CD36 deficiency in mice impairs lipoprotein lipase-mediated triglyceride clearance

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Abstract **CD36 is involved in high-affinity peripheral FFA uptake. CD36-deficient (*cd36*$^{-/-}$) mice exhibit increased plasma FFA and triglyceride (TG) levels. The aim of the present study was to elucidate the cause of the increased plasma TG levels in *cd36*$^{-/-}$ mice. *cd36*$^{-/-}$ mice showed no differences in hepatic VLDL-TG production or intestinal [^{3}H]TG uptake compared with wild-type littermates. *cd36*$^{-/-}$ mice showed a 2-fold enhanced postprandial TG response upon an intragastric fat load ($P < 0.05$), with a concomitant 2.5-fold increased FFA response ($P < 0.05$), suggesting that the increased FFA in *cd36*$^{-/-}$ mice may impair LPL-mediated TG hydrolysis. Postheparin LPL levels were not affected. However, the in vitro LPL-mediated TG hydrolysis rate as induced by postheparin plasma of *cd36*$^{-/-}$ mice in the absence of excess FFA-free BSA was reduced 2-fold compared with wild-type plasma ($P < 0.05$). This inhibition was relieved upon the addition of excess FFA-free BSA. Likewise, increasing plasma FFA in wild-type mice to the levels observed in *cd36*$^{-/-}$ mice by infusion prolonged the plasma half-life of glycerol tri[^{3}H]oleate-labeled VLDL-like emulsion particles by 2.5-fold ($P < 0.05$).** **We conclude that the increased plasma TG levels observed in *cd36*$^{-/-}$ mice are caused by decreased LPL-mediated hydrolysis of TG-rich lipoproteins resulting from FFA-induced product inhibition of LPL.**—Goudriaan, J. R., M. A. M. den Boer, P. C. N. Rensen, M. Febbraio, F. Kuipers, J. A. Romijn, L. M. Havekes, and P. J. Voshol. **CD36 deficiency in mice impairs lipoprotein lipase-mediated triglyceride clearance.** ***J. Lipid Res.*** **2005.** 46: **2175–2181.**

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CD36, also known as fatty acid translocase (1), is a receptor for several ligands, including oxidized LDL and long-chain FFAs (1–5). Abumrad et al. (1) showed that CD36 is abundant in peripheral tissues active in FFA metabolism, such as adipose tissue, skeletal muscle, and cardiac muscle, where it is involved in high-affinity uptake of FFA (1, 6, 7). To directly investigate a role for CD36 in lipid metabolism, mice lacking CD36 were generated by gene targeting (8). These CD36-deficient (*cd36*$^{-/-}$) mice exhibited increased plasma FFA and triglyceride (TG) levels (8). Coburn et al. (9) showed that FFA uptake was considerably impaired in muscle and adipose tissue of *cd36*$^{-/-}$ mice. Febbraio et al. (8) further showed that the increase in plasma TG levels in the absence of CD36 was primarily attributable to an increase in VLDL-sized particles. Although these data suggest a role for CD36 in TG metabolism in addition to FFA metabolism, the exact mechanisms underlying the increased TG levels in *cd36*$^{-/-}$ mice are unknown. It has been discussed by Hajri et al. (10) that the VLDL production rate may be enhanced in *cd36*$^{-/-}$ mice, but the increased plasma TG levels may also be attributable to increased intestinal lipid absorption or to decreased LPL-mediated TG clearance from the circulation.

Therefore, the aim of the present study was to elucidate the cause of the hypertriglyceridemia in *cd36*$^{-/-}$ mice in vivo. Our results show that the increased plasma TG levels in *cd36*$^{-/-}$ mice are caused by a decreased TG hydrolysis rate rather than by differences in the production of hepatic VLDL-TG or intestinal lipid absorption. From the present study, we conclude that the hypertriglyceridemia observed in *cd36*$^{-/-}$ mice is caused by decreased LPL-mediated hydrolysis of TG-rich lipoproteins resulting from FFA-induced product inhibition.

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Abbreviations: apoC-I, apolipoprotein C-I; $AUC_{0–8.5\ h}$, area under the curve at 0–8.5 h; *cd36*$^{-/-}$, CD36-deficient; TG, triglyceride.

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MATERIALS AND METHODS

Animals

cd36$^{-/-}$ mice were generated by targeted homologous recombination and crossed back six times to the C57Bl/6 background (8). Male and female *cd36*$^{-/-}$ mice (4–6 months of age) were used with wild-type littermates (*cd36*$^{+/+}$) as controls. They were housed under standard conditions with free access to water and food (standard rat-mouse chow diet; Standard Diet Services, Essex, UK). Principles of laboratory animal care were followed, and the animal ethics committee of our institute approved all animal experiments.

Plasma TG and FFA analysis

To determine plasma lipid levels, tail vein blood was collected from male *cd36*$^{-/-}$ and *cd36*$^{+/+}$ mice, after 4 and 16 h of fasting, into chilled paraoxon-coated capillary tubes to prevent ongoing lipolysis (11). These tubes were placed on ice and immediately centrifuged at 4°C. Plasma levels of TG (without free glycerol) and FFA were determined using the commercially available 337-B Sigma GPO-Trinder kit (Sigma, St. Louis, MO) and the Nefa-C kit (Wako Chemicals GmbH, Neuss, Germany), respectively.

Hepatic VLDL production

After an overnight fast, *cd36*$^{-/-}$ and *cd36*$^{+/+}$ male mice were anesthetized [0.5 ml/kg hypnorm (Janssen Pharmaceutical, Beerse, Belgium) and 12.5 mg/kg midazolam (Roche, Mijdrecht, The Netherlands)] and injected intravenously into the tail vein with 500 mg of Triton WR1339 per kilogram of body weight as a 10% solution in 0.9% NaCl, which virtually completely inhibits serum lipoprotein clearance (12). Blood samples were drawn at 0, 15, 30, 60, and 90 min after the Triton injection, and TG concentrations were determined in plasma as described above and related to the body mass of the mice.

Intestinal lipid absorption

To study the intestinal lipid uptake, *cd36*$^{-/-}$ and *cd36*$^{+/+}$ female mice were injected intravenously with 500 mg of Triton WR1339 per kilogram of body weight as a 10% solution in 0.9% NaCl. Directly after the Triton injection, mice were given an intragastric 200 μl olive oil bolus with 7 μCi of glycerol-tri[^{3}H]oleate ([^{3}H]triolein; Amersham, Little Chalfont, UK). Blood samples were drawn at 30, 60, 90, 120, 180, and 240 min after bolus administration, and the amount of ^{3}H radioactivity was determined in plasma. TLC analysis revealed that >90% of the label appeared in the TG fraction. Plasma volumes were calculated according to Rensen et al. (13).

Intragastric fat load

To investigate the handling of postprandial TG, male *cd36*$^{-/-}$ and *cd36*$^{+/+}$ mice, after 2 weeks on a high-fat diet and an overnight fast, were given an intragastric 200 μl olive oil bolus. Blood samples were drawn at 0, 1, 2, 4, 6, and 8.5 h after bolus administration, and FFA and TG concentrations were determined in plasma as described above and corrected for the plasma FFA and TG levels at time zero.

Plasma LPL and hepatic lipase levels

Plasma was obtained from male *cd36*$^{-/-}$ and *cd36*$^{+/+}$ mice, after 2 weeks on a high-fat diet (46.2% of the calories as fat; Hope Farms, Woerden, The Netherlands) and an overnight fast, at 10 min after a tail vein injection of heparin (0.1 U/g body weight; Leo Pharma BV, Weesp, The Netherlands). To prevent excessive plasma lipolysis, the capillaries we used to sample the postheparin plasma were kept on ice, spun immediately at 4°C, and snap-frozen in liquid nitrogen. Plasma LPL and HL levels were determined in postheparin plasma as described (14). In short, the lipolytic activity of plasma was assessed by determination of [^{3}H]oleate production upon incubation of plasma with a substrate mix containing an excess of both [^{3}H]triolein and FFA-free BSA as FFA acceptor. HL and LPL activities were distinguished in the presence of 1 M NaCl, which specifically blocks LPL.

Modulated plasma LPL and HL activities

Plasma was obtained from male *cd36*$^{-/-}$ and *cd36*$^{+/+}$ mice, after 2 weeks on a high-fat diet and an overnight fast, at 10 min after a tail vein injection of heparin (0.1 U/g). The effect of the FFA content of plasma on the activity of LPL and HL in postheparin plasma was determined by [^{3}H]oleate production during incubation of plasma with [^{3}H]triolein-labeled 75 nm VLDL mimicking protein-free emulsion particles essentially as described previously (15). Hereto, mouse plasma [final concentration, 2.5% (v/v)] was incubated with emulsion particles (final concentration, 0.5 mg TG/ml) in the absence and presence of excess FFA-free BSA (final concentration, 60 mg/ml) in a total volume of 200 μl of 0.1 M Tris, pH 8.5. The generated [^{3}H]oleate was quantified after extraction (15). Under these assay conditions, TG derived from mouse plasma contributed only marginally to the total TG present in the incubations (~1%).

Clearance of TG-rich VLDL-like emulsion particles

[^{3}H]triolein-labeled VLDL-like emulsion particles were prepared as described previously (15). FFA (oleate) in toluene was evaporated to dryness under N_2 and redissolved in 0.9% NaCl solution containing 2 mg/ml FFA-free BSA (pH 8.0). The vehicle consisted of 0.9% NaCl solution with 2 mg/ml FFA-free BSA (pH 8.0). Fed wild-type male mice were anesthetized, and an infusion needle was placed into the tail vein. The infusion of FFA (0.75 μmol oleate/min/mouse) or vehicle was started, and after 30 min and 1 h, blood samples were drawn to determine plasma FFA and TG. One hour after the start of infusion of FFA or vehicle, a bolus of [^{3}H]triolein-labeled VLDL-like emulsion particles was injected. At 2, 5, 10, and 15 min after the bolus injection, blood samples were drawn and the clearance of ^{3}H activity from the plasma was determined by scintillation counting and corrected for plasma volumes (13).

Statistical analysis

The Mann-Whitney nonparametric test for two independent samples was used to define differences between *cd36*$^{-/-}$ and *cd36*$^{+/+}$ mice. The criterion for significance was set at $P < 0.05$. All data are presented as means ± SD.

RESULTS

Increased plasma TG levels in *cd36*$^{-/-}$ mice

In accordance with previously published data (8, 9), *cd36*$^{-/-}$ mice bred at our local facility exhibited significantly increased fasting plasma FFA levels compared with wild-type littermates (0.89 ± 0.07 and 0.52 ± 0.09 mM, respectively; $P < 0.05$). **Table 1** summarizes the plasma TG levels in male *cd36*$^{-/-}$ and wild-type mice as observed by us and others (8, 10). On average, *cd36*$^{-/-}$ mice exhibited significantly 1.3- to 1.4-fold increased plasma TG levels compared with wild-type mice after various fasting periods and dietary treatments (Table 1).

Hepatic VLDL-TG production is not affected in CD36 deficiency

The increased plasma TG levels in *cd36*$^{-/-}$ mice can be attributable to *i*) increased hepatic VLDL-TG production,

TABLE 1. Effect of CD36 deficiency on plasma triglyceride levels

Mouse	Diet and Fasting Period					
	Chow, 4 h	Chow, 16 h	Chow, 8–12 h (8)	Chow, 16 h (10)	Fructose, 16 h (10)	High Fat, 16 h (10)
cd36$^{+/+}$	0.32 ± 0.08 (7)	0.16 ± 0.04 (7)	1.12 ± 0.21 (10)	0.56 ± 0.18 (7)	0.61 ± 0.13 (7)	0.40 ± 0.03 (7)
cd36$^{-/-}$	0.41 ± 0.03[a] (7)	0.39 ± 0.10[a] (7)	1.58 ± 0.38[a] (11)	0.76 ± 0.16[a] (7)	0.88 ± 0.17[a] (7)	0.50 ± 0.03[a] (7)

Triglyceride levels were measured in plasma of *cd36*$^{+/+}$ and *cd36*$^{-/-}$ male mice after various fasting periods and compared with data obtained by Febbraio et al. (8) and Hajri et al. (10) after correction for molecular weight (870) and conversion of SEM into SD values. The fructose diet consisted of 60% fructose, 20% protein, and 7% fat as soybean oil (10). The high-fat diet contained 18.2% sucrose, 33% casein, and 32% safflower oil (10). Mice were fed fructose and high-fat diets for 12 and 16 weeks, respectively (10). Values (mmol/l) represent means ± SD of n (shown in parentheses) mice per group.

[a] $P < 0.05$.

ii) increased intestinal lipid absorption, or *iii*) decreased lipolysis and/or clearance of TG from the circulation. To evaluate the effect of CD36 deficiency on hepatic VLDL production, *cd36*$^{-/-}$ mice and wild-type mice were injected with Triton WR1339 to block LPL-mediated TG hydrolysis, and the accumulation of endogenous VLDL-TG in plasma was monitored over time. **Figure 1** shows that CD36 deficiency did not affect the VLDL-TG production rate (40.9 ± 12.9 vs. 40.2 ± 1.9 μmol TG/kg/h). Consistently, we did not observe any difference in the composition of nascent VLDL that was isolated at 90 min after Triton WR1339 treatment (data not shown).

Intestinal lipid absorption is not affected in CD36 deficiency

We next investigated whether the increased plasma TG levels in CD36 deficiency could be attributable to increased intestinal lipid absorption. Hereto, *cd36*$^{-/-}$ and wild-type mice were administered an intragastric load of [^{3}H]triolein-containing olive oil after injection of Triton WR1339, and the appearance of ^{3}H label in plasma was monitored over time (**Fig. 2**). It appeared that, after a lag phase of ~30 min, ^{3}H label gradually appeared in plasma of *cd36*$^{-/-}$ and wild-type mice at similar rates (4.1 ± 1.4% and 3.5 ± 1.3% bolus/h, respectively; NS).

Increased postprandial TG response in *cd36*$^{-/-}$ mice

Apparently, the increased TG levels in *cd36*$^{-/-}$ mice cannot be explained by increased VLDL-TG production or intestinal TG absorption. Therefore, to get more insight into the underlying mechanism, we severely stressed TG metabolism by giving mice an intragastric fat load and monitored the appearance of TG and FFA in plasma (**Fig. 3**). Remarkably, the postprandial TG response was 2-fold enhanced in *cd36*$^{-/-}$ mice compared with wild-type littermates [area under the curve at 0–8.5 h ($AUC_{0-8.5\ h}$), 13 ± 6 and 7 ± 2 mM/h, respectively; $P < 0.05$], which suggests that CD36 deficiency results in impaired lipolytic conversion of postprandial TG in plasma (Fig. 3A). Interestingly, FFA levels were also 2.5-fold increased compared with those in wild-type littermates ($AUC_{0-8.5\ h}$, 20 ± 6 and 8 ± 1 mM/h, respectively; $P < 0.05$) (Fig. 3B).

CD36 deficiency does not modulate plasma LPL levels

Because the increased plasma TG levels in *cd36*$^{-/-}$ mice may thus be explained by decreased LPL-mediated TG hydrolysis, the levels of LPL and HL were measured in post-heparin plasma of *cd36*$^{-/-}$ and wild-type mice (**Fig. 4**). However, CD36 deficiency did not affect the total plasma LPL or HL levels, as determined by their TG hydrolase activity.

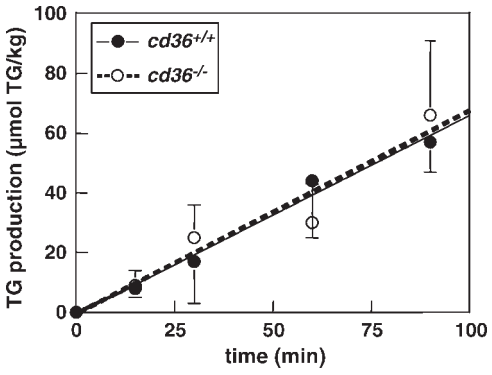

Fig. 1. Effect of CD36 deficiency on VLDL-triglyceride (TG) production rate. Triton WR1339 (500 mg/kg body weight) was injected intravenously into mice that had fasted overnight. Plasma TG levels were determined at 15, 30, 60, and 90 min and related to the body mass of the mice. Values represent means ± SD of four CD36-deficient (*cd36*$^{-/-}$) mice and three *cd36*$^{+/+}$ mice.

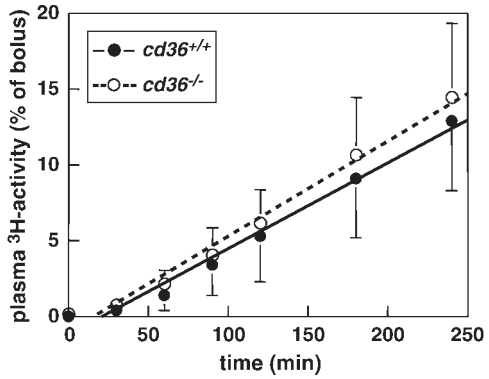

Fig. 2. Effect of CD36 deficiency on intestinal lipid absorption. Triton WR1339 (500 mg/kg body weight) was injected intravenously into mice that had fasted overnight. Directly after the Triton injection, mice were given an olive oil bolus including [^{3}H]triolein by intragastric gavage. The amount of plasma ^{3}H radioactivity was determined and is depicted as a percentage of the given bolus. Values represent means ± SD of five *cd36*$^{+/+}$ and four *cd36*$^{-/-}$ mice.

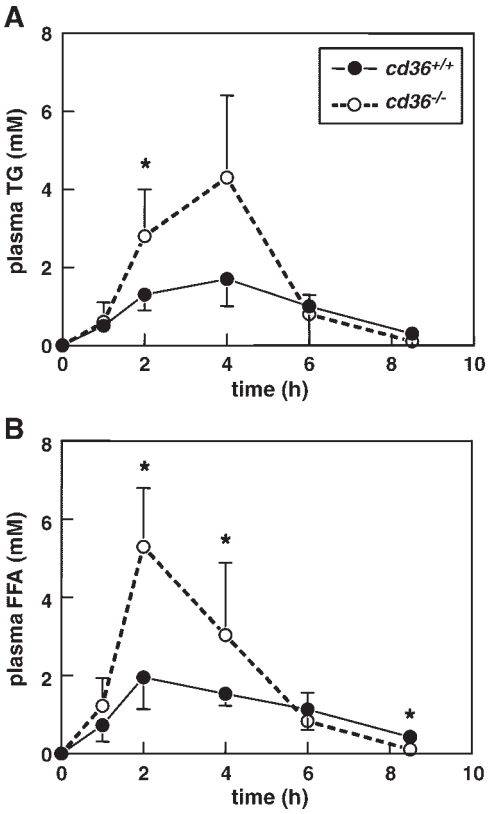

Fig. 3. Effect of CD36 deficiency on postprandial response. After 2 weeks on a high-fat diet and an overnight fast, *cd36*$^{+/+}$ and *cd36*$^{-/-}$ mice were given an intragastric olive oil bolus. Blood samples were drawn at 0, 1, 2, 4, 6, and 8.5 h after the bolus, and plasma TG (A) and FFA (B) concentrations were determined in plasma and corrected for plasma TG or FFA concentrations at time zero. Values represent means ± SD of six mice per group. * $P < 0.05$.

Increased plasma FFA levels in CD36 deficiency decrease LPL activity

Because *cd36*$^{-/-}$ mice have increased FFA levels, which are severely increased to ~5 mM after an intragastric fat load (Fig. 3), we speculated that these increased FFAs might interfere with the activity of LPL in plasma. Therefore, we determined the FFA-modulated LPL and HL activities of plasma from *cd36*$^{-/-}$ and wild-type mice in the absence of excess albumin (**Fig. 5A**). In this setting, although the total lipolysis of [^{3}H]triolein-labeled emulsion particles as induced by plasma of *cd36*$^{-/-}$ mice was not significantly decreased, the LPL activity was indeed decreased by 51% (0.13 ± 0.06 vs. 0.27 ± 0.07 nmol oleate/ml/min; $P < 0.05$). However, as shown in Fig. 5B, the addition of excess FFA-free albumin relieved this inhibition of LPL activity in *cd36*$^{-/-}$ mice (1.31 ± 0.32 vs. 0.80 ± 0.40 nmol oleate/ml/min; $P = 0.055$). *cd36*$^{-/-}$ mice even showed increased total TG hydrolase activity, probably as a result of the increased plasma TG levels (1.54 ± 0.25 vs. 1.10 ± 0.32 nmol oleate/ml/min; $P < 0.05$). Collectively, these data suggest that the increased (postprandial) TG levels are caused by a decreased TG hydrolysis rate in vivo attributable to product inhibition of LPL resulting from increased plasma FFA levels, rather than by altered production of hepatic VLDL-TG or intestinal lipid absorption.

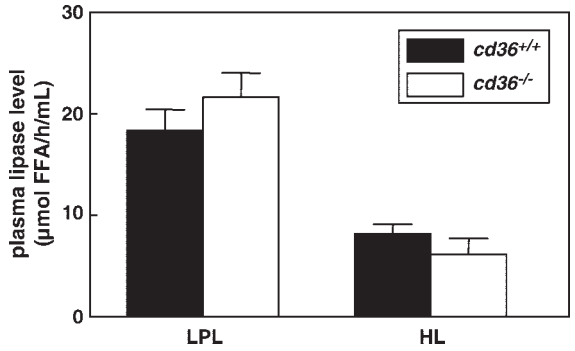

Fig. 4. Effect of CD36 deficiency on plasma LPL and HL levels. After 2 weeks on a high-fat diet, postheparin plasma was obtained after an overnight fast from *cd36*$^{+/+}$ and *cd36*$^{-/-}$ mice. Total TG hydrolase activity was measured in the absence (i.e., LPL and HL) or presence (i.e., HL) of 1 M NaCl. Values represent means ± SD of five mice per group.

Increased plasma FFA levels in wild-type mice decrease LPL-mediated TG clearance

To provide direct in vivo evidence that increased FFA levels indeed cause a decrease in LPL-dependent plasma TG clearance independent of *cd36*$^{-/-}$ background, we increased plasma FFA levels by FA infusion in fed wild-type male mice and determined the clearance of TG-rich VLDL-like emulsion particles. After 1 h of infusion, plasma FFAs were steadily increased ~1.4-fold compared with those in vehicle-infused animals (1.93 ± 0.41 vs. 1.38 ± 0.16 mM), whereas plasma TG levels were not yet increased. In mice with increased plasma FFA levels, the plasma half-life of [^{3}H]triolein-labeled TG-rich VLDL-like particles was increased 2.5-fold (17.5 ± 10.4 vs. 7.0 ± 2.6 min; $P < 0.05$) compared with that in mice infused with vehicle (**Fig. 6**), indicating a profound in vivo effect of plasma FFA levels

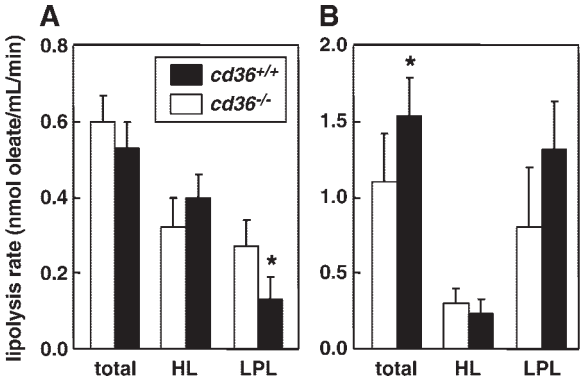

Fig. 5. Effect of CD36 deficiency on the TG hydrolase activity of plasma. After 2 weeks on a high-fat diet, postheparin plasma was obtained after an overnight fast from *cd36*$^{+/+}$ and *cd36*$^{-/-}$ mice. LPL and HL activities were determined by [^{3}H]oleate production during incubation of plasma with [^{3}H]triolein-labeled 75 nm VLDL mimicking protein-free emulsion particles in the absence (A) and presence (B) of excess FFA-free BSA. Values represent means ± SD of five mice per group. * $P < 0.05$.

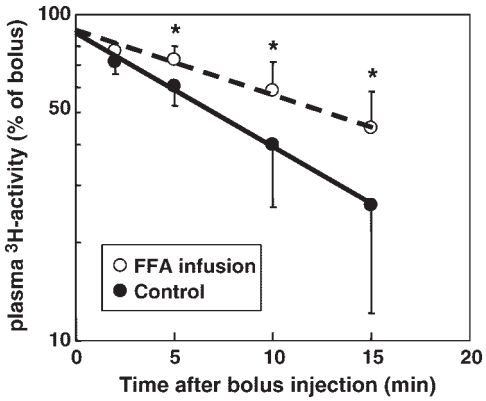

Fig. 6. Effect of increased plasma FFA on the clearance of [^{3}H]TG-labeled VLDL-like emulsion particles. Fed male wild-type mice were infused with FFA or vehicle to increase plasma FFA. During steady-state plasma FFA conditions, [^{3}H]triolein-labeled VLDL-like emulsion particles were injected and the clearance of ^{3}H activity from the plasma was followed in time. Values represent means ± SD of five mice in the FFA-infused group and four mice in the vehicle-infused group. * $P < 0.05$.

on LPL-dependent clearance of TG-rich lipoprotein particles.

DISCUSSION

In agreement with observations by others (8–10), we have shown that the absence of the fatty acid translocase CD36 in mice leads to increased plasma FFA levels concomitant with 30–40% increased TG levels. The effect of CD36 deficiency on increased plasma FFA levels can easily be explained by impaired peripheral uptake (9). Although it has been postulated that the VLDL production rate may be enhanced in *cd36*$^{-/-}$ mice (10), the mechanism underlying the effect of CD36 on TG metabolism had not been addressed. The results of the present study clearly show that the hypertriglyceridemia observed in *cd36*$^{-/-}$ mice is caused by a decreased LPL-mediated TG hydrolysis rate induced by increased plasma FFA levels, rather than by an increased production of hepatic VLDL-TG or increased intestinal lipid absorption.

Recently, we showed that the increased plasma FFA levels in *cd36*$^{-/-}$ mice lead to an enhanced FA flux toward the liver, resulting in increased TG storage (hepatic steatosis) (16). Hepatic VLDL production is thought to be primarily a substrate-driven process, regulated by the availability of FFA [reviewed by Lewis et al. (17)]. Furthermore, acute increase of plasma FFA levels stimulates VLDL production in humans (18). Therefore, the increased FFA flux to the liver in CD36 deficiency (16) may result in enhanced hepatic VLDL-TG production. Hajri et al. (10) hypothesized that such a mechanism may account for the hypertriglyceridemic effect of CD36 deficiency, but no experimental proof has been provided. Although we have observed the occurrence of increased plasma FFA levels and hepatic steatosis in *cd36*$^{-/-}$ mice, we did not detect any effect of CD36 deficiency on the expression of genes involved in transcriptional regulation (*pparα*, *pparγ*, *srebp1c*) or VLDL synthesis (*apob*, *apobec*, *apoe*, *mttp*) (data not shown). Importantly, CD36 deficiency did not affect the actual VLDL production rate or composition of nascent VLDL. Like *cd36*$^{-/-}$ mice, genetically obese *ob/ob* mice (19) and human apolipoprotein C-I (apoC-I)-overexpressing mice (20) also have increased plasma FFA levels and hepatic steatosis, but they display normal hepatic VLDL-TG production. Apparently, increased plasma FFA levels and hepatic steatosis per se do not necessarily lead to increased VLDL production.

CD36 is highly expressed in the apical membrane of enterocytes in the intestinal jejunal villi (1, 21). Because this location is the main site of FFA (lipid) absorption and CD36 does act as a FFA transporter, CD36 is thought to play a role in the intestinal uptake of FFA (21, 22). Therefore, increased intestinal lipid absorption as a result of CD36 deficiency seemed highly unlikely. Indeed, the present study showed that in the absence of CD36, lipid absorption is not affected in vivo in mice, confirming observations from our earlier study (23).

To gain more insight into the mechanism underlying the observed hypertriglyceridemia in *cd36*$^{-/-}$ mice, we severely stressed TG metabolism by giving mice an intragastric fat load, resulting in a rapid and extensive generation of chylomicrons. Remarkably, the postprandial TG response was 2-fold enhanced in *cd36*$^{-/-}$ mice compared with wild-type littermates. Concomitantly, the plasma FFA concentrations also increased to ~5 mM in *cd36*$^{-/-}$ mice, compared with only 2 mM in control littermates. During a hyperinsulinemic euglycemic clamp experiment (16), we found that plasma FFAs, which are derived from the adipose tissue by lipolysis through hormone-sensitive lipase, were equally suppressed by insulin. This finding indicates that insulin can inhibit hormone-sensitive lipase, showing that the adipose tissue in *cd36*$^{-/-}$ mice remains insulin sensitive although the liver has become insulin resistant. Therefore we know that the FFA excursion that occurs after an intragastric olive oil load is caused by the accumulation of lipolysis products by LPL in the circulation and not by an increased release of FFA from the adipose tissue. Mouse plasma contains ~0.5 mM albumin, which under normal circumstances carries the major part of plasma FFA. Because albumin has four high-affinity binding sites for FFA (24), albumin is capable of binding ~2 mM FFA in plasma. Apparently, the dramatically increased FFA levels upon the intragastric fat load in *cd36*$^{-/-}$ mice to a maximum of 5 mM exceed the maximum albumin binding capacity. Because the amphiphilic nature of FFA precludes its presence in plasma in an unbound state, it is likely that the FFA generated by TG hydrolysis will accumulate in the lipoprotein shell and interfere with LPL-mediated lipolysis. Indeed, it appeared that, although the total levels of LPL (and HL) were not affected by CD36 deficiency, LPL in postheparin plasma obtained from *cd36*$^{-/-}$ mice was less able to lipolyze VLDL-like emulsion particles in the absence of excess BSA as FFA acceptor. Upon addition of an excess of FFA-free BSA, the inhibition of LPL-mediated

lipolysis was relieved. These in vitro data thus confirm that the increased plasma TG levels in the absence of CD36 are caused by the inhibition of lipases (mainly LPL) attributable to increased plasma FFA levels. We have indeed observed that a reduction of LPL activity in heterozygous LPL-deficient mice (i.e., 40%) markedly increased the postprandial TG response after an intragastric olive oil load compared with wild-type littermates (AUC_{0-6}, 43 ± 27 vs. 3.5 ± 0.6, respectively; $P < 0.05$). In our study, we also show in vivo that in wild-type mice, 1.4-fold increased plasma FFA levels lead to a decreased capacity of LPL to lipolyze VLDL-TG. In the short time frame in which the experiment was performed, it is very unlikely that other LPL modulators, such as apoC-II or apoC-III, had changed between groups and impaired the LPL-mediated TG clearance. Slight changes in plasma concentrations of these modulators cannot be excluded in the case of the *cd36*$^{-/-}$ mice. However, our collective findings that *i*) the inhibition of LPL activity by plasma from *cd36*$^{-/-}$ mice is relieved by the addition of the FFA-sequestrant BSA, and *ii*) the increase of plasma FFA levels by infusion impairs TG clearance strongly suggest that the hypertriglyceridemic phenotype of *cd36*$^{-/-}$ mice is indeed explained mainly by increased FFA levels.

These effects of increased plasma FFA on tissue LPL activity may be explained by several mechanisms. Binding of FFA to the active site of LPL might cause classical product inhibition of LPL activity. We and others (25) showed in vitro that the rate at which LPL hydrolyzes TG in lipoproteins or emulsion particles decreases sharply with the amount of FFA formed unless albumin is present. An alternative mechanism has been proposed by Saxena and Goldberg (26), who showed in vitro that plasma FFA levels may be important modulators of LPL interaction with the endothelial cell surface and apoC-II. In vivo evidence for a role of plasma FFA in the control of LPL was proposed in humans. Peterson et al. (27) suggested that LPL is subject to feedback control by FFA, involving an unusual mechanism by which FFA may regulate not only the catalytic activity of the enzyme but also its distribution between endothelial sites (27).

In summary, in the present study, we show that the increased plasma TG levels in CD36 deficiency are not attributable to a previously hypothesized enhancing effect on VLDL production or to an effect on intestinal lipid absorption. Instead, CD36 deficiency resulted in hypertriglyceridemia caused by decreased LPL-mediated hydrolysis of TG-rich lipoproteins resulting from FFA-induced product inhibition.

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