

# Mouse CD36 Has Opposite Effects on LDL and Oxidized LDL Metabolism In Vivo

Vilayphone Luangrath, Mathieu R. Brodeur, David Rhainds, Louise Brissette

**Objective**—The cluster of differentiation-36 (CD36) is a multifunctional protein which is recognized for its in vitro ability to take up oxidized low-density lipoproteins (oxLDL) in macrophages and is therefore considered atherogenic. It also binds LDL. Our objective was to define the physiological role of CD36 in both native LDL and oxLDL metabolism in mice.

**Methods and Results**—Clearance studies of labeled LDL and oxLDL were conducted in wild-type, CD36 knockout (KO), scavenger receptor class B, type I (SR-BI) KO, and SR-BI/CD36 double KO mice. We found that CD36 impedes the disappearance of native LDL and favors that of oxLDL. This was confirmed by association and degradation assays with primary cultures of hepatic cells from wild-type and CD36 KO mice. In addition, our in vivo work indicates that neither SR-BI nor CD36 plays a significant role in cholesteryl esters (CE) selective uptake (SU) from oxLDL, whereas CD36, in absence of SR-BI, can selectively take CE from LDL.

**Conclusion**—Our investigation showed for the first time that CD36 plays a significant role in oxLDL uptake in vivo in the mouse. As CD36 also retards LDL clearance, its atherogenic character may also relate to its negative effect on LDL catabolism. (*Arterioscler Thromb Vasc Biol.* 2008;28:1290-1295)

**Key Words:** CD36 ■ SR-BI ■ LDL ■ mouse ■ cholesterol

It is well established that high levels of plasma cholesterol associated with LDL increase the risk of developing atherosclerosis. The level of LDL in blood circulation is related to the synthesis rate of their precursors and their uptake by the liver.<sup>1</sup> A large part occurs through LDL-receptors (LDLr) which take up the entire LDL particles and lead to their complete degradation, a mechanism referred to as the holoparticle pathway. LDL can also selectively transfer their CE without concomitant degradation of their apolipoproteins, a process termed selective uptake (SU).

SR-BI is a cell surface receptor recognized for its ability to selectively take up CE from high-density lipoproteins (HDL)<sup>2</sup> and LDL.<sup>3</sup> Our in vivo studies<sup>4</sup> showed that ablation of SR-BI expression in mice leads to the complete loss of LDL-CE SU early after injection. However, after an hour, an acceleration of CE disappearance was obvious in SR-BI KO mice, indicating that a SR-BI-independent pathway can rescue SR-BI deficiency. A potential candidate for the SR-BI-independent pathway is CD36, another class B scavenger receptor. This is supported by studies showing that COS cells overexpressing either SR-BI or CD36 are equipotent toward LDL-CE uptake.<sup>5</sup> However the physiological importance of CD36 in LDL-CE SU remains to be established in vivo.

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LDL can undergo oxidative modification, and these are detectable in circulation. Many studies have used standard conditions generating fully oxidized LDL (oxLDL), which we refer simply as to oxLDL. Although oxLDL cannot interact with LDLr, it is known that injected oxLDL disappear faster than native LDL in rodents and that this uptake involves the liver.<sup>6</sup> In vivo studies by Ling et al<sup>7</sup> showed that the scavenger receptor class A type I/II (SR-A) is not implicated. Endemann et al<sup>8</sup> have revealed the importance of CD36 as an oxLDL receptor in vitro. Moreover we have demonstrated<sup>3</sup> that CD36 is partially responsible for oxLDL uptake in the human hepatic parenchymal cell model: HepG2. However, the role of CD36 in oxLDL clearance in vivo was never addressed. Inasmuch as it is generally accepted that mildly oxLDL are rather present in blood circulation, these particles also deserve consideration.

Little work was conducted on CE-SU from oxLDL. Whereas our group has reported this pathway in HepG2 cells,<sup>9</sup> using SR-BI-transfected Chinese hamster ovary (CHO) cells, Gillotte-Taylor et al<sup>10</sup> did not, but demonstrated greater oxLDL degradation compared to control cells. Recently, we have shown<sup>11</sup> in situ experimentations that both mouse

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From the Département des Sciences Biologiques (V.L., M.R.B., L.B.), Université du Québec à Montréal, and the Faculté de Pharmacie (D.R.), Université de Montréal, Québec, Canada.

V.L. and M.R.B. contributed equally to this study.

Correspondence to Louise Brissette, Département des Sciences Biologiques, Université du Québec à Montréal, C.P. 8888, Succursale Centre-ville, Montréal, Québec, Canada H3C 3P8. E-mail brissette.louise@uqam.ca

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parenchymal and nonparenchymal liver cells selectively take CE from mildly oxLDL and oxLDL. However, we could determine a role of SR-BI only for oxLDL by nonparenchymal cells. Thus another receptor or pathway needs to be defined for mildly oxLDL-CE SU in both parenchymal and nonparenchymal cells as well as for parenchymal cell CE-SU from oxLDL.

The aim of this study was to define the role of CD36 in native LDL, mildly oxLDL, and oxLDL holoparticle and CE-selective uptake. Clearance studies were therefore conducted with CD36 KO and wild-type mice. Our results show that CD36 impedes the disappearance of native LDL and favors that of mildly oxLDL and oxLDL. Also, our *in vivo* studies reveal that neither SR-BI nor CD36 plays a significant role in oxLDL-CE SU, whereas CD36 significantly shows LDL-CE SU activity in absence of SR-BI.

## Methods

### Animals

CD36 wild-type or deficient mice (backcrossed 7 times to C57BL/6J mice)<sup>12</sup> were obtained from Dr Maria Febbraio (Cleveland, Ohio). Heterozygous B6/129S-*Srb1*<sup>tm1kr13</sup> breeding pairs (backcrossed 8 times to C57BL/6J mice) were obtained from Jackson Labs (Bar Harbor, Me). For breeding and genotyping protocols please see supplemental materials (available online at <http://atvb.ahajournals.org>). Six- to 12-week-old male mice were used. This study was conducted according to protocols approved by the Animal Care Use Committee of Université du Québec à Montréal.

### Lipoprotein Profile and Cholesterol Assay

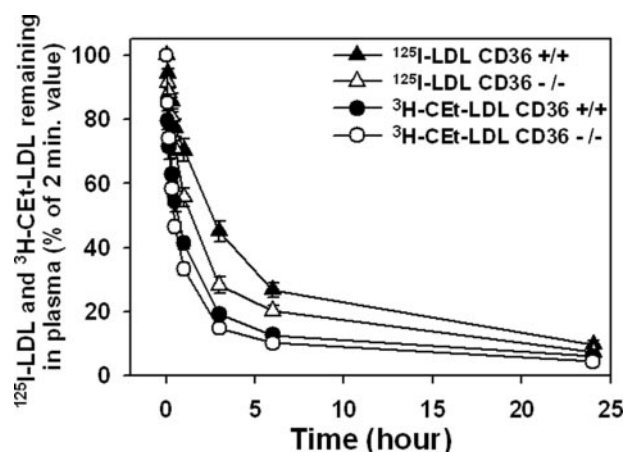
Three mice of all genotypes were fasted for 18 hours and bled through their vena cava. Lipoprotein fractionation of pooled plasma (400  $\mu$ L) was accomplished by fast protein liquid (FPLC) separation on a Superose 6 column HR13/50 (GE Healthcare). Total cholesterol, triglycerides, as well as free fatty acids were measured with commercial kits (Wako, Richmond, Va).

### Lipoprotein Isolation, Modification and Labeling

Human LDL (density 1.025 to 1.063 g/mL) were isolated from plasma as described in.<sup>4</sup> oxLDL were prepared as in Loughheed and Steinbrecher.<sup>14</sup> oxLDL typically resulted in a 2.8-fold increase in the electrophoretic mobility relative to native LDL on 0.5% agarose/barbital gels. Mildly oxLDL were obtained by incubation under similar conditions but for 4 hours and show a 1.5-fold increase in the electrophoretic mobility relative to native LDL. The 3 types of LDL were labeled with iodine-125 and in [<sup>3</sup>H]-cholesteryl oleoyl ether (CEt) as described previously.<sup>4</sup> Detailed characterization is available in supplemental Table I, and supplemental Figures I and II. Overall, labeling does not change the properties of LDL particles.

### Serum Decay and Cellular Distribution of LDL and Oxidized LDL in Liver

Mice were injected by the tail vein with a bolus of human LDL (either native or mildly oxLDL or oxLDL) containing 480  $\mu$ g of nonradiolabeled lipoproteins and 20  $\mu$ g of lipoproteins radiolabeled with <sup>125</sup>I or [<sup>3</sup>H]CEt in 150  $\mu$ L of saline. At the indicated time, blood samples were collected in microvette tubes coated with heparin from exposed saphenous veins and centrifuged at 10 000g for 5 minutes at 4°C. [<sup>3</sup>H]CEt was directly radioassayed from plasma, as it is not hydrolysable in cells and therefore free <sup>3</sup>H-cholesterol cannot return in plasma. Differently, <sup>125</sup>I was measured in the trichloroacetic acid (TCA)-precipitable fraction of plasma to eliminate the contribution of protein degradation. Disappearance curves of LDL were generated by dividing the plasma radioactivity at each point by the radioactivity determined 2 minutes after tracer injection, whereas that of oxLDL were based on the theoretical values.



**Figure 1.** Clearance of <sup>125</sup>I- and <sup>3</sup>H-CEt-labeled LDL in wild-type or CD36 KO mice. 480  $\mu$ g of nonradiolabeled human LDL and 20  $\mu$ g of LDL labeled with <sup>125</sup>I or with <sup>3</sup>H-CEt were injected in wild-type (+/+) or CD36 KO (-/-) mice. Each point represents the mean  $\pm$  SEM from 6 mice.

### Lipoprotein Cell Association Assays on Primary Cultures of Mouse Hepatic Cells

The isolation of hepatic cells from mouse liver and the association assay were done as described in Bourret et al<sup>11</sup> at the difference that primary cultures were used after a 4-hour adherence period.

## Results

### Role of CD36 in Native LDL Metabolism In Vivo in the Mouse

Our previous study in mice<sup>4</sup> showed that a SR-BI-independent pathway can rescue SR-BI deficiency for LDL-CE SU. To define this alternative pathway and to allocate it or not to a scavenger receptor, LDL-CE disappearance was followed in wild-type and SR-BI KO mice in absence or presence of mildly oxLDL and oxLDL that are recognized to bind to scavenger receptors. Supplemental Figure III (please see <http://atvb.ahajournals.org>) reveals that oxLDL retard the clearance of LDL-CE in wild-type mice and that both types of oxLDL exert that effect in SR-BI KO mice. Thus, at least in absence of SR-BI, the other pathway involved in LDL-CE clearance is by definition a scavenger receptor. CD36 became therefore a likely candidate, and LDL clearance was followed over a period of 24 hours in wild-type and CD36 KO mice. It can be observed in Figure 1 that in mice of both genotypes LDL-CE disappear faster than proteins, revealing CE-SU. The FCR data appearing in supplemental Table II (please see <http://atvb.ahajournals.org>) confirm these findings. Although no significant difference was detectable between the CE-SU FCR of wild-type and CD36 KO mice, it is however clear from Figure 1 and supplemental Table II that the most important effect of CD36 gene KO is to accelerate LDL-protein and -CE clearances. As supplemental Figure IV shows that abolishing CD36 expression does not affect the expression levels of hepatic LDLr and SR-BI, it is concluded that CD36 impedes LDL holoparticle uptake in wild-type mice.

The next approach we used to highlight the contribution of CD36 in LDL-CE clearance was to study clearance in the absence of both CD36 and SR-BI activities; the premise

**Table. Plasma Lipid Levels of Wild-Type, CD36 KO, SR-BI KO, and SR-BI/CD36 dKO Mice**

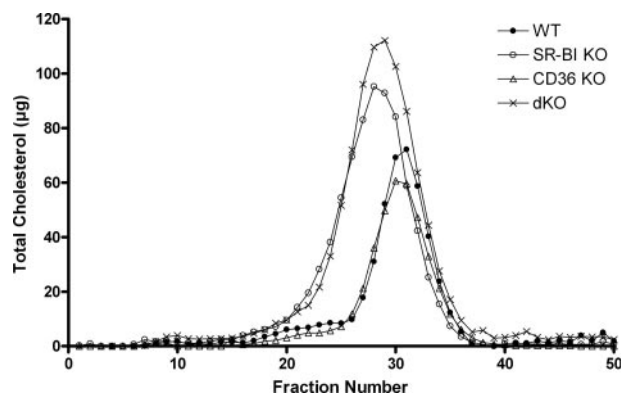
	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Free Fatty Acids mM
Wild-type	120.6±2.9 (10)	34.9±4.4 (8)	0.67±0.058 (6)
CD36 KO	88.2±4.2* (11)	32.4±3.0 (8)	1.21±0.039* (4)
SR-BI KO	208.3±10.7*,† (4)	35.3±2.2 (4)	0.62±0.065† (4)
CD36/SR-BI dKO	236.5±2.4*,† (4)	35.8±1.1 (4)	1.37±0.22* (2)

Values are mean±SEM from the No. of mice indicated in parenthesis.  
\*Statistically significant difference ( $P<0.005$ ) from wild-type mice. †Statistically significant difference ( $P<0.0001$ ) from CD36 KO mice.

being that if the CE-SU activity we measured in absence of SR-BI<sup>4</sup> is attributable to CD36, then no SU should be detectable. SR-BI/CD36 double (d) KO mice were therefore generated. Plasma cholesterol, triglyceride, and free fatty acid concentrations of wild-type, CD36 KO, SR-BI KO, and SR-BI/CD36 dKO mice are shown in the Table and their lipoprotein cholesterol patterns in Figure 2. Our cholesterol results for SR-BI KO mice are very similar to those of Rigotti et al,<sup>13</sup> as a higher plasma cholesterol level and a greater HDL-cholesterol peak were observed. However differently from Febbraio et al,<sup>12</sup> we have not detected a 30% increase in HDL cholesterol in CD36 KO mice, but rather a 27% decrease. Others<sup>15</sup> have seen no difference between the 2 genotypes. Thus we conclude that this modest discrepancy may relate to the starving period or the method of blood collection. Indeed, differently from Febbraio et al<sup>12</sup> our mice were starved for a longer period. The dKO mice show slightly higher levels of plasma cholesterol and a bigger HDL-cholesterol peak than those of SR-BI KO mice. As seen in the Table, no significant differences were detected between the triglyceride levels of the 4 genotypes. However, as shown by Febbraio et al,<sup>12</sup> our CD36 KO mice have higher levels of free fatty acids than wild-type mice. This 70% increase is also found in SR-BI/CD36 dKO mice. LDL-protein and CE clearance studies were conducted in wild-type and SR-BI/CD36 dKO mice. Figure 3 demonstrates that CE-SU is abolished in the dKO mice, indicating that in absence of SR-BI activity, CD36 is able to selectively take up CE from LDL.

### Role of CD36 in oxLDL Metabolism In Vivo in the Mouse

Experiments were conducted to define the role of CD36 in oxLDL metabolism in vivo. Clearance was followed for 1 hour, as oxLDL disappear rapidly. The comparison of protein to CE disappearance curves reveals that in wild-type mice mildly oxLDL are subjected to CE-SU (Figure 4A) but not oxLDL (Figure 4B). Thus in the mouse, the extent of LDL oxidation is negatively correlated with the magnitude of CE-SU. Importantly, CD36 deficiency does not accelerate decay as for LDL but delays protein and CE disappearances of both mildly oxLDL (Figure 4A) and oxLDL (Figure 4B). As both protein and CE clearances are delayed, we conclude that CD36 plays a significant role in the holoparticle uptake of both types of oxLDL. These data are supported by the



**Figure 2.** Fast protein liquid chromatography of plasma lipoproteins from wild-type, CD36 KO, SR-BI KO, and SR-BI/CD36 dKO mice. Plasma was collected by heart puncture from 3 animals of each genotype and was subjected to fast pressure liquid chromatography. Total cholesterol was measured in each fraction.

half-life data shown in supplemental Figure V. The effect of CD36 being incomplete, we looked for an implication of SR-BI by conducting the same experiment but with wild-type and SR-BI KO mice. The results presented in supplemental Figure V and VI are clearly against a role of SR-BI in both types of oxLDL holoparticle or selective uptake.

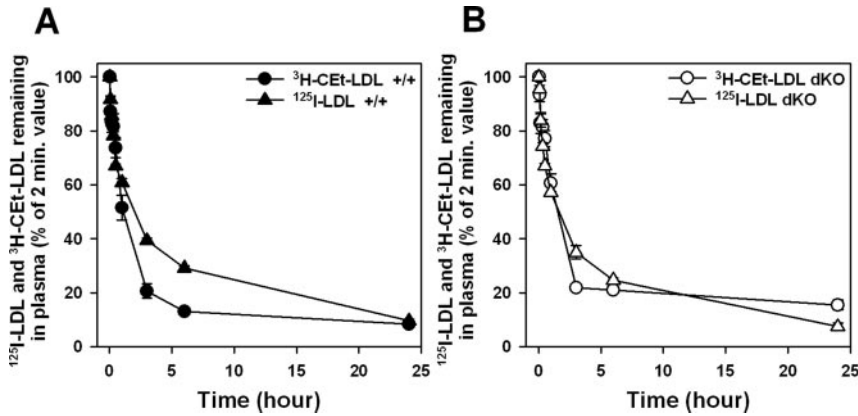
### Role of Mouse Hepatic Cell CD36 in Native LDL and oxLDL Metabolism In Vitro

To assess the involvement of hepatic CD36, experiments were conducted with primary cultures of hepatic cells from wild-type and CD36 KO mice. Supplemental Figure IV shows that these liver cells, which contain 90% hepatocytes, express CD36. Figure 5 reveals that although CD36 deficiency reduces LDL association, it increases LDL degradation by 110%. Thus, CD36 also impairs LDL uptake/degradation in primary cultures of mouse hepatic cells. Interestingly, mildly oxLDL degradation was raised by 55%, whereas oxLDL degradation was reduced by 60% in accordance with clearance studies. Differently, CE association and CE-SU were not significantly affected by the CD36 deficiency, except for oxLDL, where for an unknown reason a reduction by half of the CE association and CE-SU was detectable. It is however likely that this apparent discrepancy is simply linked to the fact that oxLDL disappear so quickly from mouse blood circulation that CE-SU does not occur at a significant level in vivo or is barely detectable. If true this would mean that in vitro, hepatic CD36 can selectively take up CE from oxLDL.

### Discussion

Although the role of SR-BI in HDL-CE SU is well accepted, in vitro assays using CHO<sup>16</sup> and Y1 2/3 adrenal cells<sup>17</sup> have rather suggested SR-BI-independent pathways for LDL-CE SU. The first study showed the importance of lipoprotein lipase, and the last of LDLr-related protein (LRP) and apoE. Later, we showed<sup>4</sup> by in vivo clearance studies in wild-type and SR-BI KO mice that SR-BI is responsible for LDL-CE SU but that a SR-BI-independent pathway can rescue SR-BI deficiency. The clearance studies in SR-BI/CD36 dKO mice





**Figure 3.** Clearance of  $^{125}\text{I}$ - and  $^3\text{H}$ -CEt-labeled LDL in wild-type or SR-BI/CD36 double KO mice.  $480\ \mu\text{g}$  of nonradiolabeled human LDL and  $20\ \mu\text{g}$  of LDL labeled with  $^{125}\text{I}$  or with  $^3\text{H}$ -CEt were injected in wild-type (A) or SR-BI/CD36 double KO (B) mice. Each point represents the mean  $\pm$  SEM from 6 mice.

(Figure 3) presented in this study directly pointed out CD36 as being responsible for the SR-BI-independent pathway *in vivo*. As the cholesterol lipoprotein patterns do not differ substantially between SR-BI KO and SR-BI/CD36 dKO mice (Figure 2 and Table), it is unlikely that the complete loss of CE-SU encountered in the dKO mice is a consequence of the endogenous pool of lipoproteins of these mice.

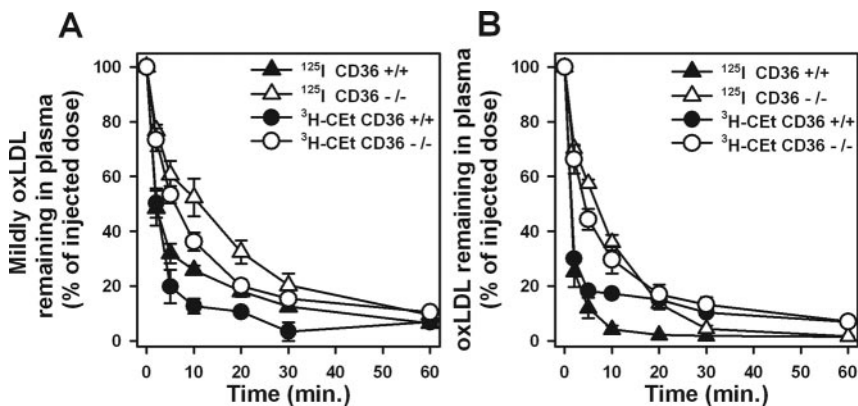
As CD36 plays a role in LDL-CE SU in absence of SR-BI and no SR-BI deficiency has yet been described in humans, the physiological importance of CD36 in LDL clearance is uncertain. On the other hand, if loss-of-function mutations are to be discovered in the SR-BI gene, it will become important to address in more details the effect of CD36-mediated CE-SU on LDL metabolism and to define whether the pathway is anti- or proatherogenic. Indeed, although HDL-CE SU has been for many years considered as beneficial, more investigations are needed before such a conclusion can be drawn for LDL-CE SU either by SR-BI or CD36, as it is possible that small dense LDL are generated by CE depletion and such a subclass of LDL is positively associated with coronary heart diseases (CHD).<sup>18</sup>

Importantly, we found *in vivo* (Figure 1) and *in vitro* (Figure 5) that murine CD36 impairs uptake/degradation of LDL-protein. The *in vitro* study revealed that mildly oxLDL are also more degraded in CD36 deficient cells, but not oxLDL. It is likely that in absence of CD36, the association of mildly oxLDL to the LDLr is favored because these may also interact with LDLr.<sup>19</sup> As the LDLr level is not different between wild-type and CD36 KO mice (supplemental Figure IV) and as both receptors have similar affinities for human

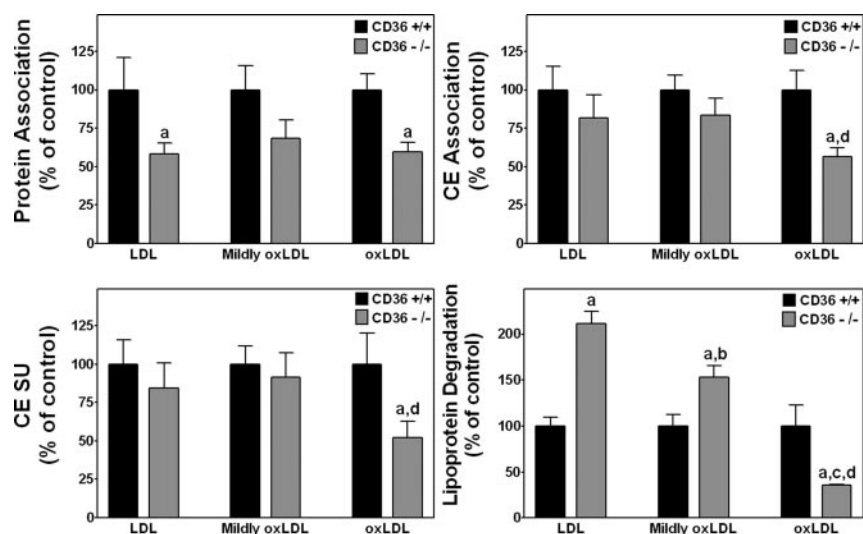
LDL,<sup>20,21</sup> we suggest that CD36, by tethering native LDL particles to the cell surface, reduces the association of LDL to the LDLr and retards their clearance.

We also showed *in vivo* that the extent of LDL oxidation is negatively correlated with CE-SU (Figure 4). It can be speculated that when oxidized, LDL particles lose their ability to deliver as much CE by the selective uptake pathway as native LDL. Alternatively, the receptor(s) involved may have a lower potential toward them than for native LDL. It is also possible that oxLDL are too readily taken up by hepatic cells for ultimate degradation to allow a CE-SU process. Moreover, we herein demonstrate by *in vivo* clearance studies that neither CD36 nor SR-BI is a mediator of oxLDL-CE SU (Figure 4 and supplemental Figure VI). Because we have shown that CE-SU can occur from mildly oxLDL, this means that the receptor or the mechanism involved requires identification.

Interestingly, CD36 deficiency, although it does not accelerate decay as for LDL, delays both protein and CE disappearances of mildly oxLDL and oxLDL (Figure 4), indicating that CD36 significantly, although not completely, mediates oxLDL holoparticle uptake. The partial implication of CD36 in oxLDL global uptake was already shown *in vitro*.<sup>3</sup> Nonetheless, we provide the first evidence of the importance of CD36 *in vivo*. It is unlikely that SR-AI/II is the other receptor partially involved because Ling et al<sup>7</sup> have compared the plasma clearance of oxLDL in SR-AI/II KO and wild-type mice and found no difference. The same conclusion applies to SR-BI as we have seen no impact in oxLDL clearance in SR-BI KO mice (supplemental Figure VI). Thus, more



**Figure 4.** Clearance of  $^{125}\text{I}$ - and  $^3\text{H}$ -CEt-labeled mildly oxLDL or oxLDL in wild-type or CD36 KO mice.  $450\ \mu\text{g}$  of nonradiolabeled lipoprotein and  $50\ \mu\text{g}$  of the same lipoprotein labeled with  $^{125}\text{I}$  or with  $^3\text{H}$ -CEt-injected in wild-type (+/+) or CD36 KO (-/-) mice. A, Mildly oxLDL. B, oxLDL. Each point represents the mean  $\pm$  SEM from 3 mice.



**Figure 5.** <sup>3</sup>H-CE and <sup>125</sup>I-protein association and protein degradation of native LDL and mildly oxLDL or oxLDL in primary cultures of wild-type and CD36 KO mouse hepatic cells. Wild-type (black bars) and CD36 KO (gray bars) mouse hepatic cells were incubated with 20 μg/mL radiolabeled LDL or mildly oxLDL or oxLDL. Results are mean ± SEM of 5 to 11 experiments. <sup>a</sup>Statistically significant difference ( $P < 0.05$ ) between the proper association of a lipoprotein with wild-type and CD36 KO hepatocytes. <sup>b</sup>Mildly oxLDL and LDL. <sup>c</sup>oxLDL and LDL. <sup>d</sup>Mildly oxLDL and oxLDL.

investigations are also required to identify the other mouse oxLDL receptor/mediator leading as CD36 to holoparticle uptake.

The matter of the tissue(s) from which CD36 exerts its effect on native and oxLDL metabolism in vivo deserves some attention, as it is frequently assumed that CD36 is barely present in liver and our results suggest that the effect that we observed is attributable to hepatic CD36. Firstly, it is well accepted that when oxLDL are injected in rodents, they are readily cleared by the liver which is also the major tissue involved in native LDL uptake.<sup>7</sup> Secondly, in the present study we show a detectable level of CD36 in mouse hepatic cells and we were able to detect, with primary cultures of these cells, a very similar impact of CD36. However, this does not completely exclude that part of the effect we observed in vivo is attributable to another tissue.

It is important to discuss our findings in relation to the association of CD36 with CHD. Indeed, as recently reviewed by Febbraio and Silverstein,<sup>22</sup> CD36 can be viewed as “The Good and The Bad”, depending on its very many roles. Very elegant studies were conducted in hyperlipidemic mouse models and all<sup>23–25</sup> but one of them<sup>26</sup> revealed a proatherosclerotic role of CD36. Based on our results, CD36 by retarding LDL disappearance in the circulation can be regarded as proatherosclerotic. In opposition, it can be viewed as antiatherosclerotic by being involved in oxLDL clearance by the liver. As oxLDL are usually found at a much lower concentration than LDL in blood circulation, our data rather suggest a proatherosclerotic role of murine CD36. How can our results be extrapolated to humans? Human CD36 deficiency exists and has been divided into 2 subgroups according to the phenotypes. In type I deficiency, neither platelets nor monocytes express CD36. CD36 is expressed in monocytes but not in platelets of type II deficient individuals. This type is very rare in Whites (0.3% of the population) but more frequent in Asians and African Americans (3 to 4%).<sup>27</sup> Interestingly, evidence is growing that CD36 might be beneficial in humans. Indeed, abnormalities of glucose and lipid metabolism were shown in CD36 deficiency including increased plasma triglycerides, decreased HDL-cholesterol,

increased LDL-cholesterol, impaired glucose tolerance, and delayed response of insulin secretion.<sup>28–29</sup> Moreover, the frequency of CD36 deficiency is higher in patients with CHD than in control subjects.<sup>30</sup> The apparent difference between conclusions reached in humans and rodents may be a consequence of their lipoprotein profiles, as the LDL/HDL ratio is higher in humans than in rodents. Furthermore, it is possible that the effect of a receptor such as CD36 on atherosclerosis depends on the plasma lipoprotein concentrations and compositions (normo- versus hyperlipidemic).

In conclusion, our study has demonstrated that CD36: (1) can lead to LDL-CE SU in absence of SR-BI; (2) retards LDL clearance; and (3) is responsible for a significant fraction of oxLDL particle clearance.

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## Disclosures

None.

## References

- Lilly SM, Rader DJ. New targets and emerging therapies for reducing LDL cholesterol. *Curr Opin Lipidol*. 2007;18:650–655.
- Acton S, Rigotti A, Landschulz KT, Xu S, Hobbs HH, Krieger M. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science*. 1996;271:518–520.
- Rhoads D, Brodeur M, Lapointe J, Charpentier D, Falstra L, Brissette L. The role of human and mouse hepatic scavenger receptor class B type I (SR-BI) in the selective uptake of low density lipoprotein cholesteryl esters. *Biochemistry*. 2003;42:7527–7538.
- Brodeur MR, Luangrath V, Bourret G, Falstra L, Brissette L. Physiological importance of SR-BI in the in vivo metabolism of human HDL and LDL in male and female mice. *J Lipid Res*. 2005;46:687–696.
- Connelly MA, Kellner-Weibel G, Rothblat GH, Williams DL. SR-BI-directed HDL-cholesteryl ester hydrolysis. *J Lipid Res*. 2003;44:331–341.

6. Van Berkel TJ, De Rijke YB, Kruijt JK. Different fate in vivo oxidatively modified low density lipoprotein and acetylated low density lipoprotein in rats. Recognition by various scavenger receptors on Kupffer and endothelial liver cells. *J Biol Chem.* 1991;266:2282–2289.
7. Ling W, Loughheed M, Suzuki H, Buchan A, Kodama T, Steinbrecher UP. Oxidized or acetylated low density lipoproteins are rapidly cleared by liver in mice with disruption of the scavenger receptor class A type I/II gene. *J Clin Invest.* 1997;100:244–252.
8. Endemann G, Stanton LW, Madden KS, Bryant CM, White RT, Protter AA. CD36 is a receptor for oxidized low density lipoprotein. *J Biol Chem.* 1993;268:11811–11816.
9. Rhoads D, Falstra L, Tremblay C, Brissette L. Uptake and fate of class B scavenger receptor ligands in HepG2 cells. *Eur J Biochem.* 1999;26:227–235.
10. Gillotte-Taylor K, Boullier A, Witztum JL, Steinberg D, Quehenberger O. Scavenger receptor class B type I as a receptor for oxidized low density lipoprotein. *J Lipid Res.* 2001;42:1474–1482.
11. Bourret G, Brodeur MR, Luangrath V, Lapointe J, Falstra L, Brissette L. In vivo cholesteryl ester selective uptake of mildly and standardly oxidized LDL occurs by both parenchymal and nonparenchymal mouse hepatic cells but SR-BI is only responsible for standardly oxidized LDL selective uptake by nonparenchymal cells. *Int J Biochem Cell Biol.* 2006;38:1160–1170.
12. Febbraio M, Abumrad NA, Hajjar DP, Sharma K, Cheng W, Pearce SF, Silverstein RL. A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism. *J Biol Chem.* 1999;274:19055–19062.
13. Rigotti A, Trigatti BL, Penman M, Rayburn H, Herz J, Krieger M. A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor scavenger receptor class B type I reveals its key role in HDL metabolism. *Proc Natl Acad Sci USA.* 1997;94:12610–12615.
14. Loughheed M, Steinbrecher UP. Mechanism of uptake of copper-oxidized low density lipoprotein in macrophages is dependent on its extent of oxidation. *J Biol Chem.* 1996;271:11798–11805.
15. Goudriaan JR, Dahlmans VE, Teusink B, Ouwers DM, Febbraio M, Maassen JA, Romijn JA, Havekes LM, Voshol PJ. CD36 deficiency increases insulin sensitivity in muscle, but induces insulin resistance in the liver in mice. *J Lipid Res.* 2003;44:2270–2277.
16. Seo T, Al-Haideri M, Treskova E, Worgall TS, Kako Y, Goldberg IJ, Deckelbaum RJ. Lipoprotein lipase-mediated selective uptake from low density lipoprotein requires cell surface proteoglycans and is independent of scavenger receptor class B type I. *J Biol Chem.* 2000;275:30355–30362.
17. Swarnakar S, Beers J, Strickland DK, Azhar S, Williams DL. The apo-lipoprotein E-dependent low density lipoprotein cholesteryl ester selective uptake pathway in murine adrenocortical cells involves chondroitin sulfate proteoglycans and an alpha 2-macroglobulin receptor. *J Biol Chem.* 2001;276:21121–21128.
18. Berneis K, Rizzo M. LDL size: does it matter? *Swiss Med Wkly.* 2004;134:720–724.
19. Pedreno J, de Castellarnau C, Cullare C, Ortin R, Sanchez JL, Llopart RL, Gonzalez-Sastre F. Platelet LDL receptor recognizes with the same apparent affinity both oxidized and native LDL. *Arterioscler Thromb. Biochem J.* 1996;318:841–847.
20. Brissette L, Charest MC, Falstra L. Selective uptake of cholesteryl esters of low-density lipoproteins is mediated by the lipoprotein-binding site in HepG2 cells and is followed by the hydrolysis of cholesteryl esters. *Biochem J.* 1996;318:841–847.
21. Calvo D, Gómez-Coronado D, Suárez Y, Lasunción MA, Vega MA. Human CD36 is a high affinity receptor for the native lipoproteins HDL, LDL, and VLDL. *J Lipid Res.* 1998;39:777–788.
22. Febbraio M, Silverstein RL. CD36: Implications in cardiovascular disease. *Int J Biochem Cell Biol.* 2007;39:2012–2030.
23. Febbraio M, Podrez EA, Smith JD, Hajjar DP, Hazen SL, Hoff HF, Sharma K, Silverstein RL. Targeted disruption of the class B scavenger receptor CD36 protects against atherosclerotic lesion development in mice. *J Clin Invest.* 2000;105:1049–1056.
24. Febbraio M, Guy E, Silverstein RL. Stem cell transplantation reveals that absence of macrophage CD36 is protective against atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2004;24:2333–2338.
25. Guy E, Kuchibhotla S, Silverstein R, Febbraio M. Continued inhibition of atherosclerotic lesion development in long term Western diet fed CD36<sup>0</sup>/apoE<sup>0</sup> mice. *Atherosclerosis.* 2007;192:123–130.
26. Moore KJ, Kunjathoor VV, Koehn SL, Manning JJ, Tseng AA, Silver JM, McKee M, Freeman MW. Loss of receptor-mediated lipid uptake via scavenger receptor A or CD36 pathways does not ameliorate atherosclerosis in hyperlipidemic mice. *J Clin Invest.* 2005;115:2192–2201.
27. Yamamoto N, Akamatsu N, Sakuraba H, Yamazaki H, Tanoue K. Platelet glycoprotein IV (CD36) deficiency is associated with the absence (type I) or the presence (type II) glycoprotein IV on monocytes. *Blood.* 1994;83:392–397.
28. Miyaoka K, Kuwasako T, Hirano K, Nozaki S, Yamashita S, Matsuzawa Y. CD36 deficiency associated with insulin resistance. *Lancet.* 2005;357:686–687.
29. Yanai H, Chiba H, Morimoto M, Abe K, Fujiwara H, Fuda H, Hui SP, Takahashi Y, Akita H, Jamieson GA, Kobayashi K, Matsuno K. Human CD36 deficiency is associated with elevation in low-density lipoprotein-cholesterol. *Am J Med Genet.* 2000;93:299–304.
30. Yamashita S, Hirano K, Kuwasako T, Janabi M, Toyama Y, Ishigami M, Sakai N. Physiological and pathological roles of a multi-ligand receptor CD36 in atherogenesis; insights from CD36-deficient patients. *Mol Cell Biochem.* 2007;299:19–22.