# Nitrosylated high density lipoprotein is recognized by a scavenger receptor in rat liver

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**Abstract** In order to assess the presence of specific recognition sites for high density lipoprotein (HDL) in vivo, HDL was nitrosylated with tetranitromethane and the decay and liver uptake were compared with that of native HDL. The association of intravenously injected nitrosylated HDL (TNM-HDL) with liver was greatly increased as compared to native HDL. Using a cold cell isolation method, it became evident that the liver endothelial cells were responsible for the increased uptake of the modified HDL. The involvement of the endothelial cells in the uptake of TNM-HDL from the circulation could also be demonstrated morphologically by using the fluorescent dye dioctadecyltetramethyl-indocarbocyanine perchlorate (Dil) to label HDL. In vitro competition studies with isolated liver endothelial cells indicated that unlabeled modified HDL and acetylated LDL displaced iodine-labeled TNM-HDL, while no competition was seen with LDL and a slight displacement was seen with unlabeled native HDL. Nonlipoprotein competitors of the scavenger receptor such as fucoidin and polyinosinic acid blocked the interaction of TNM-HDL with the liver endothelial cells. Also the degradation of TNM-HDL was blocked by low concentrations of chloroquine. It can be concluded that a scavenger receptor on liver endothelial cells is involved in the clearance of tetranitromethane-modified HDL, which excludes the possibility of using TNM-HDL in vivo to assess the non-receptor-dependent uptake of HDL. The use of nitrosylated HDL in vitro as a low affinity control is limited to cell types that do not possess scavenger receptors, because cell types with scavenger receptors will recognize and internalize TNM-HDL by a high affinity scavenger pathway.—**Kleinherenbrink-Stins, M. F., D. Schouten, J. van der Boom, A. Brouwer, D. L. Knook, and T. J. C. van Berkel.** Nitrosylated high density lipoprotein is recognized by a scavenger receptor in rat liver. *J. Lipid Res.* 1989. **30:** 511–520.

**Supplementary key words** native HDL • liver endothelial cells • acetylated LDL

High affinity binding sites for high density lipoproteins (HDL) have been detected on liver cells (1–9) and liver membrane preparations (10–13) of various species and peripherhal cell types (14–18). In peripheral cells the amount of high affinity sites can be up-regulated by loading the cells with cholesterol, either in the form of cholesterol–albumin complexes (13, 16, 18) or as acetylated LDL (15, 17). According to the concept of Glomset (19), HDL functions as a cholesterol acceptor of the peripheral cells and will deliver its cholesterol mainly to the liver. Several studies concerning the identification of the apolipoprotein involved in the recognition of HDL by different cell types indicated a role for apoA-I (20, 21).

Recent studies have shown that treatment of human apoE-free HDL with tetranitromethane (TNM) inhibits binding of the HDL to the high affinity HDL binding sites of fibroblasts (22), hepatic membranes (23, 24), and ovarian cells (25). It is known that tetranitromethane treatment affects the tyrosine residues on proteins (26). Two types of conditions have been used to modify HDL. Nestler, Chacko, and Strauss (25) used a 0.5–10 × molar excess of TNM over HDL, while Brinton et al. (22) used varying concentrations of tetranitromethane (TNM) (0.03–30 mM). In the present investigation we tested various nitrosylation conditions in order to use the nitrosylated HDL in vivo to assess the role of the high affinity site in the uptake of HDL by the various liver cell types. A comparison of the uptake of native HDL with that of nitrosylated HDL should then indicate the relative importance of the tentative HDL receptor in a similar way as used earlier for LDL and methylated LDL (27, 28). However, it appeared that irrespective of the nitrosylation condition the TNM-HDL disappeared more rapidly from the circulation than untreated HDL.

In the present study we determined which hepatic cells were responsible for the increased interaction of TNM-HDL with the liver. Low temperature cell isolation techniques were used (29). Furthermore, the tentative recep-

Abbreviations: HDL, high density lipoproteins; LDL, low density lipoproteins; TNM, tetranitromethane; Dil, 1,1'-dioctadecyl-3,3,3',3'-tetramethyl indocarbocyanine perchlorate; PAGE, polyacrylamide gel electrophoresis; AcLDL, acetylated LDL.

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tor responsible for the avid interaction is indicated. We also visualized the cell type responsible for the in vivo clearance of TNM-HDL by the liver and therefore used the fluorescent molecule 1,1'-dioctadecyl-3,3,3',3'-tetramethyl indocarbocyanine perchlorate (Dil) (30). Dil is a highly lipophilic molecule that can be noncovalently incorporated into lipoproteins and thus has no effect on surface charge. When the fluorescent-labeled lipoprotein is internalized and degraded within cells, the lipophilic fluorescent probe remains in the lysosomal membrane of the individual cells. Dil has been used to visualize the uptake of lipoproteins by macrophages, fibroblasts and arterial foam cells (30, 31), hepatocytes (32), endothelial cells (33, 34), and vascular endothelium (35).

## MATERIALS AND METHODS

### Materials

Collagenase (type I), bovine serum albumin (fraction V), chloroquine, and polyinosinic acid were purchased from Sigma (St. Louis, MO). Tetranitromethane (TNM) was obtained from Aldrich Chemical Company (Brussels, Belgium), metrizamide from Nyegaard & Co. (Oslo, Norway), $Na^{125}I$ from Amersham International (Amersham, UK), 1,1'-dioctadecyl-3,3,3',3'-indocarbocyanine perchlorate C18 (Dil) from Molecular Probes (Eugene, OR), fucoidine from Kochlight (Haverhill, UK), and Ham's F-10 medium from Gibco-Europe, Hoofddorp, The Netherlands. All other chemicals and solvents were of reagent grade.

### Animals

Throughout the study 3-month-old Wistar rats were used; they had free access to food and water. Prior to the experiments, the rats were anesthetized with nembutal given intraperitoneally. Radiolabeled lipoproteins were injected into the inferior vena cava. Dil-labeled lipoproteins were injected into the jugular vein of overnight-fasted rats after halothane anesthesia. The injected dose was calculated on the basis of a plasma volume of 3.75 ml per 100 g body weight for our rats (29).

### Methods

For the determinations of serum decay and liver association, serum and liver samples were taken at the several time intervals, as described by Van Berkel et al. (36, 37). The cellular distribution in the liver of the injected lipoproteins was determined by isolation of liver cells at low temperature. Parenchymal cells and nonparenchymal cells were isolated by a cold collagenase method and nonparenchymal cells were further separated into endothelial and Kupffer cells by centrifugal elutriation (29, 38).

For in vitro binding studies, the different types of liver cells were isolated using collagenase at 37°C (3, 4, 29). The purity and integrity of the parenchymal cells (99% pure) were judged by light microscopy and trypan blue exclusion. The purity of the Kupffer and endothelial cell preparations was greater than 95%, as determined using an endogenous peroxidase stain (39).

### Lipoprotein isolation, labeling and modification

Human HDL ($1.062 < d < 1.21$ g/ml) were isolated using the method of Redgrave, Roberts, and West (40). The HDL fraction was subjected to a second identical centrifugation to remove albumin and was further processed by heparin-Sepharose chromatography to remove apoE (41). The absence of apoE was checked by SDS-PAGE under reducing conditions according to Laemmli (42).

Native human HDL was radiolabeled with $^{125}I$ using the method of McFarlane (43) as modified by Bilheimer, Eisenberg, and Levy (44). The specific activities of the $^{125}I$-labeled HDL ranged from 100 to 200 cpm/ng lipoprotein.

Distribution of label in HDL was 44% ± 2% in apoA-I; 44% ± 2% in apoA-II; and 6% in apoCs; 2% was TCA-soluble and 4% was present in lipids.

For nitrosylation of radiolabeled HDL, a 0.5- to 10-fold molar excess of TNM to HDL (HDL:TNM = 1:x) was used, based on the presence of seven tyrosine residues per molecule of apoA-I as described by Nestler et al. (25). HDL was also nitrosylated using various molar solutions of TNM (TNM-HDL) as described by Brinton et al. (22).

Low density lipoprotein (LDL) was acetylated (AcLDL) with acetic acid anhydride as described by Basu et al. (45). Native and modified HDL were subjected to SDS-PAGE (42) and to agarose gel electrophoresis, according to the method of Demacker (46). HDL and TNM-modified HDL were labeled with Dil according to Pitas et al. (30), the density of the incubation mixture was subsequently raised to 1.21 g/ml with KBr, and the lipoproteins were reisolated by ultracentrifugation (39). The Dil-labeled lipoproteins were then made free of apoE as described above.

### In vitro experiments

For competition studies, freshly isolated liver cells were incubated with different amounts of unlabeled human HDL, LDL, modified HDL, or AcLDL in the presence of an indicated amount of radiolabeled native or modified HDL or AcLDL in Ham's F-10 medium, 1% BSA for the indicated periods at 37°C. Samples were centrifuged for 2 min at 600 *g*. The pellets were resuspended in 50 mM Tris-HCl (pH 7.4) 0.15 M NaCl, 1% BSA, and washed twice. A final washing was performed in a similar medium without BSA. Degradation of lipoproteins was measured as previously described by Van Berkel et al. (4). The degradation values represent radioactivity present in the

acid-soluble water phase. Inhibitors of cellular association or degradation were added prior to the lipoproteins. Protein determinations were according to the method of Lowry et al. (47) using BSA as a standard. All experiments were repeated at least two times with different cell and lipoprotein preparations.

### Light and electron microscopy

For the visualization studies, Dil-lipoproteins (5–15 μg/ml plasma) were injected into the jugular vein and allowed to circulate for the indicated periods.

At the indicated time points, rat livers were fixed with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) by a total body perfusion-fixation via the heart. Tissues were dissected and processed for light microscopical cryomicrotomy as described by Tokuyasu (48). Cryosectioning was performed on a Reichert FC4 cryomicrotome. Sections (thickness: 4 μm) were picked up with a droplet of sucrose, placed on glass slides, and mounted with glycerol. Photographs were taken with a Leitz Ortholux Microscope, using standard Rhodamine emission and excitation filter combination and Kodak 1600 Ektachrome films. For the electron microscopy, lipoprotein samples were negatively stained with phototungstate according to Forte and Nordhausen (49).

## RESULTS

### In vivo biochemical studies

The disappearance of native apoE-free HDL from serum proceeded at a low rate and less than 2% of the iodine radioactivity was found in liver up to 60 min after injection. In contrast to native HDL, nitrosylated HDL injected into rats was cleared much faster from the circulation. Within 60 min, more than 60% of the injected dose was cleared from the circulation. The association of nitrosylated HDL with the liver occurred very rapidly and at 15 min after injection, up to 37% of the injected dose was recovered in the liver. Thereafter, the amount of label associated with the liver slowly decreased (**Fig. 1**).

The excess of tetranitromethane (TNM) over HDL used for the determination of the serum decay and liver uptake with time was tenfold, an excess similar to that used by Nestler et al. (25). **Fig. 2** shows the nature of the modification in HDL which occurs under various nitrosylation conditions. SDS-PAGE showed that apoA-I was the most prominent apolipoprotein in native HDL.

Modification with HDL-TNM ratios ranging from 1:7.5 to 1:2.5 resulted in a protein band with a slightly higher molecular weight than apoA-I. Also, a distinct protein band with a molecular weight of 90,000 representing cross-linked apoA-I was observed. Upon modification

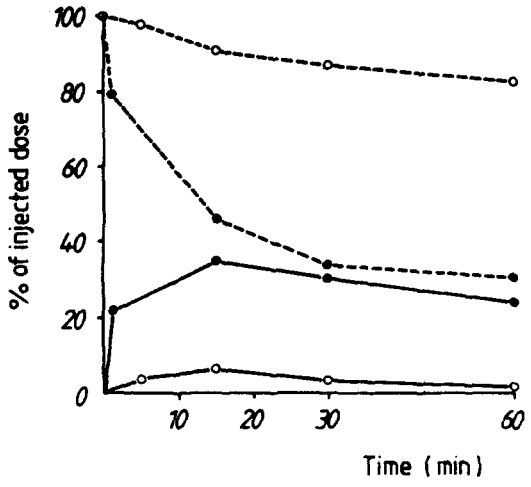


**Fig. 1.** Serum decay and uptake by liver of apoE-free $^{125}$I-labeled HDL and apoE-free $^{125}$I-labeled TNM-HDL. ApoE-free $^{125}$I-labeled HDL or apoE-free $^{125}$I-labeled TNM-HDL (100 μg of apolipoprotein) was injected into anesthetized rats and the uptake by liver and serum decay were determined. The liver was not perfused and the values are corrected for the estimated contribution of the radioactivity in serum to the liver values (36, 37). HDL was modified using HDL-TNM 1:10; (○---○), serum values of native HDL; (●---●), serum values of TNM-HDL; (○—○), uptake by liver of native HDL; (●—●), uptake by liver of TNM-HDL.

with a ratio 1:10, the apoA-I was virtually absent and a broad band with a molecular weight range from 65,000 to 95,000 was seen in the gel in accordance with the data of Chacko (13) (not shown). The modification with 3 mM TNM gave results on SDS-PAGE similar to those with a 1:10 HDL-TNM, which is comparable with the results of Chacko (13) and Brinton et al. (22). Apparently, the nitrosylation conditions that are effective in blocking the interaction with the high affinity site for HDL (either HDL-TNM of 1:10 or 3 mM TNM) lead to extensive cross-linking and the apolipoproteins are converted completely into high molecular weight polymers.

On agarose gel electrophoresis the extensively nitrosylated HDL moved ahead of native HDL which indicates an increased negative charge. In accordance with the data of Chacko et al. (13, 50) and Brinton et al. (22), the modification with tetranitromethane (TNM) did not result in an increase in size or difference in shape of the lipoprotein particles as judged by electron microscopy. An average particle size of 11.7 ± 1.8 nm and 11.7 ± 1.9 nm was found for the native and modified apoE-free human HDL, respectively (**Fig. 3**). Furthermore, no aggregates or other indications for cross-linking of the lipoproteins to each other were observed.

In order to identify the cell type(s) responsible for the increased liver uptake of nitrosylated HDL, we injected the radiolabeled modified HDL into rats and isolated parenchymal, endothelial, and Kupffer cells from the liver by a low temperature method. **Fig. 4** illustrates that, ir-

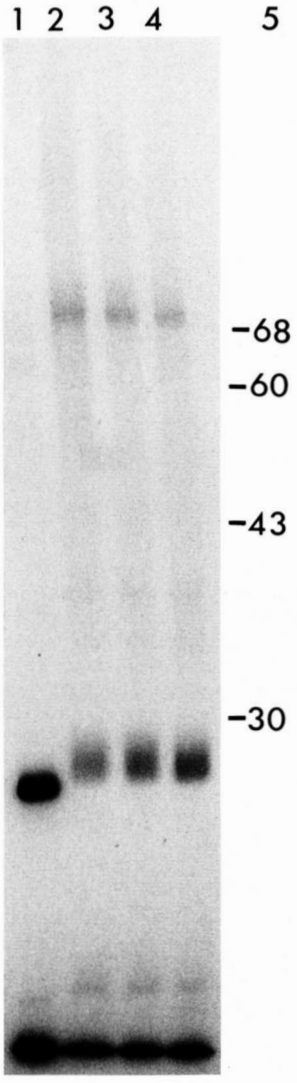


**Fig. 2.** Autoradiographic analysis of iodinated HDL and iodinated TNM-HDL in 10% SDS gels. HDL was incubated with various amounts of tetranitromethane. The ratios of HDL-TNM used in modification are: 1:0 (lane 1); 1:7.5 (lane 2); 1:5 (lane 3); 1:2.5 (lane 4); molecular weight markers (lane 5). Twenty five $\mu$g of protein was added to each well and chromatographed as described.

respective of the nitrosylation conditions, the modified HDL showed an equal or increased association to liver as compared to native HDL. The liver endothelial cells especially showed an increased uptake of nitrosylated HDL, even when nitrosylation at an HDL-TNM value of 1:2.5 was used. When the relative uptake of nitrosylated HDL in the various cell types is compared to that of acetylated LDL, a striking similarity is evident.

From these data, taking into account the amount of protein contributed by each cell type to the total liver, one can calculate the contribution of the various liver cell types to the total liver uptake of nitrosylated HDL (**Table 1**). The similarity in cellular uptake of nitrosylated HDL and acetylacted LDL may indicate that the same receptor is responsible for the uptake by the liver. To test this hypothesis, cross-competition experiments were performed with isolated liver endothelial cells.

### In vitro studies

The association of iodinated TNM-HDL to endothelial cells in vitro was effectively inhibited in the presence of an excess of unlabeled TNM-HDL and AcLDL, but not by the presence of LDL. HDL showed an intermediate behavior (**Fig. 5**). When iodinated AcLDL was used as a ligand for the scavenger receptor, it appeared however that unlabeled TNM-HDL competed only slightly compared to AcLDL (**Fig. 6**). The scavenger receptor is reported to be inhibited also by the polyanions fucoidin and polyinosinic acid (51). **Fig. 7** shows that fucoidin and polyinosinic acid are effective inhibitors of the association of TNM-HDL to endothelial cells. Addition of 10 $\mu$M of either of these polyanions decreased cell association at least 85%. Comparison of the time course of cell-association (**Fig. 8**) indicates that nitrosylated HDL associated with isolated endothelial cells to a much greater degree than native HDL. The association of TNM-HDL with the liver endothelial cells increased to 1350 ng/mg cell protein after 2 hr of incubation. The actual uptake was even higher since 900 ng TNM-HDL/mg cell had been degraded and excreted during this period. For native HDL a value of 50 ng/mg cell protein was obtained after 2 hr, with little or no degradation (not shown). During the whole time course, unlabeled acetylated LDL was very

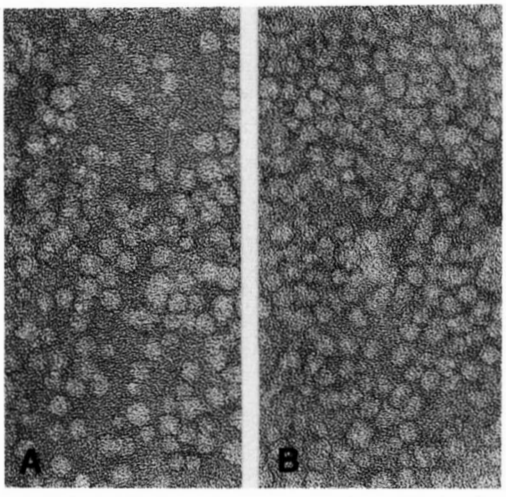


**Fig. 3.** Electron microscopy analysis of HDL (A) and of TNM-modified HDL (B). HDL was treated with 3 mM TNM, negatively stained, and analyzed by electron microscopy as indicated in Materials and Methods; magnification ×281,600.

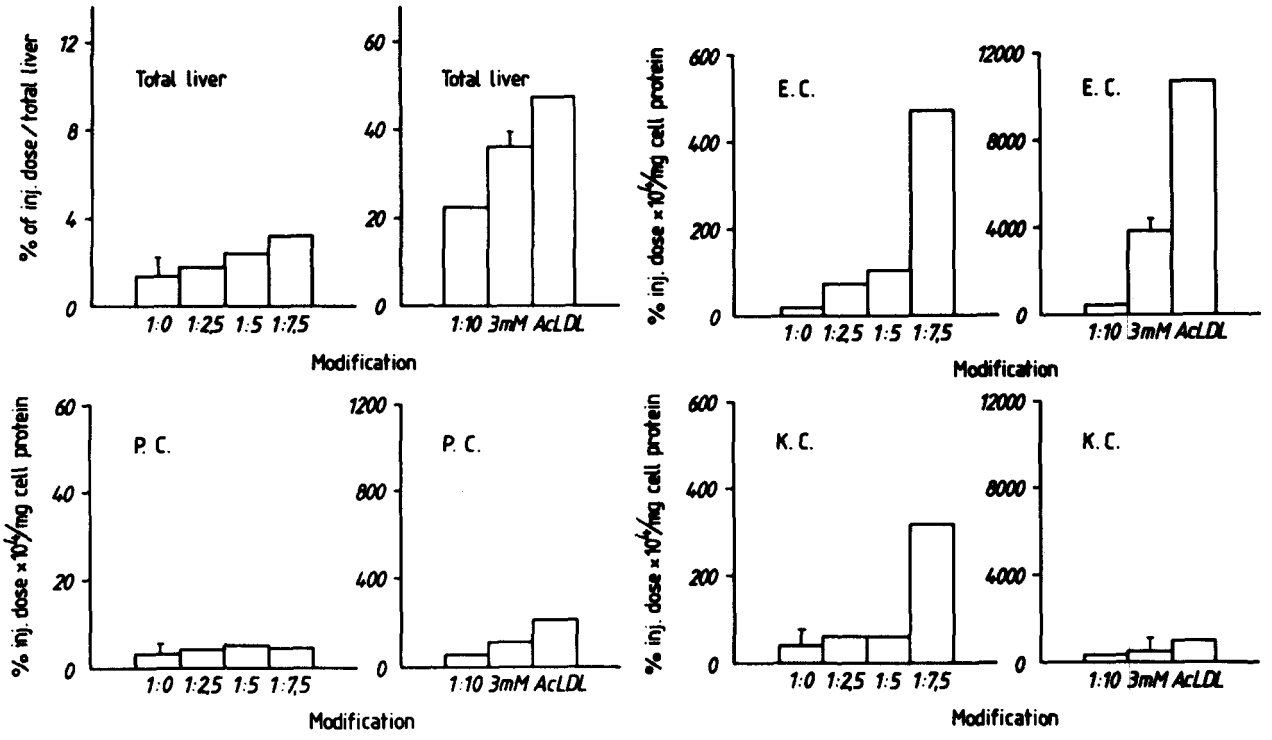


**Fig. 4.** Uptake by liver and cellular distribution after in vivo injection of native apoE-free $^{125}$I-labeled HDL, apoE-free $^{125}$I-labeled TNM-HDL with varying degrees of nitrosylation, and $^{125}$I-labeled AcLDL. Modification conditions for nitrosylation were varied with the following molar ratios of HDL-tetranitromethane 1:2.5, 1:5, 1:7.5, and 1:10 according to Chacko (13). In addition, HDL was modified with 3 mM tetranitromethane according to Brinton et al. (22). PC, parenchymal cells; KC, Kupffer cells; EC, endothelial cells.

effective in blocking the interaction of nitrosylated HDL with the endothelial liver cells. The degradation of TNM-HDL was inhibited by low concentrations of chloroquine (**Fig. 9**) implying an involvement of the lysosomes.

### In vivo visualization studies

To establish morphologically the involvement of liver endothelial cells in the clearance of TNM-HDL by a method independent from the cell isolation procedure, the cellular site of hepatic uptake of native and TNM-HDL was also studied with Dil-labeled lipoproteins.

In cryo sections of livers from rats injected with Dil-labeled TNM-HDL that was allowed to circulate for 10 min, we found a punctuate fluorescent labeling (**Fig. 10**), clearly outlining the sinusoids, which supports suggestions of an involvement of the liver endothelial cells in the clearance of TNM-HDL. Kupffer cells also showed some labeling (Fig. 10). When the labeled lipoprotein was injected and allowed to circulate for 1 hr (not shown), the labeling pattern appeared identical to that observed after 10 min. The labeling of the liver with Dil-TNM-HDL showed a striking similarity with the results of Pitas et al. (34) and Netland et al. (35) after intravenous injection of Dil-AcLDL. The endothelial cells could be distinguished from the Kupffer cells by their abundance and their uniform distribution and because they were situated along the lining of the sinusoids, whereas the Kupffer cells had a different distribution and were less abundant.

When Dil-labeled native HDL was injected, no punctuate labeling along the sinusoids was noticed after 10 min or 1 hr of circulation (not shown). These results are consistent with the slow rate of uptake of the native HDL and also serve as a control for the fluorescence exhibited with nitrosylated HDL.

TABLE 1. Relative contribution of different liver cell types to the total liver uptake of native HDL, nitrosylated HDL,[a] and acetylated LDL

| | Parenchymal Cells | Kupffer Cells | Endothelial Cells |
|---|---|---|---|
| | | % | |
| HDL | 74 | 13 | 13 |
| TNM-HDL | 41 | 4 | 55 |
| AcLDL | 33 | 5 | 61 |

Data are given as percentages based upon the amount of radioactivity/mg cell protein and multiplied by the amount of protein that each cell type contributes to total liver protein.

[a]Using 3 mM tetranitromethane.

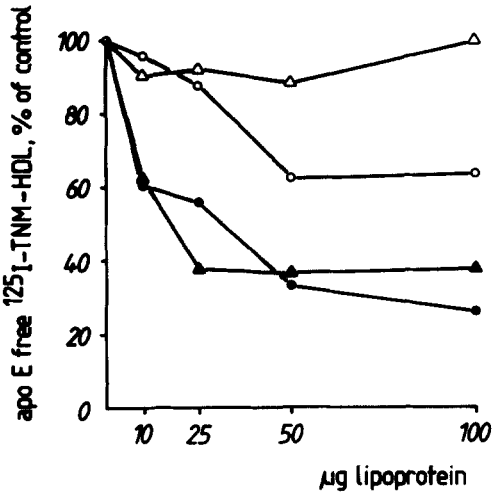


**Fig. 5.** Comparison of the ability of unlabeled lipoproteins to compete with the cell association of $^{125}$I-labeled apoE-free TNM-HDL to liver endothelial cells. Cells were incubated in vitro for 10 min at 37°C with 5 µg of $^{125}$I-labeled apoE-free TNM-HDL and with the indicated amounts of AcLDL (▲), LDL (△), HDL (○), and TNM-HDL (●). $^{125}$I-Labeled TNM-HDL apolipoprotein association is expressed as the percentage of radioactivity obtained in the absence of unlabeled lipoprotein.

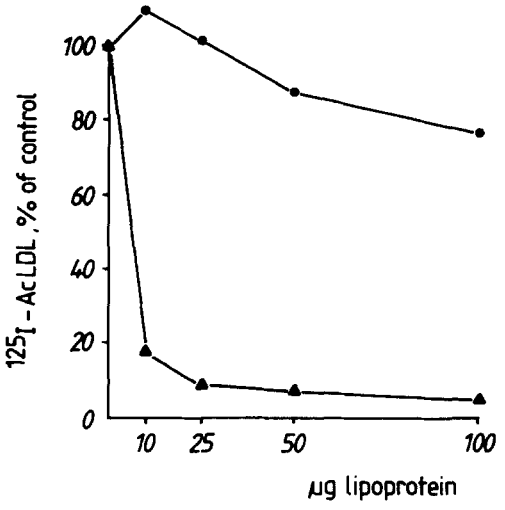


**Fig. 6.** Comparison of the ability of unlabeled apoE-free TNM-HDL (●) and AcLDL (▲) to compete with the degradation of $^{125}$I-labeled acetylated LDL by liver endothelial cells. Cells were incubated for 2 hr at 37°C with 1 µg of AcLDL and the indicated amounts of unlabeled lipoproteins. $^{125}$I-Labeled apolipoprotein degradation is expressed as the percentage of radioactivity obtained in the absence of unlabeled lipoprotein.

## DISCUSSION

Chemical modification of HDL with tetranitromethane prevents the competition of unlabeled HDL with iodinated HDL (13, 22–25). The inability of nitrosylated HDL to compete with the native iodinated HDL has been shown with fibroblasts (22), HepG2 cells (23), ovarian cells (25), and liver membranes (13, 24). In addition, it was recently shown that $^{125}$I-labeled nitrosylated HDL did not bind to liver membranes with high affinity or with saturable kinetics (13). The mechanism of the effect of nitrosylation is suggested to be the consequence of modification of tyrosine residues (52) or cross-linking of apolipoproteins to one another (13). Because TNM-mediated cross-linking is also believed to alter the secondary structure of the protein, the TNM treatment itself can affect the accessibility of many amino acid residues at some distance from the cross-linking site (22). Electron microscopy studies revealed that there was no interparticle cross-linking (13, 22) and it was shown by Chacko et al. (50) that the inactivation of the high affinity binding of HDL to liver membranes by TNM treatment is not the result of cross-linking of apoprotein to phospholipids. In the present experiments we determined the effect of gradual nitrosylation upon the in vivo interaction of apoE-free HDL with liver parenchymal, endothelial, and Kupffer cells. The data indicate that nitrosylation of HDL leads to an increased decay and increased liver association. It appears specifically that liver endothelial cells possess a recognition mechanism whereby even slightly nitrosylated HDL is readily recognized. The nitrosylation grade used to block the high affinity HDL recognition (1:10) (13) or 3 mM (22) leads, respectively, to a 10- and 20-fold increased liver uptake of HDL. Our in vitro competition experiments showed that, in addition to nitro-

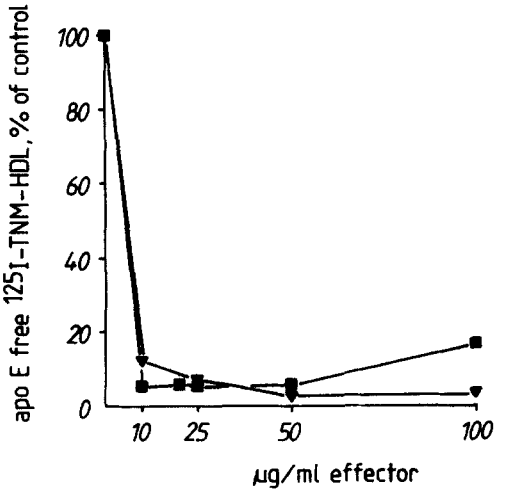


**Fig. 7.** The effect of increasing concentrations of fucoidine (■) and polyinosinic acid (▼) on the cell association of TNM-HDL. The cells were incubated for 2 hr at 37°C with 5 µg $^{125}$I-labeled TNM-HDL in the presence of the indicated amounts of fucoidine or polyinosinic acid. $^{125}$I-Labeled apolipoprotein association is expressed as the percentage of radioactivity obtained in the absence of effector.

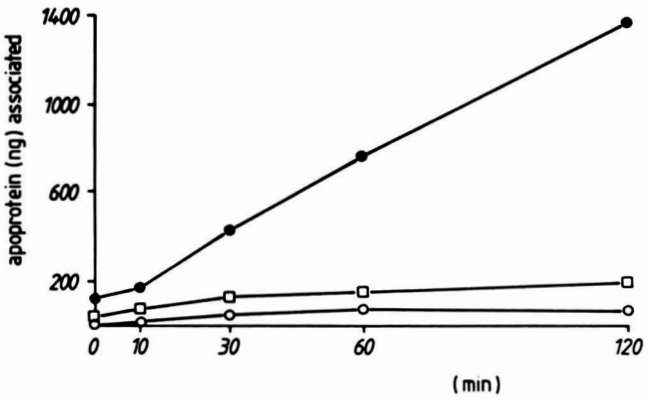


**Fig. 8.** Time course of the in vitro association of apoE-free $^{125}$I-labeled TNM-HDL in the absence (●—●) or presence (□—□) of an excess of 100 μg/ml AcLDL compared to the time course of the association of apoE-free $^{125}$I-labeled HDL (○—○) with liver endothelial cells. The cells were incubated with 10 μg/ml of apoE-free $^{125}$I-labeled HDL or apoE-free $^{125}$I-labeled TNM-HDL at 37°C and the values are expressed as ng apolipoprotein associated to the cells per mg cell protein.

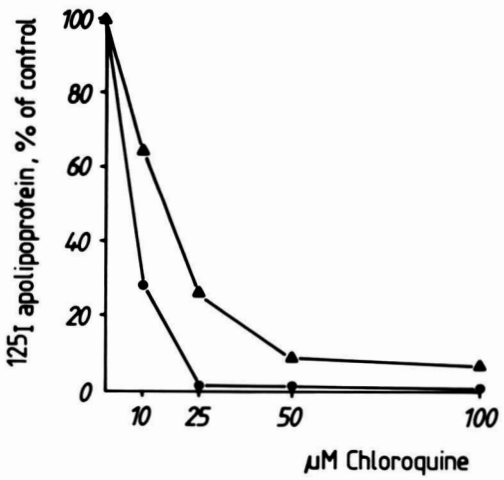


**Fig. 9.** The effect of increasing concentrations of chloroquine on the degradation of apoE-free $^{125}$I-labeled TNM-HDL (●) and $^{125}$I-labeled AcLDL (▲) by isolated liver endothelial cells. The cells were incubated for 2 hr at 37°C with 10 μg of apoE-free $^{125}$I-labeled TNM-HDL or $^{125}$I-labeled AcLDL and the indicated amounts of chloroquine. $^{125}$I-Labeled apolipoprotein degradation is expressed as the percentage of radioactivity obtained in the absence of chloroquine.

sylated HDL itself, acetylated LDL was also a competitor for the association of nitrosylated HDL to endothelial cells, while LDL was ineffective. The decrease in cellular association of TNM-HDL by AcLDL and nonlipoprotein competitors such as fucoidin and polyinosinic acid indicate that the association of TNM-HDL with the liver endothelial cells is mediated by the scavenger receptor. The partial inhibition of TNM-HDL association by HDL indicates that a minor portion of the uptake of TNM-HDL may be mediated by a receptor other than the scavenger receptor.

High affinity recognition appears to be coupled to uptake and degradation of the TNM-HDL in the lysosomes because chloroquine appears to be an effective inhibitor of the degradation. Such an intracellular route is comparable to evidence obtained with other substrates for the scavenger receptor (53).

However, in experiments testing the ability of TNM-HDL to inhibit the degradation of acetylated $^{125}$I-labeled LDL, TNM-HDL was a poor competitor. A similar poor competition was recently described for endothelial cell-modified LDL or enzymatically modified LDL, although a clear-cut scavenger receptor interaction of these substrates is established (54). This difference in cross-

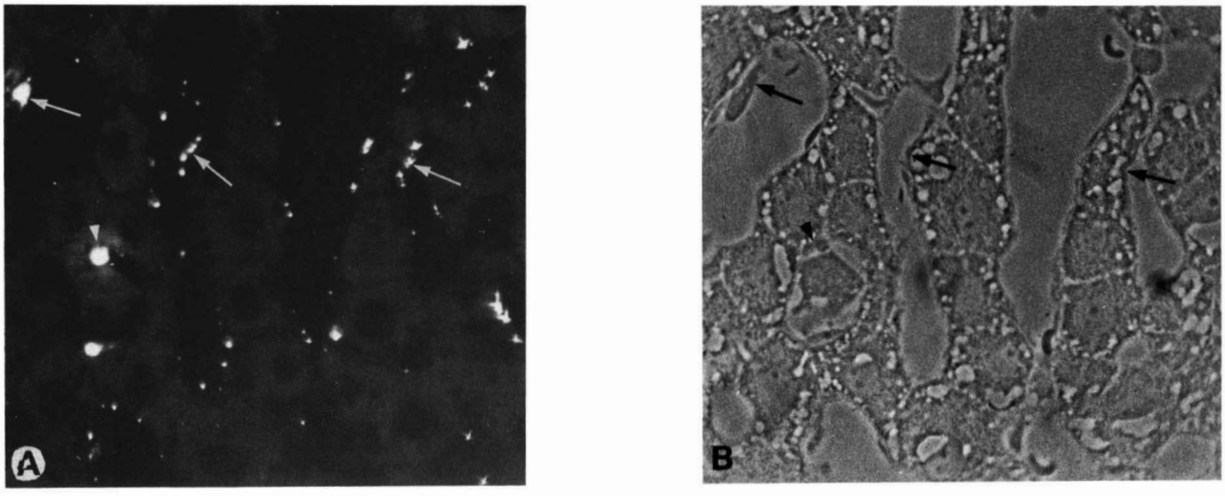


**Fig. 10.** Liver association of DiI-labeled TNM-HDL at 10 min after intravenous injection. A: Fluorescence of DiI-TNM-HDL in endothelial liver cells is indicated by arrows, while Kupffer cells are indicated by an arrow head; magnification ×992. B: Same section as shown in A using phase contrast; magnification ×992.

competition may either be explained by a difference in affinity of the scavenger receptor for AcLDL as compared to TNM-HDL or by the existence of more than one class of scavenger receptor as suggested in recent publications (54–56).

The charge of the nitrosylated HDL is affected by the degree of nitrosylation and results in a more negative charge than for native HDL as also showed by Chacko (13) and Brinton et al. (22). The increased negative charge may be responsible for the induced recognition by the scavenger receptor (51). Scavenger receptor-mediated uptake of modified lipoproteins is dependent on the grade of modification as originally shown with malondialdehyde-modified LDL (57). We could not demonstrate a threshold modification degree with TNM in which the tyrosine-induced receptor recognition was abolished and no scavenger receptor activity was induced. A shift in configuration in the apolipoprotein, as a result of the introduction of negatively charged groups—as in the case in nitrosylation—may, with HDL, unavoidably express parts of the apolipoprotein that are recognized by the scavenger receptor. Evidence for such a mechanism was presented by Haberland and Fogelman (58) with maleylated serum albumin.

Chemically modified LDL has been used extensively in vivo to characterize the tissue sites for the receptor-dependent uptake (27, 28). Furthermore, the regulation of the LDL-receptor-dependent uptake was studied by comparing the in vivo fate of native and either arginine- or lysine-modified LDL (59, 60). The present experiments show that a similar approach to study the HDL-receptor-dependent uptake and degradation in vivo is not possible with nitrosylated HDL because even the lowest degree of nitrosylation induces recognition by a scavenger receptor. This property not only limits the possibility of using nitrosylated HDL as an experimental tool in vivo, but also may influence the interpretation of in vitro data. For instance, Nestler et al. (25) showed that in the rat ovarian cell steroidogenesis is stimulated by nitrosylated HDL to a degree at least equal to that of native HDL. They concluded that high affinity binding is not an essential event in the HDL pathway and that HDL can deliver its sterol through low-affinity cellular associations. However, Pitas et al. (34) showed that at least ovarian endothelial cells do possess scavenger receptors, and it may be possible that although nitrosylated HDL does not follow the high affinity pathway of HDL to deliver its cholesterol intracellularly, it utilizes the scavenger receptor. Because scavenger receptors are present on macrophages and endothelial cells in spleen, bone marrow, adrenal, and ovary (34), this property may be relevant for a variety of cell types.

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