to normal rabbit fibroblasts was lower than that of NZW LDL [17]. Furthermore, changes in the chemical composition of LDL, induced by partial ileal bypass, influenced their removal rate [18,19].

The transport of dietary lipids by the apo B-containing lipoproteins is challenged in the cholesterol-fed NZW rabbits. As in the WHHL rabbits, retinyl palmitate in the $d < 1.019$ fraction and in the LDL fraction was elevated in NZW rabbits after fat loading, but the intensity of the apo B-48 band did not increase. Apparently, in the case of down-regulation of the LDL receptor, removal of apo B-48-containing particles is unaffected.

The drawing of conclusions concerning the presence or absence of the apo B-48 band in the $d < 1.019$ fraction, but especially in the LDL fraction, is hampered by the presence of additional minor bands in the apo B-48 region, despite the use of a protein inhibitor during the isolation procedure. In normal fed NZW rabbits these bands were not present, in agreement with Havel *et al.* [13], who described that the major B apoprotein of VLDL, IDL and LDL is apo B-100. They also observed small amounts

of protein (< 5% of total) with the electrophoretic mobility of apo B-48 in IDL and LDL, which they considered to originate from the liver on the basis of liver-perfusion experiments.

The fact that in WHHL rabbits and cholesterol-fed rabbits no clear accumulation of apo B-48 occurred to a value higher than 5% of apo B-100 indicates that the clearance of chylomicrons in these rabbits is not impaired, in agreement with results of Kita *et al.* [1]. If the chylomicron remnants contain sufficient amounts of apo E, they may be eliminated from the circulation by way of the defective LDL receptor, which hypothetically remains able to eliminate VLDL remnants [20,21]. Note that less than 5% of binding capacity of the LDL receptors is needed to remove the B-48-containing particles. Otherwise, the LDL-related protein, which may function as the chylomicron receptor, may be involved [22].

Our results on the time-dependent distribution of retinyl palmitate over the lipoprotein fractions are similar to those reported by Beaumont & Assadollahi [2]. However, owing to the concentration-dependent transfer of retinyl palmitate, it is very unlikely that the accumulation of retinyl palmitate in the LDL fraction at later stages reflects accumulation of very small chylomicron remnants [2]. This is supported by the absence of B-48 in our study in those LDL fractions with huge amounts of retinyl palmitate. Bowler *et al.* [3] studied chylomicron metabolism in WHHL rabbits after intravenous administration of washed heterologous radiolabelled rat chylomicrons. Most of the labelled lipids were removed from the circulation very rapidly. This raises the question whether these particles indeed mimic the kinetic behaviour of chylomicron remnants. Another approach to study the metabolism of chylomicrons was the use of artificial lipid emulsions [23]. This method appeared valid, from studies in rat and man [24,25]. However, rats and man have only a limited lipid transfer capacity [14]. After injection of the emulsion it was found that the triolein component was cleared normally in WHHL rabbits, but the cholesteryl oleate clearance was delayed. This was ascribed to accumulation of chylomicron-remnant-like particles [23]. However, it can be questioned whether there is sufficient time to acquire apo E from donor lipoproteins, if most of the lipids are already removed within 3–10 min. In comparison with the apo E content, which we found in the $d < 1.019$ fraction in our WHHL rabbits, the content found by the authors by incubation *in vitro* for 15 min was very low in relation to the intensity of the apoprotein B-100 band [23]. It is therefore possible that most of the lipid particles are removed by other ways, for example by means of the scavenger receptor. In addition, the preferential binding of cholesteryl oleate to triolein could also be explained by transfer reactions, with a preferential transfer of triolein to those particles with the shortest half-lives. Because in these studies the occurrence of any lipid transfer was completely neglected, the conclusions drawn by the authors [3,24] about removal rate of chylomicrons in WHHL rabbits are questionable.

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