Hepatic steatosis : metabolic consequences

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Chapter 4

CD36 Deficiency in Mice Impairs Lipoprotein Lipase-Mediated Triglyceride Clearance

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Abstract

CD36 is involved in high affinity peripheral fatty acid (FA) uptake. Mice lacking CD36 exhibit increased plasma FA and triglyceride (TG) levels. The aim of the present study was to elucidate the cause of the increased plasma TG levels in CD36-deficient (*cd36* $^{-/-}$) mice. *Cd36* $^{-/-}$ mice showed no differences in hepatic VLDL-TG production or intestinal [^{3}H]TG uptake as compared to wild type littermates. Importantly, the postprandial TG response upon an intragastric fat load was enhanced 2-fold in *cd36*$^{-/-}$ mice compared to wild type mice (13 ± 6 vs 7 ± 2 mM.h; $P < 0.05$), with a concomitant 2.5-fold increased FA response (20 ± 6 vs 8 ± 1 mM.h; $P < 0.05$), suggesting that the elevated FA in *cd36* $^{-/-}$ mice may impair LPL-mediated TG hydrolysis. Postheparin plasma lipoprotein lipase (LPL) levels were not different between *cd36* $^{-/-}$ and wild type mice. However, the *in vitro* LPL-mediated TG-hydrolysis rate as induced by postheparin plasma of *cd36* $^{-/-}$ mice in absence of excess FA-free BSA was reduced by 51% compared to wild type littermates (0.13 ± 0.06 vs 0.27 ± 0.07 nmol oleate/mL/min $P < 0.05$). This inhibition was relieved upon addition of excess FA-free BSA. To study whether LPL activity can be decreased *in vivo* via product inhibition by FA, we increased plasma FA in wild type mice by infusion and showed that the plasma half-life of glycerol tri[^{3}H]oleate-labeled VLDL-like emulsion particles was increased 2.5-fold (t½ = 17.5 ± 10.4 vs 7.0 ± 2.6 min, $P < 0.05$) as compared to vehicle-infused mice.

We conclude that the increased plasma TG levels observed in *cd36* $^{-/-}$ mice do not result from an increased hepatic VLDL-TG production or intestinal lipid absorption, but are caused by decreased LPL-mediated hydrolysis of TG-rich lipoproteins resulting from FA-induced product inhibition of LPL.

Introduction

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

Therefore, the aim of the present study was to elucidate the cause of the hypertriglyceridemia in CD36-deficient 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 FA-induced product inhibition.

Materials and Methods

Animals

CD36-deficient mice were generated by targeted homologous recombination and crossed back 6 times to 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 FA analysis

To determine plasma lipid levels, tail vein blood was collected from male *cd36* $^{-/-}$ and *cd36* $^{+/+}$ mice, after 4 h and 16 h 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 FA were determined using the commercially available kits #337-B Sigma GPO-Trinder kit (Sigma, St. Louis, MA, USA) and Nefa-C kit (Wako Chemicals GmbH, Neuss, Germany), respectively.

Hepatic VLDL-TG 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 Triton WR1339 per kg 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 Triton WR 1339 per kg 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 glycerol-tri[^{3}H]oleate ([^{3}H]triolein; Amersham, Little Chalfont, United Kingdom). 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 FA and TG concentrations were determined in plasma as described above and corrected for the plasma FA and TG levels at t=0.

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 hepatic lipase (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 FA-free BSA as FA-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 FA 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-sized 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 FA-free BSA (final concentration 60 mg/mL) in a total volume of 200 µL of 0.1 M Tris pH 8.5. 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 (approx. 1%).

Clearance of TG-rich VLDL-like emulsion particles

[^{3}H]Triolein-labeled VLDL-like emulsion particles were prepared as described previously.[15] Fed wild type male mice were anaesthetized and an infusion needle was placed into the tail vein. The infusion of FA (0.75 µmol [^{3}H]oleate/min/mouse) or vehicle was started and after 30 min and 1 h blood samples were drawn to determine plasma FA and TG. One hour after the start of infusion of FA 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 2 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 FA levels compared to 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 1.3-1.4-fold increased plasma TG levels compared to wild type mice after various fasting periods and dietary treatments (Table 1).

Table 1. Effect of CD36-deficiency on plasma TG levels (mM)

Diet	**chow**	**chow**	**chow** [8]	**chow** [10]	**fructose** [10]	**high fat** [10]
Fasting period	**4h**	**16h**	**8-12h**	**16h**	**16h**	**16h**
cd36 $^{+/+}$	0.32 ± 0.08	0.16 ± 0.04	1.12 ± 0.21	0.56 ± 0.18	0.61 ± 0.13	0.40 ± 0.03
cd36 $^{-/-}$	0.41 ± 0.03*	0.39 ± 0.10*	1.58 ± 0.38*	0.76 ± 0.16*	0.88 ± 0.17*	0.50 ± 0.03*

Triglyceride (TG) 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 (*MW* 870) and conversion of SE 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 represent the mean ± SD per group, *$P < 0.05$

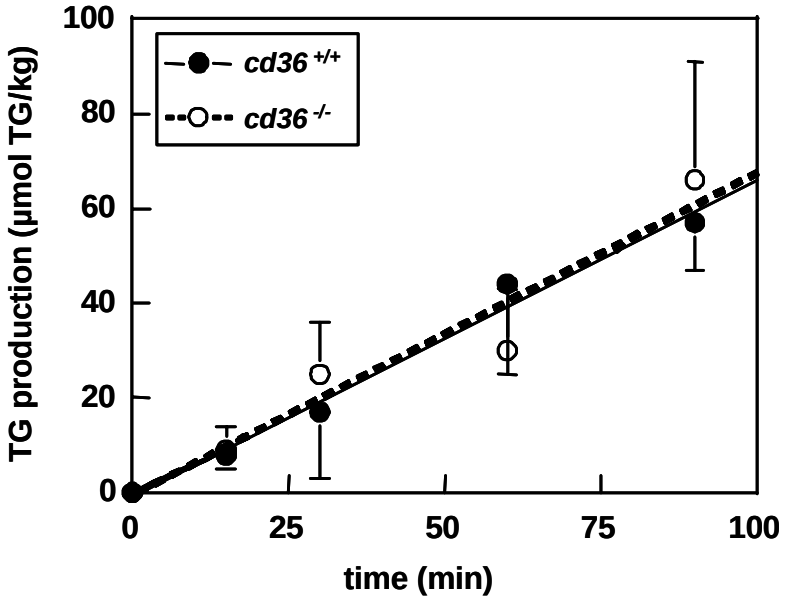

Figure 1. Effect of CD36 deficiency on VLDL-TG production rate. Triton WR 1339 (500 mg/kg body weight) was injected iv into mice which had fasted overnight. Plasma triglyceride (TG) levels were determined at 15, 30, 60, and 90 minutes and related to the body mass of the mice. Values represent means ± SD of 3 *cd36* $^{+/+}$ and 4 *cd36* $^{-/-}$ mice.

Hepatic VLDL-TG production is not affected in CD36 deficiency

The increased plasma TG levels in *cd36* $^{-/-}$ mice can be due either to i) increased hepatic VLDL-TG production, 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-TG 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 versus 40.2 ± 1.9 µmol TG·kg^{-1}·h^{-1}). Consistently, we did not observe any difference in the composition of nascent VLDL-TG that was isolated at 90 min after Triton WR1339 treatment (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 due 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 (Figure 2). It appeared that, after a lag-phase of approximately 30 min, ^{3}H-label gradually appeared in plasma of *cd36* $^{-/-}$ and wild type mice at a similar rate of 4.1 ± 1.4 and 3.5 ± 1.3% of bolus·h^{-1}, respectively.

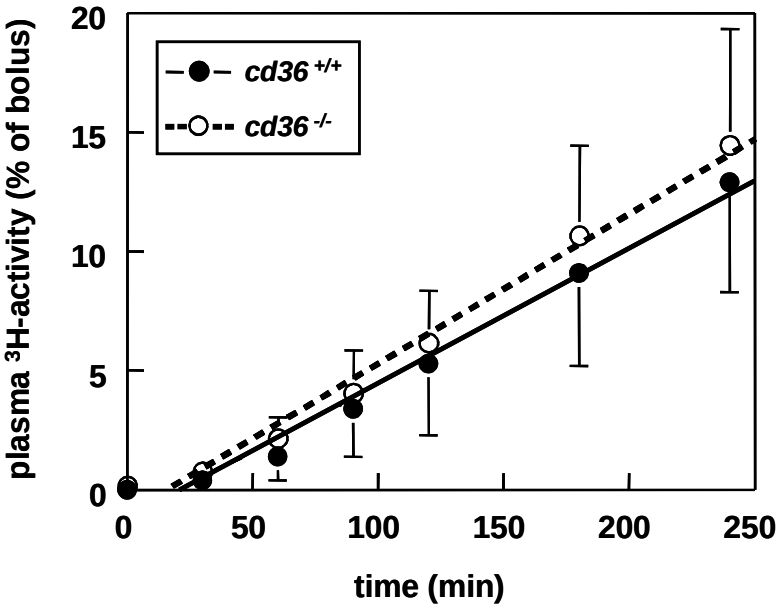

Figure 2. Effect of CD36 deficiency on intestinal lipid absorption. Triton WR 1339 (500 mg/kg body weight) was injected i.v. into mice which were fasted overnight. Directly after the Triton injection, mice were given an olive oil bolus including [^{3}H]triolein by intragastric gavage. The amount of plasma ^{3}H-radioactivity was determined, and depicted as a percentage of the given bolus. Values represent means ± SD of 5 *cd36* $^{+/+}$ and 4 *cd36* $^{-/-}$ mice.

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

Apparently, the elevated TG levels in *cd36* $^{-/-}$ mice cannot be explained by an 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 FA in plasma (Figure 3). Remarkably, the postprandial TG response was 2-fold enhanced in *cd36* $^{-/-}$ mice as compared to wild type littermates ($AUC_{0\text{-}8.5\ h}$: 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 (Figure 3A). Interestingly, FA levels were also 2.5-fold elevated as compared to wild type littermates ($AUC_{0\text{-}8.5\ h}$: 20 ± 6 and 8 ± 1 mM·h, respectively; $P < 0.05$; Figure 3B).

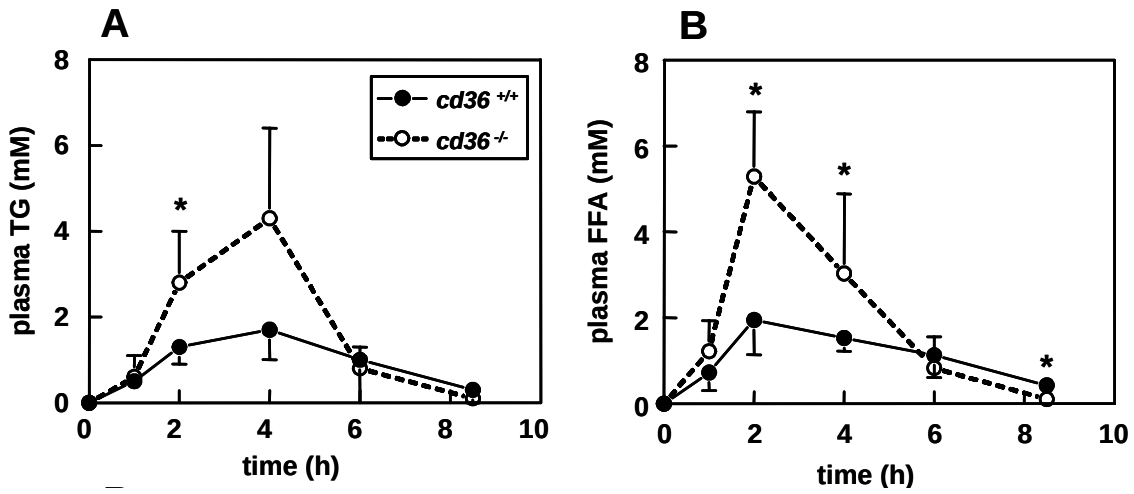

Figure 3. Effect of CD36 deficiency on postprandial response. After 2 weeks on a high fat diet and an overnight fast, *cd36* $^{+/+}$ and *cd36* $^{-/-}$ mice were given an intragastric olive oil bolus. Blood samples were drawn at 0, 1, 2, 4, 6 and 8.5 h after the bolus and plasma triglyceride (A) and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

CD36 deficiency does not modulate plasma LPL levels

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

Increased plasma FA levels in CD36 deficiency decreases LPL activity

Since CD36-deficient mice have elevated FA levels, which are severely increased to approximately 5 mM after an intragastric fat load (Figure 3), we speculated that these elevated FA might interfere with the activity of LPL in plasma. Therefore, we determined the FA-modulated LPL and HL activities of plasma from *cd36* $^{-/-}$ and wild type mice in the absence of excess albumin (Figure 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 Figure 5B, the addition of excess FA-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 show an increased total TG hydrolase activity probably due to 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* caused by product-inhibition of LPL resulting from increased plasma FA levels, rather than by an altered production of hepatic VLDL-TG or intestinal lipid absorption.

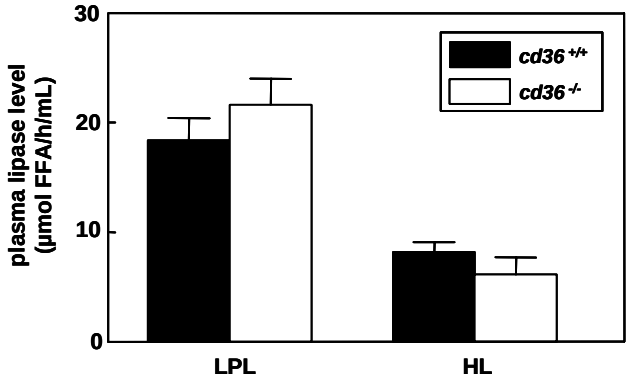

Figure 4. Effect of CD36 deficiency on plasma LPL and hepatic lipase (HL) levels. After 2 weeks on a high fat diet, postheparin plasma was obtained after an overnight fast from *cd36* $^{+/+}$ and *cd36* $^{-/-}$ mice. Total triglyceride hydrolase activity was measured in the absence (i.e. LPL and HL) or presence (i.e. HL) of 1 M NaCl. Values represent means ± SD of 5 mice per group, **P* < 0.05.

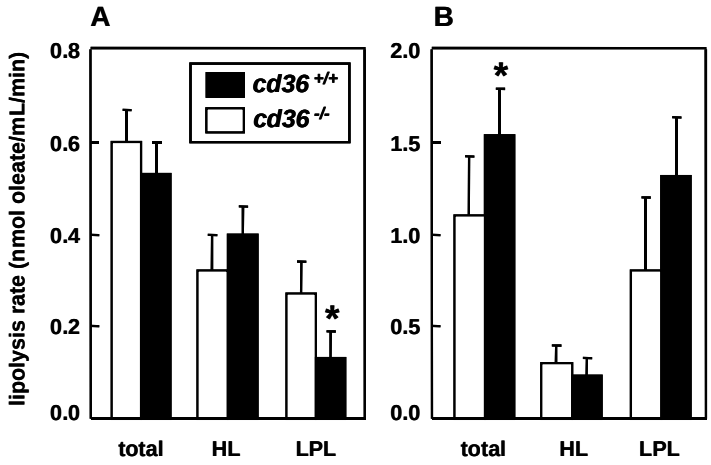

Figure 5. Effect of CD36 deficiency on the TG hydrolase activity of plasma. After 2 weeks on a high fat diet, postheparin plasma was obtained after an overnight fast from *cd36* $^{+/+}$ and *cd36* $^{-/-}$ mice. LPL and HL activities were determined by [^{3}H]oleate production during incubation of plasma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

Increased plasma FA levels in wild type mice decreases LPL-mediated TG clearance

To provide direct *in vivo* evidence showing that increased FA levels indeed cause a decrease in LPL-dependent plasma TG clearance independent of *cd36*$^{-/-}$ background, we increased plasma FA 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 FA were steadily increased approximately 1.4-fold compared to vehicle infused animals (1.93 ± 0.41 vs 1.38 ± 0.16 mM) while plasma TG levels were not increased yet. In mice with increased plasma FA levels the plasma half-life of [^{3}H]triolein-labeled TG-rich VLDL-like particles was 2.5-fold increased ($t_{½}$ = 17.5 ± 10.4 vs 7.0 ± 2.6 min, $P < 0.05$) compared to mice infused with vehicle (Figure 6) indicating a profound *in vivo* effect of plasma FA levels on LPL-dependent clearance of TG-rich lipoprotein particles.

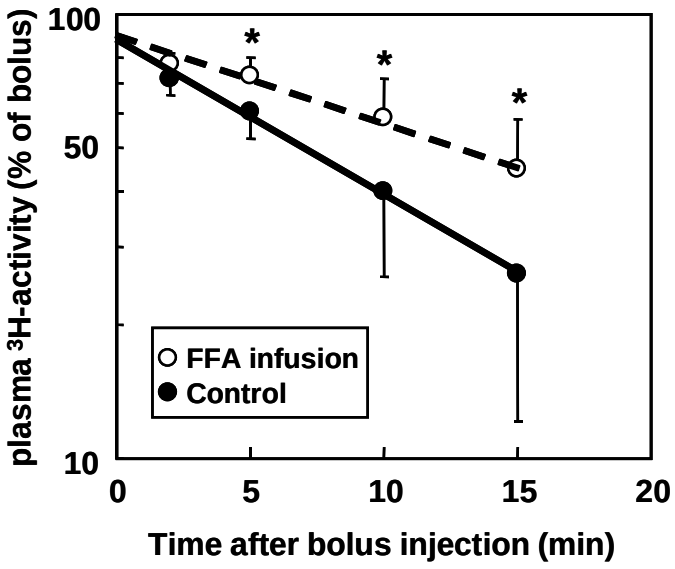

Figure 6. Effect of increased plasma FA on the clearance of [3H]TG-labeled VLDL-like emulsion particles. Fed male wild type mice were infused with FA or vehicle to increase plasma FA. During steady state plasma FA levels [^{3}H]triolein-labeled VLDL-like emulsion particles were injected and the clearance of ^{3}H-activity from the plasma was followed in time. Values represent means ± SD of 5 mice in the FA-infused group and 4 mice in the vehicle-infused group, *$P < 0.05$.

Discussion

In agreement with observations by others[8-10], we have shown that absence of the fatty acid translocase CD36 in mice leads to increased plasma FA levels concomitant with 30-40% increased TG levels. The effect of CD36 deficiency on increased plasma FA levels can easily be explained by an impaired peripheral uptake.[9] Although it has been postulated that the VLDL-TG production rate may be enhanced in CD36-deficient mice[10], the mechanism underlying the effect of CD36 on TG metabolism had not been addressed yet. 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 FA levels, rather than by an increased production of hepatic VLDL-TG or increased intestinal lipid absorption.

Recently, we have shown that the increased plasma FA levels in CD36-deficient mice lead to an enhanced FA flux towards the liver, resulting in increased TG storage (hepatic steatosis).[16] Hepatic VLDL-TG production is thought to be primarily a substrate-driven process, regulated by the availability of FA (reviewed by Lewis *et al.*[17]). Furthermore, acute elevation of plasma FA levels stimulates VLDL-TG production in humans.[18] Therefore, the increased FA flux to the liver in CD36 deficiency[16] may result in an 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 elevated plasma FA levels and hepatic steatosis in *cd36* $^{-/-}$ mice, we did not detect any effect of CD36 deficiency on expression of genes involved in transcriptional regulation (*pparα*, *pparγ*, *srebp1c*) or VLDL-TG synthesis (*apob*, *apobec*, *apoe*, *mttp*) (not shown). Importantly, CD36 deficiency did not affect the actual VLDL-TG production rate or composition of nascent VLDL-TG. Similar to CD36-deficient mice, genetically obese *ob/ob* mice[19] and human apoCI-overexpressing mice[20] also have increased plasma FA levels and hepatic steatosis, but display normal hepatic VLDL-TG production. Apparently, increased plasma FA levels and hepatic steatosis *per se* do not necessarily lead to increased VLDL-TG production.

CD36 is highly expressed in the apical membrane of enterocytes in the intestinal jejunal villi.[1,21] Since this location is the main site of FA (lipid) absorption and CD36 does act as a FA transporter, CD36 is thought to play a role in the intestinal uptake of FA.[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 get more insight into the mechanism underlying the observed hypertriglyceridemia in CD36-deficient 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 as compared to wild type littermates. Concomitantly, the plasma FA concentrations also increased to approximately 5 mM in *cd36*$^{-/-}$ mice, as compared to only 2 mM in control littermates. Mouse plasma contains approximately 0.5 mM albumin, which under normal circumstances carries the major part of plasma FA. Since albumin has 4 high-affinity binding sites for FA[24], albumin is capable of binding about 2 mM FA in plasma. Apparently, the dramatically increased FA levels upon the intragastric fat load in *cd36*$^{-/-}$ mice to a maximum of 5 mM exceed the maximum albumin-binding capacity. Since the amphiphilic nature of FA precludes its presence in plasma in an unbound state, it is likely that the FA 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 lipolyse VLDL-like emulsion particles in the absence of excess BSA as FA acceptor. Upon addition of an excess of FA-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 inhibition of lipases (mainly LPL) due to elevated plasma FA levels. We have indeed observed that a reduction of LPL activity in heterozygous LPL-deficient (*lpl* $^{+/-}$) mice (i.e. 40%) markedly elevated the postprandial TG response after an intragastric olive oil load as compared to wild type littermates ($AUC_{0\text{-}6}$: 43 ± 27 vs 3.5 ± 0.6, *lpl* $^{+/-}$, respectively; $P < 0.05$).

In our study we also show *in vivo* that in wild type mice 1.4-fold increased plasma FA 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 apoCII or apoCIII have changed between groups and impair 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 1) the inhibition of LPL activity by plasma from CD36-deficient

mice is relieved by addition of the FA-sequestrant BSA, and 2) elevation of plasma FA levels by infusion impairs TG clearance, strongly suggest that the hypertriglyceridemic phenotype of CD36-deficient mice is indeed mainly explained by increased FA levels.

These effects of increased plasma FA on tissue LPL activity may be explained by several mechanisms. Binding of FA 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 emulsions particles decreases sharply with the amount of FA formed unless albumin is present. An alternative mechanism has been proposed by Saxena and Goldberg[26] who showed *in vitro* that plasma FA levels may be important modulators of LPL interaction with the endothelial cell surface and apoCII. *In vivo* evidence for a role of plasma FA in the control of LPL was proposed in humans. Peterson ***et al.***[27] suggested that LPL is subject to feedback control by FA, involving an unusual mechanism that FA 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 due to a previously hypothesized enhancing effect on VLDL-TG 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 FA-induced product inhibition.

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