Oxidized VLDL Induces Less Triglyceride Accumulation in J774 Macrophages Than Native VLDL Due to an Impaired Extracellular Lipolysis

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Abstract—The present study examined the relative contributions of the different pathways by which oxidatively modified VLDL (oxVLDL) promotes the uptake and intracellular accumulation of lipids in J774 macrophages. VLDL was oxidized for a maximum of 4 hours, resulting in an increase in thiobarbituric acid–reactive substances and an increased electrophoretic mobility on agarose gel. The lipid composition of the relatively moderately oxidized VLDL samples did not differ significantly from that of nonoxidized VLDL samples. The uptake of 125I-labeled VLDL by the J774 cells increased with oxidation time and was completely blocked on coincubation with polyinosinic acid (PolyI), indicating that oxVLDL is taken up by the cells via the scavenger receptor only. Despite the 2-fold increased uptake of oxVLDL protein, the cell association of triglyceride (TG)-derived fatty acids by the J774 macrophages after incubation with oxVLDL was only 50% of that with native VLDL. In line with these observations, the induction of de novo synthesis of TG by J774 cells was ~3-fold less efficient after incubation with oxVLDL than after incubation with native VLDL. The induction of de novo synthesis of TG with oxVLDL was even further decreased on simultaneous incubation with PolyI, whereas PolyI did not affect the native VLDL-induced TG synthesis. These results indicate that oxVLDL induces endogenous TG synthesis predominantly through particle uptake via the scavenger receptor and much less via the extracellular lipoprotein lipase (LPL)–mediated hydrolysis of TG, as is the case for native VLDL. In line with these observations, we showed that the suitability of VLDL as a substrate for LPL decreases with oxidation time. Addition of oxVLDL to the LPL assay did not interfere with the lipolysis of native VLDL. However, enrichment of the oxidized lipoprotein particle with native apoC2 was able to fully restore the impaired lipolysis. Thus, from these studies it can be concluded that on oxidation, VLDL becomes less efficient in inducing TG accumulation in J774 cells as a consequence of a defect in apoC2 as an activator for the LPL-mediated extracellular lipolysis. (Arterioscler Thromb Vasc Biol. 2000;20:144-151.)

Key Words: oxidized VLDL ■ lipolysis ■ lipid accumulation ■ macrophages

The role of triglyceride (TG)-rich lipoproteins in foam cell formation is currently under investigation. Several groups of investigators have shown that human VLDL is capable of inducing cholesteryl ester and TG accumulation in different macrophage culture systems.1–4 It has been postulated that the mechanisms by which VLDL stimulates cellular lipid accumulation involve at least 2 different pathways2,4: (1) the receptor-mediated uptake of intact VLDL particles and (2) the direct uptake of free fatty acids (FFAs) as generated by the extracellular lipoprotein lipase (LPL)–mediated hydrolysis of VLDL-TG, followed by intracellular reesterification into lipids. The resulting cholesterol-enriched remnant particles are thereafter taken up via a receptor-mediated process.

In analogy with LDL,5,6 it has been shown that in vitro exposure of β-VLDL to endothelial cells causes oxidation of this lipoprotein.7,8 This results in a 2- to 3-fold increased degradation by mouse peritoneal macrophages7 and rabbit smooth muscle cells8 compared with unoxidized β-VLDL and in a 2- to 3-fold increased intracellular cholesterol esterification rate. Isolated human VLDL, like human LDL, was shown to be effectively oxidized in vitro on incubation with free radicals.9 The degradation of oxidized human VLDL (oxVLDL) by mouse peritoneal macrophages was also increased 2-fold compared with native VLDL.10 More recently, it was shown that oxVLDL causes greater accumulation of cholesteryl ester in J774 macrophages than oxLDL.11 Altogether, these data suggest that oxidation of VLDL augments its atherogenic potential and may contribute to foam cell formation in humans.

So far, little is known with respect to the pathways by which oxVLDL promotes uptake and intracellular accumulation of lipids in macrophages. Recent studies by Whitman et
al showed a decrease in cellular TG levels on incubation with oxidized hypertriglyceridemic VLDL, strongly pointing to a different routing for the uptake of oxidized lipids compared with their nonoxidized counterparts. In the present study, we examined more closely the processing of oxidatively modified forms of human VLDL in the murine macrophage cell line J774. Despite the enhanced cellular (protein) uptake, we found that normolipidemic human oxVLDL induces less TG accumulation in these macrophages than native VLDL. The difference between oxVLDL and normal VLDL in this respect appeared to be due to an impaired LPL-mediated extracellular lipolysis of oxVLDL-TG as a consequence of defective apoC2.

Methods

Cells
Murine macrophage-like J774 cells were cultured in 75-cm² flasks in DMEM supplemented with 10% (vol/vol) FCS, 0.85 g/L NaHCO₃, 4.76 g/L HEPES, 100 U/mL penicillin, 100 mg/mL streptomycin, and 2 mmol/L glutamine. The cells were cultivated at 37°C in an atmosphere containing 5% CO₂ and 95% air. For each experiment, the cells were plated in 6- or 24-well plates. The cells were fed every 3 days and were used for experiments within 7 days after plating. Twenty-four hours before each experiment, the cells were washed with DMEM containing 1% (wt/vol) BSA and further incubated with DMEM containing 5% (vol/vol) of lipoprotein-deficient human serum (d>1.21 g/mL) instead of FCS. The experiments were subsequently conducted in DMEM containing 1% (wt/vol) BSA.

Lipoproteins
Human VLDL, LDL, and HDL were isolated according to Redgrave et al. by density-gradient ultracentrifugation of pooled plasma obtained from healthy normolipidemic volunteers after an overnight fast. Immediately after isolation, the lipoprotein fractions were extensively dialyzed against PBS (pH 7.4) containing 10 mmol/L EDTA at 4°C. The protein contents of the VLDL and LDL samples were determined by the method of Lowry et al. After isolation, part of the VLDL sample was labeled with glycerol tril[14C]oleate (Amersham; specific activity, 61 mCi/mmol) as described by Groener et al. In brief, 5 µCi glycerol tril[14C]oleate was added to 12.8 µL phosphatidylcholine (100 mg/mL) and 10 µL butyral hydroxystearate in chloroform (1 mmol/L). After the evaporation of the chloroform under a stream of nitrogen, 1 mL of 50 mmol/L Tris/HCl, pH 7.5, containing 0.27 mmol/L EDTA was added. The suspension was sonicated twice for 5 minutes under nitrogen with a Lasonic 1510 sonicator. The sonicated lipids were added to a mixture of 6.24 mL human lipoprotein-deficient serum, 0.22 mL, 0.13-mol/L EDTA, and 0.94 mL 10-mmol/L DTNB. Subsequently, 5 mg of VLDL-TG was added, and the mixture was incubated for 40 hours at 37°C. The VLDL was reisolated by ultracentrifugation as described above. The degree of oxidation was determined by measuring thiobarbituric acid–reactive substances (TBARS) with thiobarbituric acid (in 20% trichloroacetic acid) with fresh malonaldehyde-tetramethylethelal as a standard. Furthermore, the degree of oxidation was determined by agarose gel electrophoresis (100 V, 30 minutes, Paragon Lipoprotein Electrophoresis kit, Beckman Instruments), and the electrophoretic mobility relative to native VLDL of the different oxVLDL fractions was calculated.

The LDL fraction was acetylated by repeated additions of acetic anhydride as described by Basu et al. The conversion of LDL into acetylated LDL (acLDL) was confirmed by agarose gel electrophoresis as described above.

Interaction of VLDL and oxVLDL with J774 Macrophages

Lipoprotein Uptake
The J774 cells were cultured in 24-well plates as described above. The association and degradation of 125I-labeled VLDL and oxVLDL was determined after a 4-hour incubation at 37°C with 10 µg/mL 125I-labeled lipoprotein in a final incubation volume of 0.5 mL, either in the absence or in the presence of a 20-fold excess of the respective unlabeled lipoprotein. The receptor-mediated (specific) cell association and degradation was calculated by subtracting the amount of labeled lipoproteins that was associated or degraded after incubation in the presence of an excess of unlabeled lipoprotein (nonspecific) from the amount of labeled lipoprotein that was cell-bound after incubation in the absence of unlabeled lipoprotein (total). At the end of the incubation period, a fraction of the medium was removed to determine the amount of lipoprotein degraded as described previously. After the remaining portion of the medium had been removed, the cells were washed 4 times with ice-cold PBS containing 0.1% (wt/vol) BSA and subsequently with PBS without BSA. To measure the cell-associated lipoprotein fraction, the washed cells were dissolved in 1 mL 0.2-mmol/L NaOH, and an aliquot of the cell lysate was counted for radioactivity. Another aliquot was used for protein determination according to Lowry et al. In the respective figures, lipoprotein uptake is expressed as the sum of cell-associated and degraded lipoproteins.

The effect of acLDL and polyinosinic acid (PolyI) on the receptor-mediated uptake of 125I-labeled oxVLDL was determined in competition experiments. Therefore, J774 cells were incubated for 4 hours at 37°C with 10 µg/mL of 125I-labeled oxVLDL in the presence of different concentrations of unlabeled oxVLDL, acLDL, or PolyI, as described in the text and the figure legends. Thereafter, the association and degradation of 125I-oxVLDL were determined exactly as described above.

Effect on Cellular and Medium Lipids
J774 cells were cultured in 6-well plates, and VLDL was labeled in the TG with glycerol tril[14C]oleate and subsequently oxidized as described above. The effect of [14C]-TG-VLDL and [14C]-TG-oxVLDL

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In Vitro Lipolysis of VLDL and oxVLDL

In vitro lipolysis experiments were performed as previously described. Briefly, VLDL samples were incubated at 37°C in 0.1 mol/L Tris/HCl, pH 8.5, in the presence of 1% (wt/vol) albumin (essentially FFA-free) and 0.2 U of commercially available bovine LPL (Sigma Chemical Co). After 10 minutes, the reaction was stopped by the addition of 50 mmol/L KH₂PO₄, 0.1% Triton-X100, 20% (vol/vol) glycerol, pH 8.5) for 1 hour at 4°C. Unbound LPL was removed by washing the plates 3 times with 0.1 mol/L Tris. To examine the effect of oxidized lipoproteins on LPL activity, HSPPG-LPL–coated plates were first incubated for 1 hour at 37°C with either native VLDL or oxVLDL4 (see below) (0.3 mmol/L TG). Thereafter, plates were washed 3 times with 0.1 mol/L Tris to remove all VLDL. Subsequently, the catalytic activity of the remaining HSPPG-bound LPL was assayed upon the addition of nonoxidized VLDL-TG samples (ranging from 0.1 to 0.5 mmol/L TG) to the wells and incubation of the plate for 20 minutes at 37°C. The reaction was terminated by the addition of 1% (vol/vol) Triton X-100 in 0.1 mmol/L Tris and cooling on ice. The FFA release by LPL bound to HSPPG was measured enzymatically as described above and was measured for 20 minutes assayed.

The ability of oxVLDL to inhibit LPL activity was further examined on the addition of a constant amount of oxVLDL4 (0.2 mmol/L TG) to a range of native VLDL samples (0.1 to 0.5 mmol/L TG) in the HSPPG-LPL assay solution. The rate of FFA release was measured as described above.

Statistical Analysis

The statistical significance between native VLDL and oxVLDL mean values was assessed with Student’s unpaired t test. A value of *P<0.05 was considered statistically significant.

Results

Characterization of VLDL Samples

Human VLDL was incubated with the peroxyl radical generator AAPH for different time periods (0, 1, 2, 3, 4, and 5 hours of incubation: VLDL, oxVLDL1, oxVLDL2, oxVLDL3, and oxVLDL4, respectively). The oxidative properties of VLDL were analyzed by TBARS assay and electrophoretic mobility on agarose gel. As shown in the Table, the oxidative changes in VLDL were accompanied by a gradual increase in

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The free cholesterol (FC), cholesteryl ester (CE), TG, phospholipid (PL), and FFA contents of native VLDL and oxidized VLDL (the samples were oxidized for 1, 2, 3, or 4 hours, respectively) were measured as described in the Methods section and are expressed in μmol/mg VLDL protein or mmol/L. The rate of oxidation was determined by measuring the TBARS. The relative electrophoretic mobility (REM) is the mobility of the samples on agarose gel relative to that of nonoxidized VLDL. All values are mean±SD of 4 experiments. ND indicates not determined.

*P<0.05, native VLDL vs oxidized VLDL samples by unpaired Student’s t test.
TBARS/mg VLDL protein and an increase in relative electrophoretic mobility on agarose gel compared with that of native VLDL. The VLDL cholesterol, TG, phospholipid, and FFA composition did not change significantly during 4 hours of incubation with AAPH (Table). Furthermore, SDS-PAGE analysis of oxVLDL showed a significant depletion in apoB, apoE, and apoC with increasing oxidation time (Figure 1). VLDL that was oxidized for 4 hours was used in further experiments.

Interaction of VLDL and oxVLDL With J774 Cells

To investigate the effects of oxidation on the cellular processing of TG-rich lipoprotein particles by macrophages, both native and oxVLDL-induced protein uptake and lipid accumulation by J774 cells was determined after a 4-hour incubation at 37°C. As shown in Figure 2A, the uptake (expressed as the sum of the cell-associated and degraded lipoprotein) of 125I-labeled oxVLDL4 by J774 cells was 2.5-fold higher than that of 125I-labeled native VLDL. To examine the effect of oxidation on the accumulation of TG-derived lipids in J774 cells, VLDL was used that was labeled in the TG moiety with glycerol tri[1-14C]oleate and subsequently oxidized (14C-TG-VLDL and 14C-TG-oxVLDL4). As shown in Figure 2B, after incubation with oxVLDL4, the total uptake of 14C-oleate by J774 cells is significantly decreased compared with that after incubation with native VLDL (14.5±0.6% versus 27.2±2.1% of total lipid added to the cells, respectively). Lipid extraction of the cell lysate and subsequent analysis by TLC showed that the decrease in 14C-oleate uptake on incubation with oxVLDL4 was reflected by a strong decrease in intracellular TG accumulation (Figure 2B). Thus, in contrast to the protein uptake, the TG-derived fatty acid uptake by J774 cells is reduced after incubation with oxVLDL compared with native VLDL.

To investigate the mechanisms underlying the decreased cellular uptake of TG-derived oleate on incubation with oxVLDL4, the relative contributions of the different pathways through which lipid accumulation is thought to occur were assessed for oxVLDL4. First, we show that acLDL is able to compete efficiently with the uptake (association and degradation) of 125I-labeled oxVLDL4 (Figure 3A). These results suggest that oxVLDL4 is taken up via the macrophage scavenger receptor and not via LDL or VLDL receptors. Furthermore, we show that the uptake (association and degradation) of 125I-labeled oxVLDL4 was completely inhibited by 100 μg/mL of PolyI (Figure 3B). Thus, through a complete inhibition of the scavenger receptor–mediated uptake of oxVLDL4 via PolyI, the role of the second pathway involved in the intracellular lipid accumulation, ie, the extracellular VLDL-TG lipolysis with subsequent cellular FFA uptake, can be determined.

Therefore, the oxVLDL-induced intracellular lipid accumulation was investigated by measuring the de novo synthesis of TG by J774 macrophages after incubation with VLDL.
and oxVLDL4, either in the absence or in the presence of PolyI. In this experimental approach, newly formed FFAs from either extracellular lipolysis or intracellular lysosomal hydrolysis of VLDL-TG are reesterified with \(^{[3]}\)Hglycerol into \(^{[3]}\)HTG. In accordance with the decreased TG accumulation after incubation with oxVLDL4 (Figure 4B), Figure 4 shows that the induction in the de novo synthesis of TG by J774 macrophages after incubation with oxVLDL4 is \(\approx 7\)-fold compared with control incubations without VLDL, whereas incubation with native VLDL induced the de novo TG synthesis by \(\approx 20\)-fold. This lower induction in TG synthesis upon incubation with oxVLDL4 compared with incubation with native VLDL cannot be explained by differences in TG content of the respective VLDL preparation (Table). Nearly complete inhibition of the receptor-mediated uptake of oxVLDL4 by PolyI caused a further lowering in the uptake of oxVLDL4 by PolyI vs without PolyI in unpaired Student’s t test.

**Lipolysis of oxVLDL**

To investigate whether the extracellular lipolysis of oxVLDL4 is hampered, the amount of FFA released into the medium was measured during a 4-hour incubation of the J774 cells with \(^{14}\)C-TG-VLDL and \(^{14}\)C-TG-oxVLDL4 (labeled in olate). Significantly lower amounts of \(^{14}\)C-oleate appeared in the medium after incubation with oxVLDL4 than with native VLDL (8.2±0.5 versus 28.2±1.8 \(\mu\)mol/mL), indeed suggesting an impaired extracellular lipolysis of oxVLDL-TG. However, \(^{14}\)C-oleate present in medium can be derived either from extracellular hydrolysis of VLDL-TG or intracellular hydrolysis of lipid pools. Thus, to directly investigate the effect of oxidation on the LPL-mediated hydrolysis of VLDL-TG, different VLDL samples with various degrees of oxidation were incubated with purified bovine LPL. Figure 5 shows that the amount of FFA released after the addition of LPL decreases with oxidation time. Thus, oxidation decreases the suitability of VLDL as substrate for LPL, leading to a defective TG hydrolysis.

To investigate whether oxidation of VLDL has a direct inhibitory effect on the enzyme activity of LPL, we performed 2 different in vitro lipolysis assays with the system in which LPL was bound to HSPG. In the first assay, we tested whether oxidized lipoproteins would irreversibly damage LPL activity by measuring the FFA release of native VLDL after preincubation of the HSPG-LPL–coated wells with oxidized lipoproteins. As shown in Figure 6, preincubation with oxVLDL4 did not significantly alter the HSPG-LPL–mediated FFA release of VLDL-TG compared with preincubation with native VLDL, indicating that LPL is still catalytically active after withdrawal of oxidized lipoproteins. In the

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**Figure 4.** Effect of inhibition of receptor-mediated uptake of oxVLDL on de novo TG synthesis in J774 cells. J774 cells were incubated for 4 hours at 37°C with DMEM/1% BSA containing \(\left(10^{-3}\right)\)\(^{3}\)Hglycerol (4.4 \(\mu\)g/mL, 25 \(\mu\)mol/L), either in the absence of lipoproteins (control) or in the presence of VLDL or oxVLDL4 (100 \(\mu\)g TG/mL medium), with (solid bars) or without (open bars) the addition of PolyI (100 \(\mu\)g/mL). After incubation, the J774 cells were washed and resuspended in 1 mL PBS. Subsequently, lipids were extracted from the cell suspension, and TG was determined as described in Methods. The values represent the mean ± SD of 4 different experiments. * \(p<0.05\), native VLDL vs oxVLDL4; ** \(p<0.05\), incubation with PolyI vs without PolyI by unpaired Student’s t test.

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**Figure 5.** In vitro lipolysis of VLDL and oxVLDL. VLDL was oxidized for 0, 1, 2, 3, or 4 hours as described in Methods and dialyzed against 0.1 mol/L Tris/HCl, pH 8.5. VLDL-TG samples, ranging from 0.1 to 0.5 mmol/L, were incubated at 37°C in the presence of 2% (wt/vol) FFA-free albumin and 0.2 U of LPL. The FFA release is expressed per unit LPL and represents the mean ± SD of at least 4 experiments. The results for 0.3 mmol/L TG are shown; similar results were obtained with other TG concentrations. * \(p<0.05\), native VLDL vs oxVLDL samples by unpaired Student’s t test.

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**Figure 6.** Effect of oxVLDL on the enzyme activity of LPL. LPL was bound to HSPGs in a 96-well microtiter plate and preincubated with native VLDL (left) or oxVLDL4 (middle). Thereafter, VLDL samples were removed, and the remaining activity of the HSPG-bound LPL was assayed as described in Methods. The lipolysis of native VLDL was also assayed in the presence of oxVLDL4 (0.2 mmol/L) (right). Depicted values represent the mean ± SD of 3 experiments with FFA release at a TG concentration of 0.3 mmol/L. Similar results were obtained with VLDL-TG concentrations at 0.2 and 0.5 mmol/L.
second in vitro lipolysis assay, we carried out a substrate curve for native VLDL in the presence or absence of oxVLDL4 (0.2 mmol/L TG). The LPL-mediated FFA release of native VLDL-TG in the absence of oxVLDL4 was not significantly different from that in the presence of the oxidized lipoprotein fraction (Figure 6), providing further evidence that oxidative modification of the lipoprotein particle does not directly inhibit LPL. To examine whether oxidation of VLDL modifies apoC2 in a manner that prevents it from acting as a cofactor for the LPL-mediated hydrolysis of TG, oxVLDL4 fractions were incubated with HDL to allow supplementation with native apoC2. As shown by Western blot analysis, the amount of human apoC2 on native VLDL and oxVLDL4 fractions remained unaltered upon incubation with HDL (see inset in Figure 7A). In line with these results, preincubation with HDL did not affect the lipolysis rate of native VLDL, nor did it restore the impaired LPL-mediated FFA release of oxVLDL4 (Figure 7A). From these data, it can thus be hypothesized that either (1) native apoC2 is unable to bind to the oxVLDL particle or (2) oxidative modification of VLDL lipid decreases its suitability as substrate for LPL independent of apoC2. To discriminate between these 2 possibilities, apoC2-deficient (apoC2−/−) VLDL was isolated from a hypertriglyceridemic patient, oxidized, and incubated with HDL before the in vitro LPL-mediated lipolysis assay. As shown in the inset of Figure 7B, both native and oxidized apoC2−/− VLDLs were enriched with human apoC2 on incubation with HDL. Furthermore, incubation of apoC2−/− VLDL and oxidized apoC2−/− VLDL with HDL effectively restored the LPL-mediated release of FFA to control levels (native VLDL) (Figure 7B), indicating that native human apoC2 binds to the oxVLDL particle and is able to overcome the inhibitory action of oxidation on VLDL lipolysis.

Discussion
In the present study, we examined the effect of oxidative modification on the processing of human VLDL-TG in the murine macrophage cell line J774. Previous studies have shown that J774 cells secrete LPL,23 but not apoE,24 into the culture medium. We showed that the uptake of oxVLDL protein by J774 macrophages was significantly increased compared with native VLDL (Figure 2A). The uptake of oxVLDL was competitively inhibited by an excess of unlabeled acLDL and completely blocked by coinubcation with Polyl. Since Polyl is known to efficiently block ligand binding to the scavenger receptor class A,25 we conclude that the uptake of oxVLDL is mediated primarily via the scavenger receptor. Similar data on the enhanced uptake of oxVLDL have previously been published by other groups.7,10 However, despite this increased protein (ie, particle number) uptake, we showed that incubation of J774 cells with oxVLDL resulted in a 2- to 3-fold less efficient accumulation of TG compared with native VLDL (Figures 2B and 4). This apparent paradox can be explained by earlier observations that the uptake of VLDL-TG components by macrophages, in contrast to VLDL-protein uptake, involves 2 different pathways,2,4,23,26: (1) receptor-mediated uptake of the intact VLDL particle and (2) uptake of FFAs generated by the extracellular LPL-mediated lipolysis of VLDL-TG, followed by intracellular reesterification into TG. Thus, in addition to its so-called “bridge function,” through which LPL can enhance the cellular uptake of lipoproteins,27–31 LPL also plays an important role in the cellular lipid accumulation by mediating the extracellular lipolysis of TG-rich lipoproteins.4

We present evidence that oxidation of VLDL results in a less efficient intracellular TG accumulation because of a defect in the second pathway, ie, a decreased suitability of oxVLDL as a substrate for LPL. Although the LPL-mediated lipolysis of VLDL-TG gradually decreased with oxidation time (to 25% of control levels), some residual lipolysis activity could still be observed for the maximally oxidized VLDL fraction (oxVLDL4) (Figure 5). In agreement with these observations, simultaneous incubation of oxVLDL4 with Polyl was not able to completely eliminate the de novo synthesis of TG to control values (Figure 4). As discussed earlier, Whitman et al12 showed that oxidation of apoE2/E2 β-VLDL isolated from type III hyperlipidemic patients resulted in a decreased accumulation of TG in J774 cells compared with native type III VLDL. Because oxidized apoE2/E2 VLDL is taken up at an enhanced rate via the scavenger receptor, whereas the receptor-mediated uptake of native apoE2/2 VLDL is severely hampered by a defect of apoE2 in binding to the LDL receptor, their results support our present data and further sustain the important role of extracellular LPL-mediated lipolysis in the intracellular accumulation of TG.

An impaired LPL-mediated lipolysis of oxVLDL can be explained by either (1) greater amounts of FFAs associated with oxVLDL; (2) a direct effect of oxidation on the enzyme activity of LPL; (3) inactivation of apoC2, an essential cofactor for LPL activation; or (4) oxidative modification of
VLDL lipid, rendering the TG component defective as a substrate for LPL. The first possibility could be excluded, because similar levels of FFAs were associated with native VLDL and oxVLDL (Table). Furthermore, we showed that LPL enzyme activity was not directly affected upon incubation with oxVLDL (Figure 6). The possible inactivation of apoC2 upon oxidation was supported by SDS-gel electrophoresis, showing significant depletion of apoC as well as other apolipoproteins with increasing oxidation time (Figure 1). In addition, enrichment of the oxidized apoC2-deficient VLDL particle with native apoC2 restored the impaired LPL-mediated lipolysis of oxVLDL (Figure 7B). Thus, we can conclude from these studies that oxidation decreases the suitability of VLDL as a substrate for LPL because of a defective apoC2, rather than because of modification of VLDL-TG. In contrast to oxidized apoC2-deficient VLDL, we were not able to restore the lipolysis of apoC2 containing oxVLDL on incubation with HDL, most likely because oxVLDL could not be supplemented with native apoC2 (see inset in Figure 7A). Although previous studies have shown that apoCs rapidly exchange between VLDL and HDL, the VLDL size range have been isolated from the human aortic intima and human aortic atherosclerotic plaques and the VLDL oxidation as being relevant for the in vivo situation. We hypothesize that VLDL oxidation may indeed occur in the intima in vivo. Whether this oxidative modification is severe enough to inhibit the LPL-mediated lipolysis is at present subject to speculation. In addition, because it may be expected that in the intima, the VLDL particle will be lipolyzed immediately after entry, it may be suggested that the oxidative modification becomes manifest only after lipolysis of most of the VLDL-TG, ie, after VLDL particles have been converted into VLDL remnant particles. Recently, it was shown that oxVLDL remnant particles are most effective in the accumulation of cholesterol ester in macrophages. Taking all these considerations into account, we suggest that in the intima, LPL-mediated lipolysis of VLDL TGs and oxidative modification of VLDL (remnant) particles represent 2 proatherogenic steps: (1) on entry into the intima, the action of LPL leads to enhanced TG accumulation in macrophages, and subsequently, (2) oxidative modification of VLDL remnant promotes cholesterol ester accumulation in macrophages.

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References
12. Whitman SC, Miller DB, Wolfe BM, Hegele RA, Huff MW. Uptake of type III hypertriglyceridemic VLDL by macrophages is enhanced by
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