Role of the Low Density Lipoprotein (LDL) Receptor Pathway in the Metabolism of Chylomicron Remnants

A QUANTITATIVE STUDY IN KNOCKOUT MICE LACKING THE LDL RECEPTOR, APOLIPOPROTEIN E, OR BOTH*

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Two receptor pathways are thought to mediate the hepatic clearance of chylomicron remnants, (i) the low density lipoprotein receptor (LDLR) pathway and (ii) non-LDLR pathway. The current study was undertaken to quantitatively assess the contribution of each receptor pathway to hepatic catabolism of chylomicron remnants, by using mice that are deficient in apolipoprotein E (apoE) (apoE(−/−)), the LDLR (LDLR(−/−)), and both (apoE(−/−);LDLR(−/−)). Vitamin A fat tolerance tests showed that the area under the curves of the plasma excursions of retinyl ester in the LDLR(−/−), apoE(−/−), and apoE(−/−);LDLR(−/−) mice were 4, 12, and 12 times larger than those in wild-type mice. The retinyl ester accumulated in the plasma of the LDLR(−/−) mice was distributed in larger subfractions of triglyceride-rich lipoproteins, chylomicrons through very low density lipoprotein-C. These results indicate that the LDLR constitutes the major pathway for the clearance of retinyl ester.

In support of this, agarose gel electrophoresis revealed that an oral fat load resulted in retention of chylomicrons in the LDLR(−/−) mice, which was not seen in wild-type mice. The observation that the apoE(−/−) mice showed larger retinyl ester excursion than LDLR(−/−) mice indicates that an apoE-dependent non-LDLR pathway is involved in the rest of the clearance of the retinyl ester.

Together, we conclude that the LDLR pathway plays a significant role in the chylomicron remnant metabolism in mice fed a normal chow.

In mammals including humans, dietary fat is absorbed by the small intestine, packaged in the chylomicrons, and transported through the lymphatic system to the systemic circulation. Lipoprotein lipase hydrolyzes the core triglycerides of the chylomicrons, converting them to chylomicron remnants. The remnants are rapidly cleared by the liver. This step of rapid hepatic clearance has been thought to involve several different processes (for review see Refs. 1–3). The initial process is sequestration of chylomicron remnants into the space of Disse, conceivably through the interaction of apoE on the lipoprotein particles with heparan sulfate proteoglycan (HSPG) on the cell surface (4, 5). Subsequent steps might involve further lipolysis of the remnants by hepatic lipase (6, 7) and acquisition of apoE secreted from the liver (8, 9). The final step appears to involve the cellular uptake of the lipoproteins, mainly by receptor-mediated endocytosis.

apoE is essential for the hepatic removal of chylomicron remnants, as evidenced by the fact that apoE-deficient humans (10, 11) and mice (12, 13) develop severe plasma retention of chylomicron remnants. As to the role of the low density lipoprotein receptor (LDLR) in the rapid plasma clearance of chylomicron remnants, conflicting results have been reported.

Several evidences have suggested that the role of LDLR in the hepatic clearance of chylomicron remnants is marginal, if present. First, in LDLR-deficient humans (14) and rabbits (15), accumulation of chylomicron remnants in the plasma is barely detectable. In support of this, the intravenously injected chylomicrons was cleared from the plasma of LDLR-deficient rabbits at a rate similar to wild-type (15). Upon vitamin A fat tolerance tests, moreover, no significant delay of the excursion of the esterified form of vitamin A, which represents chylomicron remnants, was shown in LDLR-deficient humans (16).

On the other hand, chylomicron remnants have been demonstrated to bind to the LDLR through apoE that forms a major surface component of the lipoproteins in in vitro studies (15, 17–19). When the LDLR was blocked by its monospecific antibody in mice (20) and rats (21), the clearance of chylomicron remnants was partially delayed. Moreover, the excursion of the esterified form of vitamin A in the plasma was significantly delayed in the LDLR-deficient rabbits (22, 23), suggesting that the LDLR plays a direct role in the chylomicron remnant metabolism.

Recently, functions of either the LDLR (25) or apoE (12, 13) have been ablated in mice by means of homologous recombination. We compared the impact of each protein on the metabolism of chylomicron remnants in these mice. The plasma levels of apoB-100 were markedly elevated in the LDLR(−/−) mice, whereas they were not increased in the apoE(−/−) mice. The double mutant mice lacking both the LDLR and apoE showed elevation of the plasma level of apoB-100, indicating that the lipoproteins containing apoB-100 were cleared mainly by the LDLR, and this process is independent from apoE (26). The high density lipoproteins; R, receptors; LRP, low density lipoprotein receptor related protein; α2 M, α2-macroglobulin; RAP, receptor-associated protein; FPLC, fast performance liquid chromatography; AUC, area under the curve.
**Low Density Lipoprotein Receptor in Chylomicron Metabolism**

**FIG. 1.** [3H]Retinyl ester excursion in the d < 1.019 g/ml lipoprotein fractions. After overnight fasting, 200 µl of corn oil supplemented with 0.2 Bq of [3H]retinol was administered to 4–5 male wild-type, 5 male LDLR(−/−), 4 male apoE(−/−), and 5 male apoE(−/−); LDLR(−/−) mice without anesthesia. The blood was sampled from the retro-orbital sinus. Plasma was mixed with 0.07 g of KBr and 0.5 ml of saline, and 1 ml of 4% corn oil supplemented with 0.2 Bq of [11,12-3H]retinol and carrier-free [125I] iodide were obtained through the iodine monochloride method as described (29). The iodinated lipoproteins were used within 24 h after preparation.

**Preparation of Lipoproteins**—Male Sprague-Dawley rats weighing 250–300 g were anesthetized with pentobarbital. 45 min before surgery, 2 ml of corn oil containing 2% (w/v) cholesterol was given orally. Mesenteric lymph fistulas were prepared and intestinal lymph was drained overnight into a tube containing EDTA and NaN3 (15). Chylomicron was separated by centrifugation at 1.1 × 10^6 rpm × min (31,000 rpm × 45 min at 20 °C in Beckman SW41 rotor). Chylomicron remnants were prepared by a modified method of Redgrave and Martin (29), using functionally lipolyzed rats. The chylomicron remnants were isolated by ultracentrifugation at 39,000 rpm for 2 h at 20 °C in a Beckman SW41 rotor.

Radiiodination of lipoproteins was carried out by the iodine monochloride method as described (29). The iodinated lipoproteins were used within 24 h after preparation.

**Plasma Turnover Experiments**—Mice were injected intravenously with 125I-rat chylomicron remnants in 0.2 ml of phosphate-buffered saline containing 2 mg/ml bovine serum albumin via jugular vein. Blood was sampled at various times. The apoB-associated radioactivities were measured as described (25). The amount of tracers remaining in plasma was expressed as a percentage of the calculated initial blood concentration, assuming that plasma volume is 4.4% (v/w) of body weight. Others—Triglycerides and glycerol were determined enzymatically with assay kits obtained from Sigma.

**RESULTS**

Fig. 1 compares retinyl ester excursion curves in the mice of four different genotypes. Peaks of the excursions were reached at 4 h after injection and were identical in all four different genotypes. In wild-type mice, after reaching a peak, the value declined to negligible levels at 12 h after injection. The peak values and the area under the curve (AUC) in each mouse are summarized in Table I.

In the LDLR(−/−) mice, the peak values were 4 times larger than in wild-type mice. The AUC in the mice was also 4 times larger than in wild-type mice, indicating that the non-LDLR pathway is responsible for one-fourth of the overall clearance of retinyl ester. Thus, the significance of the LDLR pathway is 3 times larger than the non-LDLR pathway. Both the apoE(−/−) and the apoE(−/−); LDLR(−/−) double mutant mice showed more exaggerated and similar excursions. Since the two excursion curves were nearly superimposable, the contribution of the LDLR pathway to the apoE-independent clearance should be negligible. In both mutant mice, the peak values and the AUC were 7 and 12 times larger than those in wild-type mice, respectively, and were 2 and 3 times larger than those in the LDLR(−/−) mice, respectively. ApoE-independent

LDLR(−/−) mice exhibited a modest increase in the plasma level of apoB-48, in contrast to the apoE(−/−) mice that showed profound elevation of the plasma level of apoB-48, supporting the hypothesis that some additional lipoprotein receptors recognize apoE on chylomicron remnants. But these additional receptors possess only limited capacity for endocytosis of chylomicron remnants, because feeding the LDLR(−/−) mice with a high fat diet caused a profound increase in the plasma level of apoB-48 to a degree comparable with that of the apoE(−/−) mice (26).

In the present study, we have employed vitamin A fat tolerance tests to analyze the chylomicron metabolism more quantitatively in the knockout mice. The results further supported the hypothesis that at least two receptor pathways are involved in chylomicron metabolism.

**MATERIALS AND METHODS**

**Animals**—LDLR knockout mice, LDLR(−/−) (25), and apoE knockout mice, apoE(−/−) (12), were created by targeted disruption of each gene by homologous recombination as described in the indicated references. The double knockout mice homozygous for both LDLR and apoE mutant alleles (apoE(−/−); LDLR(−/−)) were generated by mating as described (26). These mice or their offspring were matched with regard to generation and obligate hybrids between C57Bl/6J and 129Sv strains. They were maintained on 12-h dark/12-h light cycles and were allowed access to food and water ad libitum. A normal chow diet (Teklad 4% mouse/rat diet 7001 from Harlan Teklad Premier Laboratory Diets, Madison, WI) was used.

**Reagents**—[11,12-3H]Retinol and carrier-free [125I] iodide were obtained from DuPont NEN. Universal Gel8 of electrophoresis systems was obtained from Ciba Corning Diagnostics Corp.

**Vitamin A Fat Tolerance Test**—1 MBq of [3H]retinol in ethanol was mixed with 1 ml of corn oil. 200 µl of the mixture was given as a bolus dose into the stomach. The blood was sampled from the retro-orbital venous plexus into tubes containing EDTA. 20 µl of the plasma was mixed with saline, and its density was adjusted to 1.019 g/ml by a potassium bromide (KBr) and centrifuged at 90,000 rpm at 20 °C for 2 h in a Beckman TL100 rotor. The upper portions of the tubes were sliced by a blade, and the radioactivity in the resulting top fractions was measured by a β-counter. More than 95% of the radioactivities was shown to be present as esterified form.

Subfractionation of the lipoproteins was carried out according to a modified method of Redgrave and Carlson (27). In brief, 0.25 ml of plasma was mixed with 0.07 g of KBr and 0.5 ml of saline, and 1 ml of d = 1.10 g/ml KBr solution was added in a centrifuge tube to which 1.2 ml d = 1.063 g/ml, 1.2 ml of d = 1.019 g/ml, and 1.36 ml of d = 1.006 g/ml KBr solutions were overlaid and centrifuged at 30,000 rpm for 19 min (chylomicron), at 30,000 rpm for 36 min (VLDL-A), at 40,000 rpm for 90 min (VLDL-B), 40,000 rpm for 95 min (VLDL-C), and 40,000 rpm for 845 min (VLDL-D) sequentially at 20 °C. 0.5 ml of the top layer was taken between each centrifugation, IDL and LDL fractions were obtained by further tube slicing, and 200 µl was used for scintillation counting.

In a separate experiment, a bolus of corn oil without [3H]retinol was given into the stomach of the mice. Blood was collected sequentially, and the pooled plasma was subjected to sequential ultracentrifugation as described above. After the subfractionation of lipoproteins, 10 µl of each lipoprotein fraction was subjected to agarose gel electrophoresis using a buffer containing 50 mM barbital and 1 mM EDTA (pH 8.6). Lipids were visualized with Fat Red 7B.

The distribution of the radioactivities over the lipoproteins was analyzed by fast performance liquid chromatography (FPLC) as described (25).

The changes in [3H]retinol in the d < 1.019 g/ml lipoproteins were analyzed by fast performance liquid chromatography (FPLC) as described (25). The results further supported the hypothesis that some additional lipoprotein receptors recognize apoE on chylomicron remnants. But these additional receptors possess only limited capacity for endocytosis of chylomicron remnants, because feeding the LDLR(−/−) mice with a high fat diet caused a profound increase in the plasma level of apoB-48 to a degree comparable with that of the apoE(−/−) mice (26).

In the present study, we have employed vitamin A fat tolerance tests to analyze the chylomicron metabolism more quantitatively in the knockout mice. The results further supported the hypothesis that at least two receptor pathways are involved in chylomicron metabolism.
cle size. 6 h after a bolus of corn oil supplemented with the origin of the gels after electrophoresis. These results indicate that the clearance of chylomicron was delayed in the large lipoproteins, we employed FPLC to see the distribution of [3H]retinyl ester in the LDL fraction was not derived from resecreted hepatogenous VLDL.

Note that these values include both esterified and free retinol. Activities in the plasma of the wild-type and III compares the mean values for the excursions of total radioactivities in the plasma of the wild-type and LDL, apoE-dependent/non-LDLR, and apoE-independent/non-LDLR pathways, respectively. y and n denote yes and no, respectively.

| Ablated genes | +/+ | −/− | +/+ | −/− |
|---------------|-----|-----|-----|-----|
| LDLR | y | n | n | n |
| ApoE | y | y | n | n |
| Available pathways | LDLR/apoE | y | y | y | y |
| | LDLR/ApoE | y | n | n | n |
| | LDLR/ApoE | y | y | n | n |
| | LDLR/ApoE | y | y | y | y |
| Peak | 5.4 ± 1.6 | 21.9 ± 5.8 | 40.0 ± 11.7 | 38.4 ± 8.7 |
| AUC | 10.9 ± 3.1 | 44.7 ± 14.5 | 131.5 ± 36.2 | 126.6 ± 21.6 |
| % Clearance | 100 | 24.4 ± 7.6 | 8.1 ± 2.2 | 8.2 ± 1.6 |

a AUC, area under curve.
b The clearance as indicated in percent of the value for the wild-type mice, which is calculated from inverse of AUC. The results are expressed as mean ± S.D. Unit for peak and AUC is thousand dpm. One-way analysis of variance reveals that the values are significantly different between every pair of the genotype both in the peak values and the AUC, except between apoE(−/−) versus apoE(−/−)/LDLR(−/−) at p < 0.05.

Table I

Plasma triglyceride levels after corn oil feeding

200 μl of corn oil was given to three female wild-type and three female LDLR(−/−) mice via gastric tubing after anesthesia with pentobarbital. The triglyceride levels were determined enzymatically. Mean age and body weight of the mice at the time of the experiment were 21.1 weeks and 27.4 g, respectively. The values are mean ± S.D.

| Time (h) | 0 | 1 | 2 | 4 | 6 |
|----------|---|---|---|---|---|
| Wild-type | 56 ± 7 | 64 ± 27 | 69 ± 27 | 67 ± 17 | 49 ± 3 |
| LDLR(−/−) | 86 ± 12∗ | 85 ± 14 | 128 ± 23∗ | 130 ± 21b | 133 ± 18a |

∗ p < 0.05, compared with wild-type mice.
b p < 0.01, compared with wild-type mice.

Fig. 4 compares agarose gel electrophoretic patterns of each lipoprotein fraction separated by sequential ultracentrifugation in the four groups of mice fed a normal chow. Although the volume of each fraction was 0.5 ml in chylomicron through VLDL-D, 1 ml in IDL, 1.2 ml in LDL, and 3 ml in HDL, the same volume (10 μl) was applied to the gel. Thus, it should be taken into account that IDL, LDL, and HDL were 2, 2.5, and 6-fold underrepresented compared with larger lipoprotein fractions, chylomicron through VLDL-D. In agreement with the FPLC profile (25), HDL was the major lipoprotein fraction in the wild-type mice. In the LDLR(−/−) mice, non-HDL lipoproteins were distributed from VLDL-D to LDL, mainly in LDL and IDL. The LDL had β mobility and the VLDL-D had pre-β mobility. The electrophoretic mobility of the IDL was between β and pre-β. In contrast, the lipoproteins of the apoE(−/−) mice were distributed only in the larger subfractions, chylomicron through VLDL-C. The mobility of the chylomicron, VLDL-A, LDL-B, and LDL-C, 2, 2.5, and 6-fold respectively.

Interestingly, chylomicron that was ultracentrifugally isolated from either the apoE(−/−) or LDLR(−/−)/apoE(−/−) mice contained appreciable amounts of lipids in the VLDL-D and IDL, in addition to the lipoproteins present in the apoE(−/−) mice. The LDL(−/−) mice contained the lipoproteins that remained in the origin of the gels after electrophoresis. These results indicate that the clearance of chylomicron was delayed in the large lipoproteins.

Table II

Available pathways

The absolute levels of triglycerides in plasma were measured successively after fat load in wild-type and the LDLR(−/−) mice in a separate experiment. In wild-type mice, the plasma levels of triglycerides rose only slightly, whereas in the LDLR(−/−) mice, the levels were increased up to 50% higher than the base line, as shown in Table II.

To estimate the changes in size of the accumulated lipoproteins after vitamin A fat loading, we undertook subfractionation of lipoproteins by sequential ultracentrifugation. Table III compares the mean values for the excursions of total radioactivities in the plasma of the wild-type and LDLR(−/−) mice. Note that these values include both esterified and free retinol. Nevertheless, the significant delay of the clearance was observed in the LDLR(−/−) mice. The equal volumes of each plasma were mixed and subjected to sequential ultracentrifugation. In wild-type mice, all subclasses of the lipoproteins showed rapid turnover as shown in Fig. 2. Peak time of chylomicron, VLDL-A, and VLDL-D was 2 h, that of VLDL-B and VLDL-C was 4 h, and that of IDL and LDL was 6 h after the load. In the LDLR(−/−) mice, the peak time of large lipoproteins (d < 1.019 g/ml) was shifted to 8 h (Fig. 2). The AUC of the excursions in the LDLR(−/−) mice was significantly increased in larger lipoproteins (chylomicron, 9.3; VLDL-A, 11.4; VLDL-B, 15.1; VLDL-C, 14.0; VLDL-D, 6.5; IDL, 2.3; and LDL, 0.96-fold). Surprisingly, there was no difference in the excursions of LDL between wild-type and the LDLR(−/−) mice (Fig. 2, LDL panel), indicating that [3H]retinyl ester in the LDL fraction was not derived from resecreted hepatogenous VLDL as was observed in dogs (30) and that transfer of [3H]retinyl ester in chylomicron to LDL is negligible in mice. Comparable amounts of fat and retinol should have been absorbed because the excursions of the radioactivities in the d > 1.063 g/ml fraction were similar (Fig. 2, last panel).

To confirm that the majority of the retinyl ester is distributed in large lipoproteins, we employed FPLC to see the distribution of [3H]retinol over the full range of lipoprotein particle size. 6 h after a bolus of corn oil supplemented with [3H]retinol was given to the LDLR(−/−) mice, the plasma was taken and subjected to FPLC. As shown in Fig. 3, 66% of the whole radioactivities were eluted in the fractions corresponding to chylomicron/VLDL, 16% in LDL, 3% in HDL, and 15% in lipoprotein-free.
Oil supplemented with 0.2 MBq of [3H]retinol was administered by gavage; female mice were anesthetized by pentobarbital, and 200 μl gastric intubation to wild-type (LDLR−/−) mice were subjected to FPLC analysis. The mean age and body weight of the mice at the time of the experiment were 21.5 weeks and 24.1 g, respectively. The mice died after 1 h of anesthesia and was excluded from the results. The radioactivities in 200 μl of plasma from each mouse were measured. The values are mean ± S.D.

**DISCUSSION**

As to the role of the LDLR in the catabolism of intestinally derived lipoproteins, conflicting results have been reported. In LDLR-deficient humans, delay of the plasma clearance of retinyl ester was not demonstrated at least in the initial 5 h after the vitamin A fat load (16). In contrast, the experiments using Watanabe heritable hyperlipidemic rabbits showed significant delay of the clearance of retinyl ester in the plasma; the peak levels were 5–15-fold higher than in New Zealand White rabbits (22, 23). Since substantial amounts of retinyl ester were shown to transfer from large chylomicron to smaller LDL fraction in rabbits, the apparent delay in the catabolism of retinyl ester was explained by the lipid transfer activities in these animals (24). Our experiments using the LDLR−/− mice also demonstrated a similar delay of the clearance of retinyl ester in the plasma. As shown in Fig. 2, however, the results of lipoprotein subfractionation indicate that there was no accumulation of retinyl ester in the LDL fraction of the LDLR−/− mice. Thus, it is unlikely that either direct lipolytic conversion of chylomicron to LDL or transfer of estinyl ester from chylomicron to LDL caused the delay in the clearance of retinyl ester. Instead, the data should be interpreted simply as indicating that the LDLR is significantly involved in the chylomicron remnant metabolism. The LDLR activities in humans might be lower than those in the rodents (31), which is conceivably responsible for the species difference in the response to vitamin A load.

Pathways for chylomicron remnant clearance can be classified into four groups according to the involvement of either apoE or the LDLR: apoE-dependent/LDLR, apoE-dependent/non-LDLR, apoE-independent/LDLR, and apoE-independent/non-LDLR pathways. Since the retinyl ester excursion curve in the apoE−/−;LDLR−/− mice was almost identical to that in the apoE−/− mice (Fig. 1), it is safely concluded that all the LDLR-dependent pathways should be also entirely dependent on apoE. In other words, there is virtually no apoE-independent/LDLR pathway for chylomicron remnant clearance. This is consistent with the notion that chylomicron remnants bind to the LDLR exclusively through apoE. The AUC of the retinyl ester excursion in the apoE−/− mice was 3 times larger than in the LDLR−/− mice, indicating the existence of an apoE-dependent/non-LDLR pathway for the chylomicron remnant removal, which is also known as “chylomicron remnant receptor(s).”

Our estimation on the contribution of the LDLR to the overall clearance of chylomicron remnants is 75%, based on vitamin A tolerance tests, and is largely consistent with the results of a study in which the amounts of the injected chylomicron remnants in the isolated endosomes were compared between the LDLR−/− and wild-type mice (32). The accumulation of en-

**TABLE III**

| Time (h) | 2     | 4     | 6     | 8     | 10    | 12    |
|---------|-------|-------|-------|-------|-------|-------|
| dpm     |       |       |       |       |       |       |
| Wild-type | 921 ± 298 | 1596 ± 635 | 1685 ± 480 | 1432 ± 325 | 1158 ± 266 | 1026 ± 311 |
| LDLR−/− | 943 ± 438 | 1901 ± 883 | 3371 ± 1407 | 3464 ± 1431a | 3353 ± 1340a | 2480 ± 485a |

* a p < 0.05, compared with wild-type mice.
dosomal chylomicron remnants in the liver was almost abolished in the \(LDLR(-/-)\) mice, whereas it was readily demonstrated in wild-type mice. The slow uptake of chylomicron remnants, which was observed even in the \(LDLR(-/-)\) mice, was further inhibited by the intravenous injection of 39-kDa receptor-associated protein (RAP). It appears that this non-LDLR pathway that is sensitive to RAP corresponds to the apoE-dependent/non-LDLR in our experiments.

Similarly, the results of agarose gel electrophoresis also support the notion that the clearance of chylomicron is blocked in the \(LDLR(-/-)\) mice as shown in Fig. 5.

Although the proportion of the apoE-dependent/non-LDLR pathway in the overall metabolism of chylomicron remnants is only \(1/6\), chylomicron remnants do not accumulate in the plasma of the LDLR-deficient humans and animals fed a normal chow (14, 15, 25). They begin to accumulate only when the function of the apoE-dependent/non-LDLR pathway is blocked either by RAP (33) or by depletion of apoE (10–13), indicating that the apoE-dependent/non-LDLR pathway serves as a backup system for chylomicron clearance and is efficient enough as long as it is not overloaded by dietary fats.

However, the difference in the clearance of \(^{125}\)I-rat chylomicron remnants between wild-type and the \(LDLR(-/-)\) mice fed a normal chow were marginal, 5–10% (Fig. 6). Similar differences were reported by Choi et al. (20) and Jackle et al. (21), who used anti-LDLR antibody to distinguish the LDLR pathway from the non-LDLR pathway. Likewise, large VLDL was reported to rapidly disappear from the plasma of LDLR-deficient rabbits (34). Apparently, these results are not in agreement with our data of the vitamin A fat tolerance tests. This disagreement might arise from inappropriate interpretation of the tracer turnover studies. Tracer studies are valid, only if the tracee is in a steady state where synthesis and catabolism are in equilibrium (35). Chylomicron remnants ap-

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**FIG. 4.** Agarose gel electrophoresis of the lipoprotein fractions from the mice fed a normal chow. Blood was collected from the wild-type, \(LDLR(-/-)\), apoE\((-/-)\) and apoE\((-/-);LDLR(-/-)\) mice fed a normal chow \((n = 3)\). Equal volume of the plasma from three mice of each genotype was mixed, and 25 \(\mu\)l of it was used for the ultracentrifugation. 10 \(\mu\)l of each lipoprotein fraction of chylomicron \(Chylo\) through HDL was subjected to agarose gel electrophoresis. The gels stained with Fat Red 7B were shown.

**FIG. 5.** Agarose gel electrophoresis of the lipoprotein fractions after the fat load. 200 \(\mu\)l of corn oil was given to the stomach of the wild-type, \(LDLR(-/-)\), apoE\(-/-\) mice \((n = 3)\). Mean age and mean body weight of the mice at the time of the experiment was 16 weeks and 22 g, respectively. Blood was collected 4 and 8 h after the fat load. Pooled plasma was subjected to sequential ultracentrifugation. Equal volume of the plasma from three mice of each genotype was mixed, and 250 \(\mu\)l of the pooled plasma was used for the ultracentrifugation. 10 \(\mu\)l of each lipoprotein fraction of chylomicron \(Chylo\) through VLDL-D was subjected to agarose gel electrophoresis. The gels stained with Fat Red 7B were shown.
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