Upregulation of liver VLDL receptor and FAT/CD36 expression in LDLR$^{-/-}$ apoB$^{100/100}$ mice fed *trans*-10,*cis*-12 conjugated linoleic acid[1]

Pascal Degrace,[2,*] Bastien Moindrot,[*] Ismaël Mohamed,[*] Joseph Gresti,[*] Zhen-Yu Du,[*] Jean-Michel Chardigny,[†] Jean-Louis Sébédio,[†] and Pierre Clouet[*]

Unité Propre de Recherche de l'Enseignement Supérieur, Lipides et Nutrition EA2422,* Faculté des Sciences Gabriel, Université de Bourgogne, 21000 Dijon, France; and Institut National de la Recherche Agronomique-Université d'Auvergne,† Unité du Métabolisme Protéino-Energétique, 63000 Clermont-Ferrand, France

Abstract This study explores the mechanisms responsible for the fatty liver setup in mice fed *trans*-10,*cis*-12 conjugated linoleic acid (t10c12 CLA), hypothesizing that an induction of low density lipoprotein receptor (LDLR) expression is associated with lipid accumulation. To this end, the effects of t10c12 CLA treatment on lipid parameters, serum lipoproteins, and expression of liver lipid receptors were measured in LDLR$^{-/-}$ apoB$^{100/100}$ mice as a model of human familial hypercholesterolemia itself depleted of LDLR. Mice were fed t10c12 CLA over 2 or 4 weeks. We first observed that the treatment induced liver steatosis, even in the absence of LDLR. Mice treated for 2 weeks exhibited hypertriglyceridemia with high levels of VLDL and HDL, whereas a 4 week treatment inversely induced a reduction of serum triglycerides (TGs), essentially through a decrease in VLDL levels. In the absence of LDLR, the mRNA levels of other proteins, such as VLDL receptor, lipoprotein lipase, and fatty acid translocase, usually not expressed in the liver, were upregulated, suggesting their involvement in the steatosis setup and lipoprotein clearance. The data also suggest that the TG-lowering effect induced by t10c12 CLA treatment was attributable to both the reduction of circulating free fatty acids in response to the severe lipoatrophy and the high capacity of liver to clear off plasma lipids.—Degrace, P., B. Moindrot, I. Mohamed, J. Gresti, Z-Y. Du, J-M. Chardigny, J-L. Sébédio, and P. Clouet. **Upregulation of liver VLDL receptor and FAT/CD36 expression in LDLR$^{-/-}$ apoB$^{100/100}$ mice fed *trans*-10,*cis*-12 conjugated linoleic acid. *J. Lipid Res.* 2006.** 47: **2647–2655.**

Supplementary key words liver steatosis • low density lipoprotein receptor • triglyceride • lipoatrophy • lipoprotein • fatty acid translocase • very low density lipoprotein receptor • hepatic lipase • lipoprotein lipase • low density lipoprotein receptor-related protein • scavenger receptor class B type I

Conjugated linoleic acids (CLAs) refer to a group of dienoic derivatives of linoleic acid. In most feeding studies, CLAs are mainly represented by *cis*-9,*trans*-11-$C_{18:2}$, the main natural isomer produced in ruminants, and by *trans*-10,*cis*-12-$C_{18:2}$ (t10c12 CLA) essentially originating from vegetable oil processes. These isomers of linoleic acid have been shown to exhibit a variety of unique properties such as anticancer (1), antiatherogenic (2), and immune response-enhancing (3) effects in animal models. CLAs have also been reported to reduce total body fat content in mice, rats, and chickens (4–6). The C57Bl6 mouse is a model largely used to study the biological effects of CLA, to which this strain is very sensitive, and in particular to t10c12 CLA, which was identified recently as the isomer affecting body lipid metabolism (7, 8). After a 4 week treatment with t10c12 CLA, C57Bl6 mice exhibit severe lipoatrophy, steatotic liver, hyperinsulinemia, and plasma triglyceride (TG) alteration (7, 9). Thus, t10c12 CLA-fed mice constitute an interesting model for the study of steatosis onset in relation to lipid metabolism dysfunctions, nonalcoholic fatty liver disease now being recognized as one of the common features of the metabolic syndrome, with visceral fat obesity, insulin resistance, dyslipidemia, and hypertension (10). In a previous work, we suggested that a high uptake of plasma lipids by the liver would explain part of the TG accumulation in this organ after t10c12 CLA feeding (7). As

Abbreviations: apoB-100, apolipoprotein B-100; CPT I, carnitine palmitoyltransferase I; FAT/CD36, fatty acid translocase; LDLR, low density lipoprotein receptor; LRP, low density lipoprotein receptor-related protein; SR-BI, scavenger receptor class B type I; t10c12 CLA, *trans*-10,*cis*-12 conjugated linoleic acid; TG, triglyceride; VLDLR, very low density lipoprotein receptor.

[1] The paper is dedicated to the memory of Ismaël Mohamed, PhD student, who died on March 5, 2005 before the end of this work.

[2] To whom correspondence should be addressed.
e-mail: pascal.degrace@u-bourgogne.fr

Manuscript received 24 March 2006 and in revised form 5 July 2006 and in re-revised form 6 September 2006.

Published, JLR Papers in Press, September 6, 2006.
DOI 10.1194/jlr.M600140-JLR200

the overexpression of hepatic low density lipoprotein receptor (LDLR) was demonstrated to increase the clearance of apolipoprotein B-100 (apoB-100)-containing lipoproteins in mice (11), and as CLA treatment was found to induce the expression of LDLR (7), liver steatosis onset could depend, at least in part, on this lipoprotein receptor. Therefore, in this study, $LDLR^{-/-}$ $apoB^{100/100}$ mice, which represent a good model of human familial hypercholesterolemia (12), were fed t10c12 CLA to induce lipoatrophy to study the consequences on liver and plasma lipid parameters. We particularly focused on the hepatic effects of both the high lipid flux originating from adipose tissue attributable to t10c12 CLA action and on the absence of LDLR in this dyslipidemic model with regard to the expression of other lipid transporters. To address this issue, we also determined the gene expression profile of untreated $LDLR^{-/-}$ $apoB^{100/100}$ mice compared with normal wild-type animals.

Our results show, first, that LDLR deficiency was unable to prevent the steatosis induced by t10c12 CLA and, second, that other proteins substitute for LDLR in lipoprotein clearance to such an extent that serum TG levels were significantly reduced in these mice usually exhibiting high levels of circulating apoB-100-rich lipoproteins.

MATERIALS AND METHODS

Animals and treatments

Official French regulations (No. 87848) for the use and care of laboratory animals were followed throughout. Control (B6129SF2) and transgenic (B6; 129S-Apobtm2SgyLdlrtm1Her) mice originated from the Jackson Laboratory. Transgenic mice are deficient in LDLR and express only apoB100 ($LDLR^{-/-}$ $apoB^{100/100}$ mice). After 1 week of adaptation to the control diet (AO4; Unité d'Appui à la Recherche, Epinay-sur-Orge, France), 7 week old male mice were housed in individual plastic cages. $LDLR^{-/-}$ $apoB^{100/100}$ mice were randomly allocated to the control or the CLA diet (n = 5 for each), consisting of a basal diet, whose detailed composition has been described (13), enriched with 1% $C_{18:1\ n\text{-}9}$ (oleic acid) or t10c12 CLA, both esterified as TG. CLA-fed $LDLR^{-/-}$ $apoB^{100/100}$ mice and their corresponding controls were euthanized after 2 or 4 weeks. Wild-type B6129SF2 mice (n = 5), fed only the control diet, were used after 4 weeks. Mice were food-deprived for 4 h before anesthetized with ketamine/xylazine (7.5 mg/100 g body weight) and euthanized. For lipid analysis, liver, heart, gastrocnemius, and blood (collected from the vena cava) were stored at −80°C. Fresh samples of liver were used for immediate FA oxidation measurements on whole liver homogenates and isolated mitochondria.

Lipid analysis

Total liver, muscle, and heart lipids were extracted according to Folch, Lees, and Sloane Stanley (14). For liver, total lipids were determined by gravimetry and lipid classes were quantified by the TLC-flame ionization detection method (15). Phospholipids, cholesteryl esters, and TG were separated by TLC on silica plates (Merck, Darmstadt, Germany). Their constitutive FAs were methylated according to the procedure of Christie, Sebedio, and Juaneda (16) and analyzed by gas-liquid chromatography as described previously (17). For skeletal muscle and heart, aliquots of total lipid extracts were resuspended in a solution of Triton X-100 as described previously (18). Then, TG contents were measured using a commercial kit from Roche Diagnostics Corp. (Indianapolis, IN). Commercial kits were also used for the determination of serum TG and glycerol concentrations (Sigma Diagnostics, Saint-Quentin-Fallavier, France) and of serum free FA (Roche Diagnostics Corp.).

Serum lipoprotein analysis

Serum lipoprotein analysis was performed by fast-performance liquid chromatography, and total cholesterol was quantified with an inline detection system as described previously (19).

Liver lipolytic activity

The procedure used was adapted from that of Iverius and Ostlund-Lindqvist (20). Lipolytic activity determined on tissue homogenates corresponding to amounts of [^{3}H]oleic acid released from radiolabeled triolein as described previously (7).

Carnitine palmitoyltransferase I activity and palmitate oxidation rate

Measurements of carnitine palmitoyltransferase I (CPT I) activity and palmitate oxidation rates were performed as described previously (13). FA oxidation was measured with whole liver homogenates using two media, the first allowing mitochondrial and peroxisomal activities to occur, the second allowing the peroxisomal activity only (21), and with liver mitochondrial fractions. Protein concentrations of mitochondrial fractions were measured using the bicinchoninic acid procedure (Sigma) (22).

Western blot analysis of the very low density lipoprotein receptor

Approximately 100 mg of frozen liver was quickly homogenized with a mini-beadbeater (BioSpec Products, Inc., Bartlesville, OK) in 10 volumes of a 20 mM Tris buffer containing sucrose (0.2 M), $MgCl_2$ (2 mM), pepstatin A (1.46 μM), leupeptin (10 μM), aprotinin (0.035 TIU/l), and E64 (1.4 μM). After centrifugation of homogenates at 12,000 *g* for10 min, supernatants were half-diluted in Laemmli buffer (Bio-Rad S.A., Ivry-sur-Seine, France) (23) without boiling, and aliquots were size-fractionated on a 7% SDS-polyacrylamide gel using a Mini-Protean 3 electrophoresis cell (Bio-Rad) at 200 V for ~70 min at room temperature. After electrophoresis, proteins were transferred to a nitrocellulose membrane (Hybond-ECL; Amersham Biosciences, Saclay, France) at 140 V for 1.5 h. For immunodetection, the blots were incubated overnight in TBST [10 mM Tris, 0.15 M NaCl, and 0.05% (v/v) Tween-20] plus 5% (w/v) BSA, for 1 h in TBST, 2% BSA, plus 0.2 μg/ml of a goat anti-mouse VLDL receptor antibody (R&D Systems, Abingdon, UK), and then for 1 h in TBST, 2% BSA, plus a 1:10,000 dilution of rabbit anti-goat IgG peroxidase conjugate antibody (Sigma). The blots were developed with chemiluminescent reagents (ECL; Amersham Biosciences) and subjected to autoradiography. The membrane was stripped using Restore Western Blot Stripping Buffer (Pierce, Rockford, IL) and reprobed in the same conditions with a mouse anti-β-actin antibody and an anti-mouse IgG peroxidase conjugate antibody (Sigma) for standardization. Spot intensities were determined by densitometric analysis with a gel documentation system (Gel Doc 2000) equipped with Quantity One software (Bio-Rad).

Protein concentrations of supernatants were measured by the bicinchoninic acid procedure after trichloroacetic acid precipitation to eliminate incompatible substances.

Gene expression

Total mRNA was extracted from liver by the Tri-Reagent method adapted from the procedure of Chomczynski and Sacchi (24). Tri-Reagent was provided by Euromedex (Souffelweyersheim, France). Total mRNA were reverse-transcripted using the Iscript cDNA kit (Bio-Rad). Real-time PCR was performed as described previously (19). Primer pairs were designed using Primers! software and were synthesized by MWG-Biotech AG (Ebersberg, Germany). The sequences of the forward and reverse primers used are as follows: 5′-aattagtagaaccgggccac-3′ and 5′-ccaactcccaggtacaatca-3′, respectively, for fatty acid translocase (FAT/CD36); 5′-ctaaggacccctgaagacaca-3′ and 5′-tctcatacattcccgttaccgt-3′ for LPL; 5′-gtgaatgtggggttagtggac-3′ and 5′-acttcgcagattcctccagc-3′ for HL; 5′-gaccgactggcgaacaaat-3′ and 5′-ctgggtgttggtcctctgta-3′ for low density lipoprotein receptor-related protein (LRP); 5′-agcaccacagatcaatgacc-3′ and 5′-ctctcgtccatttttcttcgaga-3′ for very low density lipoprotein receptor (VLDLR); 5′-tcccttcgtgcattttctca-3′ and 5′-gttcatcccaacaaacagg-3′ for scavenger receptor class B type I (SR-BI); and 5′-aatcgtgcgtgacatcaaag-3′ and 5′-gaaaagagcctcagggcat-3′ for β-actin.

Statistics

Differences in mean values between groups were tested by one-way ANOVA. Significant differences between means were tested by Student's *t*-test for an independent variable. When variances were unequal, means were tested by the Kruskal-Wallis nonparametric test.

RESULTS

Effects of t10c12 CLA feeding on body, liver, and serum parameters in LDLR$^{-/-}$ apoB$^{100/100}$ mice

Table 1 shows that dietary t10c12 CLA did not affect body weights of LDLR$^{-/-}$ apoB$^{100/100}$ mice for the two durations of treatment. The drastic reductions of epididymal adipose tissue weights and concomitant liver steatosis usually found in wild-type mice fed t10c12 CLA were also observed in the transgenic model. After 2 weeks of CLA feeding, adipose tissue and liver relative weights were already markedly altered (−62% and +56%, respectively), these effects being even more pronounced after 4 weeks (−82% and +97%, respectively) (Table 1). Liver TG content increased with treatment duration, and the TG enrichment found in mice fed t10c12 CLA for 4 weeks was even greater than that measured under the same experimental conditions with wild-type mice [13-fold vs. 7.5-fold (7), respectively]. It is worth noting that the t10c12 CLA treatment also increased liver cholesteryl ester contents but did not affect free cholesterol contents. To determine whether lipid accumulation occurred in tissues other than liver, the TG contents of heart and skeletal muscle were measured. Unlike the liver, heart and muscle did not accumulate TG in response to CLA feeding, with muscle TG levels actually being reduced by 6-fold.

In LDLR$^{-/-}$ apoB$^{100/100}$ mice, as serum lipid parameters are modified with aging, data from control and CLA-fed mice were compared for the same treatment duration. Indeed, Table 1 shows that the effect of t10c12 CLA on serum TG levels was dependent on the duration of treatment, because TGs were increased after 2 weeks and decreased after 4 weeks, relative to the control series. Levels of total cholesterol, free FA, or glycerol in serum were unaltered after 2 weeks of treatment but were decreased significantly when t10c12 CLA was administered for 4 weeks.

Lipoprotein profile analysis (**Fig. 1**) indicates that VLDL-cholesterol and HDL-cholesterol levels were increased in the serum of mice fed t10c12 CLA for 2 weeks (+165% and +22%, respectively), whereas LDL-cholesterol was decreased slightly, but not significantly. Additionally, when mice were fed for 4 weeks, cholesterol levels were decreased in all fractions, particularly in apoB-100 lipoproteins (VLDL-cholesterol, −78%; LDL-cholesterol, −41%; HDL-cholesterol, −26%, relative to controls).

TABLE 1. Body, liver, and serum parameters in LDLR$^{-/-}$ apoB$^{100/100}$ mice fed t10c12 CLA for 2 or 4 weeks

	2 Weeks		4 Weeks	
Parameter	Control	t10c12 CLA	Control	t10c12 CLA
Body weight (g)				
Organ wet weight (% body weight)	24.2 ± 0.74	23.51 ± 0.39	26.39 ± 0.70	25.17 ± 0.89
Liver	5.25 ± 0.28	8.19 ± 0.23[a]	5.09 ± 0.19	10.06 ± 0.52[b]
Periepidymal adipose tissue	1.32 ± 0.09	0.50 ± 0.07[a]	1.46 ± 0.15	0.26 ± 0.02[b]
Liver lipids (mg/g)				
TGs	21.5 ± 1.2	97.1 ± 9.8[a]	16.5 ± 5.1	215.8 ± 9.3[b]
Cholesteryl esters	3.1 ± 0.7	6.4 ± 1.2[a]	2.1 ± 0.4	4.8 ± 0.8[b]
Free cholesterol	1.4 ± 0.3	1.5 ± 0.2	1.3 ± 0.1	1.4 ± 0.1
Heart TGs (mg/g)	—	—	3.2 ± 0.6	3.7 ± 0.7
Muscle TGs (mg/g)	—	—	11.4 ± 1.4	1.9 ± 0.4[b]
Serum parameters (mg/ml)				
TGs	2.43 ± 0.21	4.04 ± 0.41[a]	7.45 ± 1.45	2.84 ± 0.17[b]
Total cholesterol	4.44 ± 0.26	4.50 ± 0.31	8.48 ± 0.78	4.89 ± 0.22[b]
Free fatty acids	0.20 ± 0.03	0.23 ± 0.02	0.33 ± 0.04	0.25 ± 0.02[b]
Glycerol	0.34 ± 0.02	0.30 ± 0.02	0.44 ± 0.01	0.36 ± 0.04[b]

t10c12 CLA, *trans*-10,*cis*-12 conjugated linoleic acid; TG, triglyceride. Results are expressed as means ± SEM (n = 5).

[a] Significant difference at $P < 0.05$, t10c12 CLA versus control after 2 weeks.

[b] Significant difference at $P < 0.05$, t10c12 CLA versus control after 4 weeks.

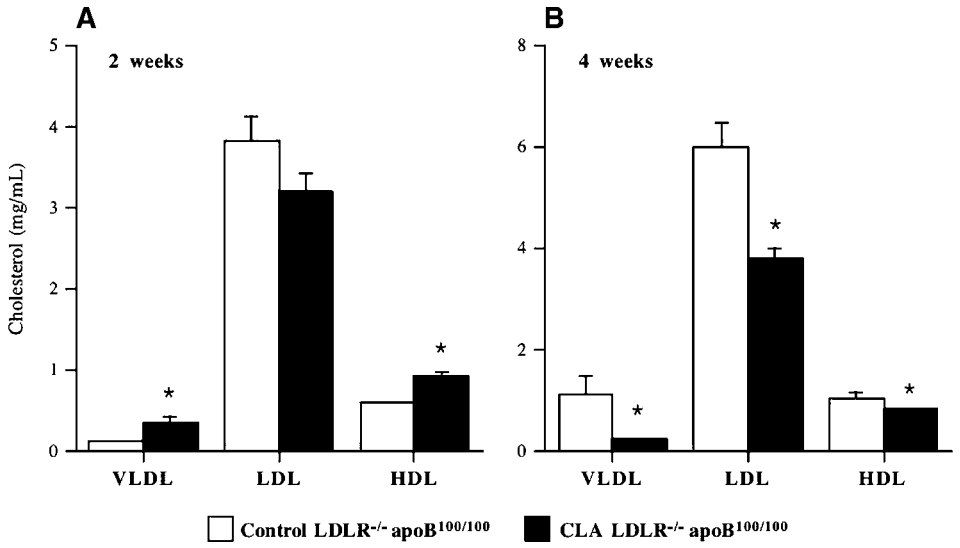

Fig. 1. Total cholesterol content of serum lipoproteins of LDLR$^{-/-}$ apoB$^{100/100}$ mice fed *trans*-10,*cis*-12 conjugated linoleic acid (t10c12 CLA) for 2 weeks (A) or 4 weeks (B). Results are expressed as means ± SEM (n = 5). * Significant difference at $P < 0.05$ versus control.

Effects of t10c12 CLA feeding on liver FA oxidation in LDLR$^{-/-}$ apoB$^{100/100}$ mice

Administration of t10c12 CLA improved both peroxisomal and mitochondrial palmitate oxidation rates as measured using liver homogenates after both 2 and 4 weeks of treatment (**Table 2**). Similarly, carnitine-dependent palmitate oxidation rates measured using isolated mitochondria were also increased, as were CPT I activities in the t10c12 CLA series (Table 2).

mRNA expression of proteins involved in liver lipid uptake in LDLR$^{-/-}$ apoB$^{100/100}$ mice compared with wild-type mice

The impact of the absence of LDLR on the mRNA expression of some proteins involved in lipid uptake (HL, LPL, SR-BI, LRP, VLDLR, FAT/CD36) was estimated in LDLR$^{-/-}$ apoB$^{100/100}$ mice in comparison with wild-type mice (**Fig. 2**). The data indicate that the liver of control transgenic mice overexpressed LPL, FAT/CD36, and VLDLR, which are usually poorly expressed in this organ. In these mice, mRNA levels of HL and two other potential candidates for lipoprotein transport, SR-BI and LRP, were not different between the two genotypes.

Effects of t10c12 CLA feeding on the mRNA expression of proteins involved in liver lipid uptake in LDLR$^{-/-}$ apoB$^{100/100}$ mice

The mechanisms of the steatosis setup in LDLR$^{-/-}$ apoB$^{100/100}$ mice fed t10c12 CLA, despite the absence of LDLR, were investigated through the estimation of mRNA levels of enzymes and receptors involved in plasma FA or lipoprotein uptake (SR-BI, LRP, HL, VLDLR and FAT/CD36). Dietary t10c12 CLA decreased mRNA expression of liver HL at both 2 and 4 weeks and increased that of LPL at 4 weeks (**Fig. 3**). Among the lipoprotein receptors studied, VLDLR was upregulated in the two CLA series, whereas mRNA levels of LRP and SR-BI were significantly upregulated and downregulated, respectively, but only after 4 weeks of CLA treatment. Feeding t10c12 CLA also strongly increased mRNA levels of FAT/CD36, which is usually poorly expressed in the liver, after 2 or 4 weeks of treatment.

TABLE 2. Activities related to fatty acid oxidation in the liver of LDLR$^{-/-}$ apoB$^{100/100}$ mice fed t10c12 CLA for 2 or 4 weeks

	2 Weeks		4 Weeks	
Activity	Control	t10c12 CLA	Control	t10c12 CLA
In liver homogenates		*nmol/min/g tissue*		
Peroxisomal FA oxidation	42.5 ± 2.7	56.7 ± 1.2[a]	60.6 ± 3.5	83.9 ± 2.5[b]
Mitochondrial FA oxidation	301 ± 10	364 ± 14[a]	231 ± 12	303 ± 14[b]
In isolated mitochondria		*nmol/min/mg protein*		
FA oxidation	5.23 ± 0.06	5.53 ± 0.04[a]	3.31 ± 0.09	3.95 ± 0.20[b]
CPT I activity	1.01 ± 0.01	1.14 ± 0.07[a]	2.22 ± 0.08	2.70 ± 0.08[b]

CPT I, Carnitine palmitoyltransferase I. Results are expressed as means ± SEM (n = 5).
[a] Significant difference at $P < 0.05$, t10c12 CLA versus control after 2 weeks.
[b] Significant difference at $P < 0.05$, t10c12 CLA versus control after 4 weeks.

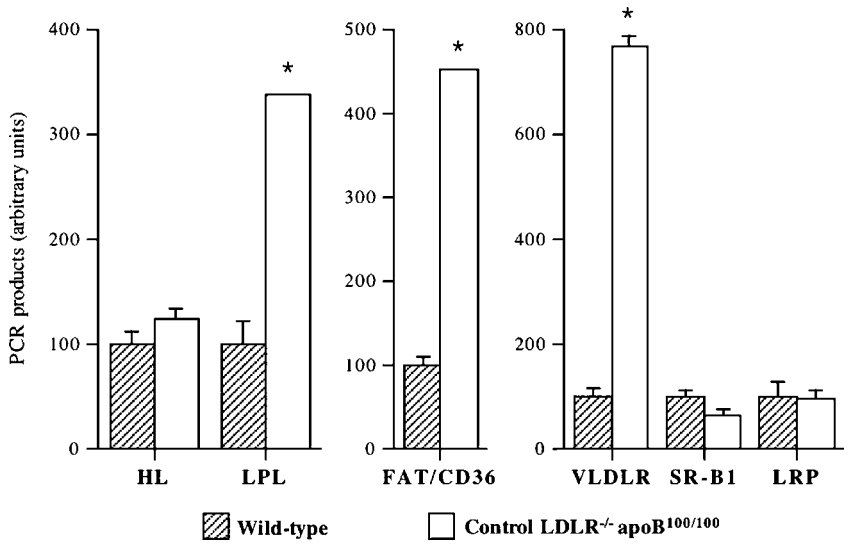

Fig. 2. Real-time PCR semiquantification of mRNA levels of proteins involved in liver lipid uptake in LDLR$^{-/-}$ apoB$^{100/100}$ mice compared with wild-type mice. Results are expressed as mean PCR product abundance ± SEM (n = 5) in LDLR$^{-/-}$ apoB$^{100/100}$ mice relative to wild-type mice after normalization to β-actin levels. * Significant difference at $P < 0.05$ versus the wild type. FAT/CD36, fatty acid translocase; LRP, low density lipoprotein receptor-related protein; SR-BI, scavenger receptor class B type I; VLDLR, very low density lipoprotein receptor.

Liver VLDLR protein levels and lipolytic activity in LDLR$^{-/-}$ apoB$^{100/100}$ mice

The apparent inductions of mRNA levels of VLDLR and LPL prompted us to study their effects on protein levels and catalytic activity, respectively. **Figure 4** indicates that VLDLR protein levels were induced concomitant with mRNA levels. As the regulation of LPL may also occur at the posttranslational level (25), we measured the actual lipolytic activity of liver extracts. The results presented in **Fig. 5** indicate that the liver capacities to hydrolyze TG were greater in LDLR$^{-/-}$ apoB$^{100/100}$ mice fed t10c12 CLA for 4 weeks than in control transgenic mice.

DISCUSSION

In wild-type mice, t10c12 CLA feeding induced severe lipoatrophy with concomitant liver steatosis, and we previously showed that mRNA levels of LDLR were induced, suggesting an increase in lipoprotein uptake by hepatocytes (7). In this study, the fact that liver TG accumulation was found even in the absence of LDLR raises the question of how hepatocytes manage to face the high flux of lipids that were not stored any longer in adipose tissues of t10c12 CLA-fed mice. Because the liver steatosis seems to be related to lipoatrophy, one could think that hepatocyte TG accumulation did not result from a direct action of t10c12 CLA on liver cells but rather as a consequence of mechanisms altering the adipose tissue.

Liver steatosis may also originate from a reduction of lipoprotein secretion rates, from an inhibition of FA β-oxidation, from high rates of de novo lipogenesis, and/or from high lipid uptake. Feeding mice with t10c12 CLA did not reduce liver lipoprotein secretion (7) or FA oxidation and CPT I activities (this study). De novo lipogenesis might be stimulated by t10c12 CLA feeding, owing to the greater [saturated + monounsaturated]/[polyunsaturated] ratios found in liver lipids of LDLR$^{-/-}$ apoB$^{100/100}$-treated mice than in those of control mice (i.e., 22.3 vs. 5.62, respectively; data not shown). Indeed, in a recent study, it was hypothesized that the conversion of excess glucose to FA and the storage as TG in the liver, rather than in adipose tissue, could be the mechanism leading to liver fat accumulation (9). Therefore, the data presented here also support the conclusion that lipids diverted from adipose tissues, and available for other organs such as the liver, might contribute to a large extent to hepatic lipid accumulation. Consistent with this hypothesis, we did not observe any lipid accumulation in the other two lipid-utilizing tissues, heart and muscle, suggesting that the liver could be the main acceptor of plasma lipids in CLA-fed mice.

Gene expression analysis of control LDLR$^{-/-}$ apoB$^{100/100}$ mice compared with wild-type mice indicated that hepatocytes of LDLR-deficient mice overexpressed other genes in response to high levels of TG-rich lipoproteins. Interestingly, LPL, FAT/CD36, and VLDLR, which are usually poorly expressed in liver (26–28), were induced. Under normal conditions, VLDLR is known to participate in the clearance of VLDL mediated by peripheral organs actively using fat, such as heart or adipose tissue, but not the liver (29). Nevertheless, it has been demonstrated that the induction of hepatic expression of VLDLR using adenoviral vectors improved lipoprotein clearance (30, 31). In this way, in the absence of LDLR, the upregulation

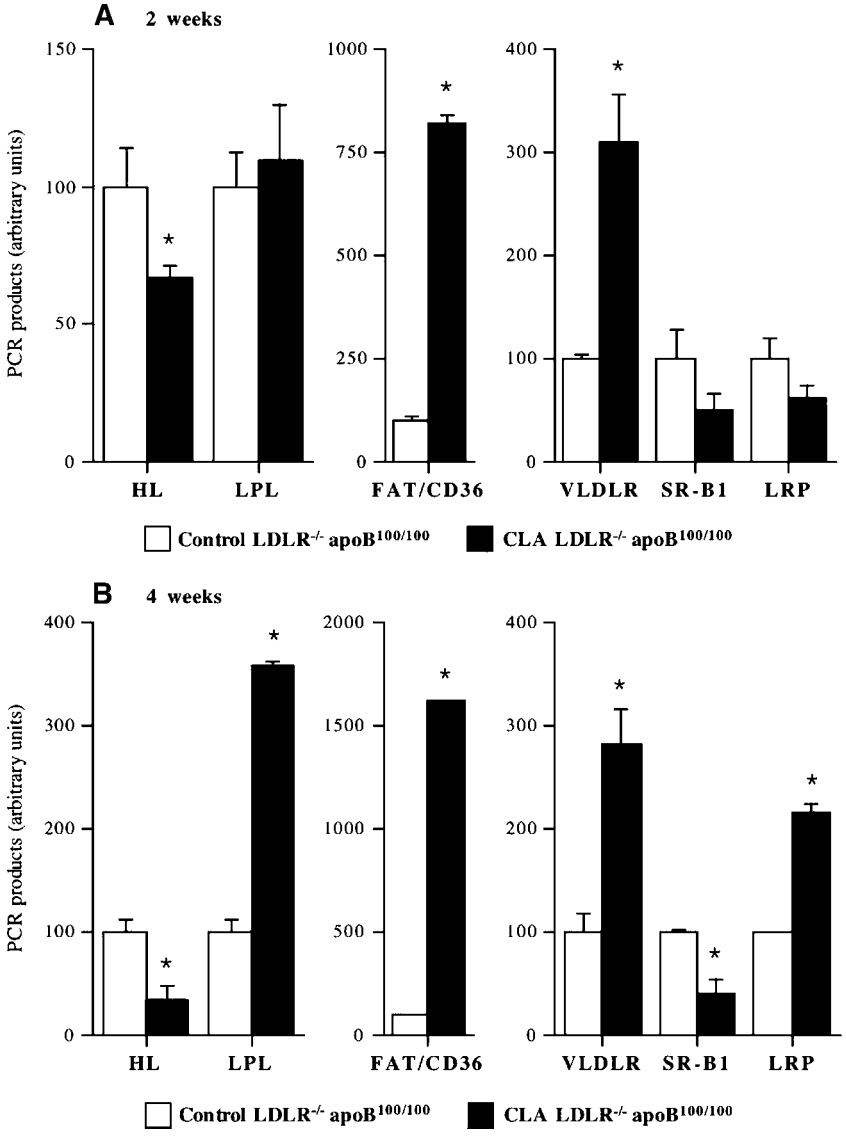

Fig. 3. Real-time PCR semiquantification of mRNA levels of proteins involved in liver lipid uptake in $LDLR^{-/-}$ $apoB^{100/100}$ mice fed t10c12 CLA for 2 weeks (A) or 4 weeks (B). Results are expressed as mean PCR product relative abundance ± SEM (n = 5) in t10c12 CLA-fed $LDLR^{-/-}$ $apoB^{100/100}$ mice relative to control mice after normalization to β-actin levels. * Significant difference at $P < 0.05$ versus control.

of VLDLR mRNA and protein levels observed in the liver of $LDLR^{-/-}$ $apoB^{100/100}$ mice compared with wild-type mice strongly suggests that VLDLR is an effective surrogate receptor for the clearance of lipoproteins.

A possible mechanism to explain the effect of VLDLR on lipoproteins has been proposed (32). VLDLR would facilitate the hydrolysis rather than the internalization of particles binding lipoproteins by maintaining them in close interaction with LPL. In our study, the concomitant upregulation of VLDLR and LPL supports this concept, and the increase in FAT/CD36 mRNA levels also supports the possible involvement of this transporter in the uptake of FAs released. Some other studies also suggest close relationships between FAT/CD36 and LPL (33) and similarly between VLDLR and LPL (34). Nevertheless, as far as we are aware, this is the first study to report a concomitant induction of the expression of VLDLR, LPL and FAT/CD36 in liver, which suggests a functional cooperation of these proteins to face the lipoprotein abundance.

LPL and FAT/CD36 are peroxisome proliferator-activated receptor γ-responsive genes (35), and recent studies have established a role for hepatic peroxisome proliferator-activated receptor γ in the development and maintenance of liver steatosis (36, 37). Therefore, the induction of FAT/CD36 and LPL could be related to the greater delivery of FA to liver cells. This seems to apply particularly to FAT/CD36, whose mRNA levels increased concomitantly with liver TG infiltration (**Fig. 6**).

Interestingly, the comparison of gene expression between wild-type and $LDLR^{-/-}$ $apoB^{100/100}$ mice indicates that LRP mRNA levels were comparable, which does not

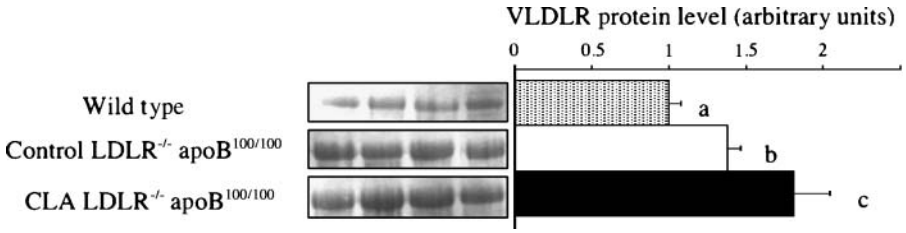

Fig. 4. Relative levels of VLDLR protein in liver of wild-type mice and LDLR$^{-/-}$ apoB$^{100/100}$ mice fed the control or t10c12 CLA diet for 4 weeks. Data from Western blot analysis are expressed as mean intensity ± SEM (n = 4) of the signal for VLDLR normalized with β-actin. Different letters indicate statistical significance between means at $P < 0.05$.

ascribe any apparent role for LRP in the metabolism of apoB-100-containing lipoproteins, even in the absence of LDLR, as was reported previously (38). However, mRNA levels of LRP increased after 4 weeks of t10c12 CLA treatment, suggesting that LRP stimulation could be secondary to the establishment of CLA-induced hyperinsulinemia (9), as has been demonstrated in adipocytes (39), and this receptor likely also participates in the clearance of lipoproteins.

Surprisingly, mRNA levels of HL, which could also provide an alternative clearance pathway for apoB-100-containing lipoproteins independent of LDLR (40), were not induced in LDLR$^{-/-}$ apoB$^{100/100}$ mice and even decreased after t10c12 CLA feeding. This supports the possibility that HL would be inversely regulated by the cholesterol supply (41). The same hypothesis could be retained to explain the SR-BI downregulation, because convergent arguments support the view that HL and SR-BI would be coexpressed to exert coordinated functions in cell cholesterol homeostasis (42).

According to our data, the overexpression of VLDLR, LPL, and FAT/CD36 observed in LDLR$^{-/-}$ apoB$^{100/100}$ mice after 4 weeks of CLA feeding accelerated liver lipoprotein clearance to such an extent that the increased serum TG levels observed after 2 weeks of CLA feeding were lower than in controls. It is worth noting that this TG lowering coincides with the nearly complete absence of adipose tissue. Under these conditions, the release of free FA from adipose tissue was necessarily decreased, reducing lipid flux to the liver and the subsequent VLDL secretion rates compared with the 2 week series. On the whole, we suggest that the t10c12 CLA-dependent TG-lowering effect was attributable to both the reduction of a source of FA for liver lipoprotein synthesis and the high capacity of liver to clear off plasma lipids. It is now well established that liver LDLR activity constitutes a key factor for the regulation of apoB-containing lipoproteins (43, 44). Therefore, this study provides evidence that, in the absence of LDLR, some efficient alternative regulatory mechanisms also occur (e.g., see control LDLR$^{-/-}$

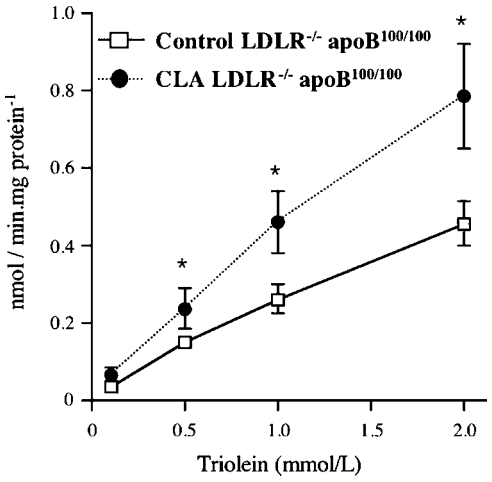

Fig. 5. Liver triglyceride (TG) lipolytic activity in LDLR$^{-/-}$ apoB$^{100/100}$ mice fed t10c12 CLA for 4 weeks. Activity was determined using liver homogenate supernatants with triolein as a substrate. Results are expressed as means ± SEM (n = 5) of nmol free FA released from [^{3}H]triolein/mg protein, and error bars indicate SEM. * Significant difference at $P < 0.05$ versus control.

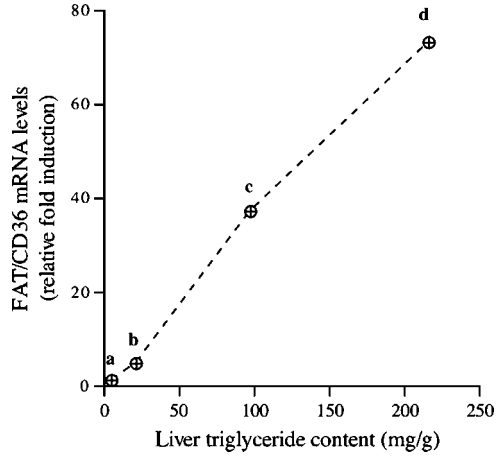

Fig. 6. Correlation between liver TG content and FAT/CD36 mRNA levels. Fold changes in PCR product abundance relative to wild-type mice were obtained from Figs. 2 and 3. TG contents were obtained from Table 1 and Ref. 7 for LDLR$^{-/-}$ apoB$^{100/100}$ mice and wild-type mice, respectively. a, Wild-type mice; b, control LDLR$^{-/-}$ apoB$^{100/100}$ mice; c, d, LDLR$^{-/-}$ apoB$^{100/100}$ mice fed t10c12 CLA for 2 weeks (c) or 4 weeks (d).

apoB$^{100/100}$ vs. wild-type mice) with convenient upregulation when the fat storage is defective in adipose tissue (e.g., after t10c12 CLA feeding).

The authors thank Mrs. Legendre for fast-performance liquid chromatography analysis and helpful discussions and Mrs. Baudoin for figure construction and typing of the manuscript. This work was supported by grants from the Ministère de la Recherche et de la Technologie and the Région Bourgogne (Dijon, France).

REFERENCES

1. Field, C. J., and P. D. Schley. 2004. Evidence for potential mechanisms for the effect of conjugated linoleic acid on tumor metabolism and immune function: lessons from n-3 fatty acids. *Am. J. Clin. Nutr.* **79 (Suppl.):** 1190–1198.
2. McLeod, R. S., A. M. LeBlanc, M. A. Langille, P. L. Mitchell, and D. L. Currie. 2004. Conjugated linoleic acids, atherosclerosis, and hepatic very-low-density lipoprotein metabolism. *Am. J. Clin. Nutr.* **79 (Suppl.):** 1169–1174.
3. Miller, C. C., Y. Park, M. W. Pariza, and M. E. Cook. 1994. Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection. *Biochem. Biophys. Res. Commun.* **198:** 1107–1112.
4. DeLany, J. P., F. Blohm, A. A. Truett, J. A. Scimeca, and D. B. West. 1999. Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake. *Am. J. Physiol.* **276:** R1172–R1179.
5. Park, Y., K. J. Albright, W. Liu, J. M. Storkson, M. E. Cook, and M. W. Pariza. 1997. Effect of conjugated linoleic acid on body composition in mice. *Lipids.* **32:** 853–858.
6. Wang, Y., and P. J. Jones. 2004. Dietary conjugated linoleic acid and body composition. *Am. J. Clin. Nutr.* **79 (Suppl.):** 1153–1158.
7. Degrace, P., L. Demizieux, J. Gresti, J. M. Chardigny, J. L. Sebedio, and P. Clouet. 2003. Association of liver steatosis with lipid oversecretion and hypotriglyceridaemia in C57BL/6j mice fed trans-10,cis-12-linoleic acid. *FEBS Lett.* **546:** 335–339.
8. Park, Y., J. M. Storkson, K. J. Albright, W. Liu, and M. W. Pariza. 1999. Evidence that the trans-10,cis-12 isomer of conjugated linoleic acid induces body composition changes in mice. *Lipids.* **34:** 235–241.
9. Ide, T. 2005. Interaction of fish oil and conjugated linoleic acid in affecting hepatic activity of lipogenic enzymes and gene expression in liver and adipose tissue. *Diabetes.* **54:** 412–423.
10. Angulo, P., and K. D. Lindor. 2002. Non-alcoholic fatty liver disease. *J. Gastroenterol. Hepatol.* **17 (Suppl.):** 186–190.
11. Murayama, T., M. Yokode, H. Horiuchi, H. Yoshida, H. Sano, and T. Kita. 2000. Overexpression of low density lipoprotein receptor eliminates apolipoprotein B100-containing lipoproteins from circulation and markedly prevents early atherogenesis in apolipoprotein E-deficient mice. *Atherosclerosis.* **153:** 295–302.
12. Sanan, D. A., D. L. Newland, R. Tao, S. Marcovina, J. Wang, V. Mooser, R. E. Hammer, and H. H. Hobbs. 1998. Low density lipoprotein receptor-negative mice expressing human apolipoprotein B-100 develop complex atherosclerotic lesions on a chow diet: no accentuation by apolipoprotein(a). *Proc. Natl. Acad. Sci. USA.* **95:** 4544–4549.
13. Degrace, P., L. Demizieux, J. Gresti, J. M. Chardigny, J. L. Sebedio, and P. Clouet. 2004. Hepatic steatosis is not due to impaired fatty acid oxidation capacities in C57BL/6J mice fed the conjugated trans-10,cis-12-isomer of linoleic acid. *J. Nutr.* **134:** 861–867.
14. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
15. Morrison, W. L., and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride methanol. *J. Chromatogr.* **5:** 600–608.
16. Christie, W. W., J. L. Sebedio, and P. Juaneda. 2001. Quantitative lipid analysis using the new Iatroscan TLC-FID system. *Inform.* **12:** 147–152.
17. Sebedio, J. L., E. Angioni, J. M. Chardigny, S. Gregoire, P. Juaneda, and O. Berdeaux. 2001. The effect of conjugated linoleic acid isomers on fatty acid profiles of liver and adipose tissues and their conversion to isomers of 16:2 and 18:3 conjugated fatty acids in rats. *Lipids.* **36:** 575–582.
18. Lund, E. G., L. B. Peterson, A. D. Adams, M. H. Lam, C. A. Burton, J. Chin, Q. Guo, S. Huang, M. Latham, J. C. Lopez, et al. 2006. Different roles of liver X receptor alpha and beta in lipid metabolism: effects of an alpha-selective and a dual agonist in mice deficient in each subtype. *Biochem. Pharmacol.* **71:** 453–463.
19. Degrace, P., B. Moindrot, I. Mohamed, J. Gresti, and P. Clouet. Moderate consumption of beer reduces liver triglycerides and aortic cholesterol deposit in LDLr(−/−) apoB(100/100) mice. *Atherosclerosis.* Epub ahead of print. February 15, 2006; doi:10.1016/j.atherosclerosis.2006.01.012.
20. Iverius, P. H., and A. M. Ostlund-Lindqvist. 1986. Preparation, characterization, and measurement of lipoprotein lipase. *Methods Enzymol.* **129:** 691–704.
21. Veerkamp, J. H., H. T. Van Moerkerk, J. F. Glatz, and V. W. Van Hinsbergh. 1983. Incomplete palmitate oxidation in cell-free systems of rat and human muscles. *Biochim. Biophys. Acta.* **753:** 399–410.
22. Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk. 1985. Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150:** 76–85.
23. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* **227:** 680–685.
24. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162:** 156–159.
25. Doolittle, M. H., O. Ben-Zeev, J. Elovson, D. Martin, and T. G. Kirchgessner. 1990. The response of lipoprotein lipase to feeding and fasting. Evidence for posttranslational regulation. *J. Biol. Chem.* **265:** 4570–4577.
26. Abumrad, N. A., M. R. el-Maghrabi, E. Z. Amri, E. Lopez, and P. A. Grimaldi. 1993. Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36. *J. Biol. Chem.* **268:** 17665–17668.
27. Kirchgessner, T. G., K. L. Svenson, A. J. Lusis, and M. C. Schotz. 1987. The sequence of cDNA encoding lipoprotein lipase. A member of a lipase gene family. *J. Biol. Chem.* **262:** 8463–8466.
28. Oka, K., K. Ishimura-Oka, M. J. Chu, M. Sullivan, J. Krushkal, W. H. Li, and L. Chan. 1994. Mouse very-low-density-lipoprotein receptor (VLDLR) cDNA cloning, tissue-specific expression and evolutionary relationship with the low-density-lipoprotein receptor. *Eur. J. Biochem.* **224:** 975–982.
29. Takahashi, S., J. Sakai, T. Fujino, H. Hattori, Y. Zenimaru, J. Suzuki, I. Miyamori, and T. T. Yamamoto. 2004. The very low-density lipoprotein (VLDL) receptor: characterization and functions as a peripheral lipoprotein receptor. *J. Atheroscler. Thromb.* **11:** 200–208.
30. Chen, S. J., D. J. Rader, J. Tazelaar, M. Kawashiri, G. Gao, and J. M. Wilson. 2000. Prolonged correction of hyperlipidemia in mice with familial hypercholesterolemia using an adeno-associated viral vector expressing very-low-density lipoprotein receptor. *Mol. Ther.* **2:** 256–261.
31. Oka, K., L. Pastore, I. H. Kim, A. Merched, S. Nomura, H. J. Lee, M. Merched-Sauvage, C. Arden-Riley, B. Lee, M. Finegold, et al. 2001. Long-term stable correction of low-density lipoprotein receptor-deficient mice with a helper-dependent adenoviral vector expressing the very low-density lipoprotein receptor. *Circulation.* **103:** 1274–1281.
32. Tacken, P. J., M. H. Hofker, L. M. Havekes, and K. W. van Dijk. 2001. Living up to a name: the role of the VLDL receptor in lipid metabolism. *Curr. Opin. Lipidol.* **12:** 275–279.
33. Febbraio, M., N. A. Abumrad, D. P. Hajjar, K. Sharma, W. Cheng, S. F. Pearce, and R. L. Silverstein. 1999. A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism. *J. Biol. Chem.* **274:** 19055–19062.
34. Yagyu, H., E. P. Lutz, Y. Kako, S. Marks, Y. Hu, S. Y. Choi, A. Bensadoun, and I. J. Goldberg. 2002. Very low density lipoprotein (VLDL) receptor-deficient mice have reduced lipoprotein lipase activity. Possible causes of hypertriglyceridemia and reduced body mass with VLDL receptor deficiency. *J. Biol. Chem.* **277:** 10037–10043.
35. Schoonjans, K., B. Staels, and J. Auwerx. 1996. The peroxisome proliferator activated receptors (PPARs) and their effects on lipid

metabolism and adipocyte differentiation. *Biochim. Biophys. Acta.* **1302:** 93–109.
36. Inoue, M., T. Ohtake, W. Motomura, N. Takahashi, Y. Hosoki, S. Miyoshi, Y. Suzuki, H. Saito, Y. Kohgo, and T. Okumura. 2005. Increased expression of PPARgamma in high fat diet-induced liver steatosis in mice. *Biochem. Biophys. Res. Commun.* **336:** 215–222.
37. Memon, R. A., L. H. Tecott, K. Nonogaki, A. Beigneux, A. H. Moser, C. Grunfeld, and K. R. Feingold. 2000. Up-regulation of peroxisome proliferator-activated receptors (PPAR-alpha) and PPAR-gamma messenger ribonucleic acid expression in the liver in murine obesity: troglitazone induces expression of PPAR-gamma-responsive adipose tissue-specific genes in the liver of obese diabetic mice. *Endocrinology.* **141:** 4021–4031.
38. Veniant, M. M., C. H. Zlot, R. L. Walzem, V. Pierotti, R. Driscoll, D. Dichek, J. Herz, and S. G. Young. 1998. Lipoprotein clearance mechanisms in LDL receptor-deficient "Apo-B48-only" and "Apo-B100-only" mice. *J. Clin. Invest.* **102:** 1559–1568.
39. Descamps, O., D. Bilheimer, and J. Herz. 1993. Insulin stimulates receptor-mediated uptake of apoE-enriched lipoproteins and activated alpha 2-macroglobulin in adipocytes. *J. Biol. Chem.* **268:** 974–981.
40. Dichek, H. L., S. M. Johnson, H. Akeefe, G. T. Lo, E. Sage, C. E. Yap, and R. W. Mahley. 2001. Hepatic lipase overexpression lowers remnant and LDL levels by a noncatalytic mechanism in LDL receptor-deficient mice. *J. Lipid Res.* **42:** 201–210.
41. Perret, B., L. Mabile, L. Martinez, F. Terce, R. Barbaras, and X. Collet. 2002. Hepatic lipase: structure/function relationship, synthesis, and regulation. *J. Lipid Res.* **43:** 1163–1169.
42. Acton, S., A. Rigotti, K. T. Landschulz, S. Xu, H. H. Hobbs, and M. Krieger. 1996. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science.* **271:** 518–520.
43. Horton, J. D., H. Shimano, R. L. Hamilton, M. S. Brown, and J. L. Goldstein. 1999. Disruption of LDL receptor gene in transgenic SREBP-1a mice unmasks hyperlipidemia resulting from production of lipid-rich VLDL. *J. Clin. Invest.* **103:** 1067–1076.
44. Twisk, J., D. L. Gillian-Daniel, A. Tebon, L. Wang, P. H. Barrett, and A. D. Attie. 2000. The role of the LDL receptor in apolipoprotein B secretion. *J. Clin. Invest.* **105:** 521–532.