Implications of endogenous and exogenous lipoprotein lipase for the selective uptake of HDL_3-associated cholesteryl esters by mouse peritoneal macrophages

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Abstract To investigate the implications of endogenous LPL on selective uptake of HDL_3-associated cholesteryl esters (HDL_3-CEs) by mouse peritoneal macrophages (MPMs), we have performed uptake experiments with MPMs obtained from control mice and transgenic knockout animals expressing LPL exclusively in muscle but not in macrophages. The capacity for HDL_3 holoparticle, total HDL_3-CE, and selective HDL_3-CEs was independent of the expression of functional endogenous LPL (161 vs. 187, 1251 vs. 1300, and 1090 vs. 1113 ng HDL_3/mg cell protein; control and LPL-deficient macrophages, respectively). Both control and LPL-deficient macrophages displayed, however, pronounced capacity for total HDL_3-CE uptake in excess of HDL_3 holoparticle uptake exceeding particle uptake by 7-fold. Despite the fact that endogenous LPL was without any effect on selective uptake, the addition of exogenous LPL led to a significant increase in cellular selective HDL_3-CE uptake. Upon addition of purified LPL, HDL_3 holoparticle (internalization and degradation), total HDL_3-CE, and selective HDL_3-CEs, was increased up to 2-fold. HDL_3 holoparticle binding to control and LPL-deficient MPMs at 4°C was enhanced 2.7- and 2.6-fold, respectively, in the presence of LPL. The present results suggest that endogenous LPL is without effect on selective uptake of HDL_3-CEs. In contrast, the addition of exogenous LPL enhanced selective uptake of HDL_3-CEs along with HDL_3 holoparticle uptake apparently by the proposed bridging function of the enzyme.—**Panzenboeck, U., A. Wintersberger, S. Levak-Frank, R. Zimmermann, R. Zechner, G. M. Kostner, E. Malle, and W. Sattler.** Implications of endogenous and exogenous lipoprotein lipase for the selective uptake of HDL_3-associated cholesteryl esters by mouse peritoneal macrophages. *J. Lipid Res.* 1997. **38**: 239–253.

Supplementary key words intracellular hydrolysis • knockout animals • holoparticle uptake • cholesterol • atherogenesis • cholesterol transport

The antiatherogenic properties of high density lipoproteins (HDL) are commonly attributed to their central role in reverse cholesterol transport (1). During this process extrahepatic cholesterol is taken up by HDL, transported to the liver, and subjected to bilary secretion. HDL is able to accept free cholesterol from cellular membranes and some of this cholesterol is subjected to esterification due to the activity of lecithin cholesterol acyltransferase (2).

Several pathways contribute to the turnover of HDL-associated cholesteryl esters (HDL-CE). Besides uptake of the whole lipoprotein particle (holoparticle uptake), cholesteryl ester transfer protein (CETP)-mediated transfer reactions and other receptor- and enzyme-independent lipid exchange mechanisms contribute to HDL lipid turnover (for review, see ref. 3). HDL-CEs are turned over in excess of holoparticles and this is a result of transfer and selective uptake mechanisms. CETP catalyzes the exchange of HDL_3-CEs to triglyceride (TG)-rich lipoproteins while the HDL_3 particle acquires TG in exchange and is transformed to a more buoyant HDL_2 particle (4). In addition, HDL-CE are taken up by cells via a mechanism termed selective uptake without concomitant lipoprotein particle uptake (5). In vivo experiments performed in rats (lacking CETP) have demonstrated that this pathway is of major importance for hepatic removal of HDL-CEs (6). Simi-

Abbreviations: apo, apolipoprotein; BSA, bovine serum albumin; CE(s), cholesteryl ester(s); CETP, cholesteryl ester transfer protein; Ch, (unesterified) cholesterol; CHO, Chinese hamster ovary cells; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; HPLC, high performance liquid chromatography; HDL, high density lipoproteins; HMM(s), human monocyte-derived macrophages(s); LDL, low density lipoproteins; LPDS, lipoprotein-deficient serum; LPL, lipoprotein lipase; MPM(s), mouse peritoneal macrophage(s); PBS, phosphate-buffered saline; TBS, Tris-buffered saline; TLC, thin-layer chromatography; TG(s), triglyceride(s).

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lar studies performed in rabbits (animals with ≈3-fold higher CETP activities as compared to humans) revealed that selective uptake of HDL-CEs might contribute as much as 25% to total CE turnover (7). In addition to in vivo experiments, selective uptake of HDL-CE was studied in different organs and various primary and permanent cell lines. From some of these studies it appears that selective uptake is a receptor-independent pathway and does not require specific apolipoprotein components. Selective uptake of HDL-CE involves two steps, i.e., uptake in a releasable, plasma membrane-associated pool and subsequent transfer into a non-releasable, cytoplasmatic pool (8–11). Recently Acton et al. (12) have shown that SR-BI, a CD36-related class B scavenger receptor is involved in selective uptake of HDL lipids. Western blot analyses of murine tissues revealed high expression of SR-BI in liver, ovaries, and adrenal glands (13), tissues with high capacity for selective HDL-CE uptake. Additional support for the role of SR-BI during selective HDL-CE uptake stems from observations that overexpression of SR-BI in Chinese hamster ovary (CHO) cells resulted in significantly increased selective uptake of HDL-associated lipids (13).

In addition to SR-BI, lipoprotein lipase (LPL; E.C.3.1.1.34) has been suggested to mediate selective uptake of liposome-associated cholesteryl esters and ether analogues to human fibroblasts and J774 macrophages (14). In vivo, circulating chylomicrons are catabolized by LPL, with muscle and adipose tissue being the major sites of LPL expression (15). LPL hydrolyzes TGs from chylomicrons thereby generating free fatty acids (FFAs) for subsequent tissue uptake and metabolism (16). In addition, LPL has been shown to anchor lipoproteins to the surface of a variety of cells (17–19). LPL increases surface binding and degradation of lipoproteins (20–22) by mechanisms involving the low density lipoprotein receptor-related protein (LRP) and the very low density lipoprotein (VLDL) receptor (23–25).

The ability of LPL to enhance lipoprotein binding and uptake as well as apoprotein-independent lipid transfer of CEs has prompted us to investigate the effect of LPL on selective uptake of HDL_3-CEs. For these studies, LPL-deficient mouse macrophages obtained from transgenic knockout mice, expressing LPL exclusively in muscle, represented an excellent tool to study the implications of endogenous LPL on selective HDL_3-CE uptake. From our data it appears that endogenous LPL is only of minor importance for selective uptake of HDL_3-CEs by macrophages. In contrast, addition of exogenous bovine LPL to control and LPL-deficient macrophages enhanced HDL_3 holoparticle, total CE, and selective CE uptake up to 2-fold.

MATERIALS AND METHODS

Generation of transgenic knockout mice

The generation of transgenic knockout mice was described recently (26). Mouse lines expressing LPL exclusively in muscle were generated by breeding a transgenic mouse line containing the muscle specific creatine kinase promoter driving a human LPL minigene (27) onto the LPL knockout background. The breeding strategy, leading to mouse lines expressing human LPL exclusively in skeletal and cardiac muscle but not in any other tissue, was described elsewhere (26).

Isolation of mouse peritoneal macrophages

Isolation of mouse macrophages from control and transgenic knockout animals was performed as described (28). Briefly, peritoneal exudate cells were elicited by i.p. injection of 2 ml thioglycollate broth (3 g/100 ml; Difco Labs, Austria) and harvested 3 days post-stimulus injection. Macrophages were plated in Dulbecco's modified Eagle's medium (DMEM, BioWhitaker, Austria) on 6- or 12-well plates (Costar, Austria) and washed after 4 h three times with phosphate-buffered saline (PBS; 10 mM, pH 7.4, 0.15 M NaCl). Subsequently cells were cultured in DMEM containing 10% FCS (Bio Whitaker, Austria). Twelve h prior to the experiments cells were incubated in DMEM containing 10% FCS or 10% LPDS as indicated.

Lipoprotein preparation

Human apoE-free HDL_3 was prepared by discontinuous density ultracentrifugation of plasma obtained from normolipemic donors (29). HDL_3 was recovered from the tubes and dialyzed against 10 mM PBS (pH 7.4). SDS/PAGE revealed the presence of apoA-I as the major apolipoprotein. LPDS was prepared by standard techniques (30).

Lipoprotein labeling

HDL_3 was labeled with [1,2,6,7-^{3}H-cholesteryl] linoleate (DuPont NEN, Austria) by CETP-catalyzed transfer from donor liposomes essentially as described previously (31). Briefly, 200 μCi of the corresponding label and 80 μg egg yolk lecithin (Sigma, Austria) were dried under argon and sonicated in 1 ml of PBS. HDL_3 (1 ml, containing 2–5 mg protein), LPDS (1 ml, as a source of CETP), and PBS (1 ml) were added. The mixture was incubated under argon at 37°C in a shaking water bath overnight. Subsequently the labeled HDL_3 was reisolated in a TLX120 bench-top ultracentrifuge in a TLA100.4 rotor (Beckman, Austria) as described (32). The HDL band was aspirated and desalted by size exclu-

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sion chromatography on PD10 columns (Pharmacia, Austria). This labeling procedure resulted in specific activities of 5–7 cpm/ng HDL_3 protein.

Iodination of HDL_3 was performed as described by Sinn et al. (33) using N-Br-succinimide as the coupling agent. Routinely 1 mCi of ^{125}INa (Amersham, Austria) was used to label 5 mg of HDL_3 protein. This procedure resulted in specific activities between 300–450 dpm/ng protein. Unspecific activity was always less than 3% of total activity. No crosslinking or fragmentation of apoA-I due to the iodination procedure could be detected by SDS-PAGE on 15% gels and subsequent autoradiography.

Analysis of cellular lipids by thin-layer and high performance liquid chromatography

Cellular lipids were extracted with hexane–2-propanol 3:2 (v/v) as described (30). The lipid extracts were dried under argon, redissolved in 100 μl of $CHCl_3$ and separated by TLC using hexane–diethylether–acetic acid 70:29:1 (v/v/v) as the mobile phase. Lipid spots were visualized with iodine vapor. Lipid identification was performed by co-chromatography with known standards, i.e., cholesterol and cholesterol linoleate. After excision of the bands the radioactivity present in the Ch and CE band was quantitated by liquid scintillation counting in a LKB β-counter. Reversed phase HPLC of cellular lipid extracts was performed on a LC-18 column (250 × 4.6 mm, Supelco, Austria) with methanol–2-propanol as the mobile phase (1 ml/min) and detection at 210 nm, exactly as described previously (32). Quantitation was performed by peak area comparison with external standards of known concentration.

Reversed phase HPLC of radiotracers present in cellular lipid extracts was performed on a Chrompack LC-18 column (250 × 2 mm, Chrompack, Austria) with methanol–2-propanol 1:1(v/v) as the mobile phase (200 μl/min). Radiolabeled cholesterol and cholesteryl linoleate were detected with a Radiomatic Flo-One radiometric HPLC detector (Packard-Canberra, Austria; channel set at 0–18.5 keV) which was preceded by a variable wavelength UV-detector to allow simultaneous detection of labeled and unlabeled compounds. Liquid scintillation fluid flow rate was set at 1 ml/min.

LPL assays

LPL activity was measured as described previously (34). Briefly, 200 μl of cell supernatant was incubated with 100 μl of a stable substrate mixture consisting of tri[9,10(n)-^{3}H] oleoyl glycerol (Amersham, Austria), 4.63 mM trioleoyl glycerol, 0.45 mM lecithin, 1% BSA, 4.6% glycerol, 50 mM Tris-HCl (pH 8.2). LPL activity was calculated from the amount of FFA released per minute after subtraction of blank values. To discriminate between membrane bound and intracellular LPL activities, the supernatant was removed and macrophages were washed, chilled on ice, and incubated in the presence of 0.1 units heparin (4°C, 30 min). Intracellular LPL activities were measured after cell lysis in 500 μl lysis buffer (50 mM NH_4Cl, pH 8.1).

RNA isolation and Northern blot analysis

To isolate macrophage RNA, adherent cells were lysed (700 μl lysis buffer) and total RNA was isolated using the RNeasy total RNA kit (Quiagen, Austria). Total RNA (5–15 μg) was separated on 1.2% agarose gels (containing 1.1% formaldehyde and 1 × MOPS), transferred to a Hybond N$^+$nylon membrane (Amersham) by vacuum blotting, and hybridized to the radiolabeled mouse LPL cDNA and human LPL exon 10 probe as described (35). The DNA probes were radiolabeled with [^{32}P]dCTP using the Promega random primer kit.

Isolation of LPL from bovine milk

Bovine LPL was isolated as described (36). Fresh, unpasteurized bovine milk (1 liter) was centrifuged (9,000 rpm, 4°C, 30 min) to separate cream. The floating cake was discarded and NaCl was added to a final concentration of 350 mM to the remaining skim milk. After filtration, 40 ml heparin-Sepharose (Pharmacia) was added and the mixture was left overnight with gentle agitation on an end-over-end shaker. The gel was collected and washed in a glass frit. Subsequent purification steps, i.e., affinity chromatography on heparin-Sepharose and hydrophobic interaction chromatography on phenyl-Sepharose (Pharmacia), were performed as described for human LPL (34). Yields for bovine LPL ranged from 500–1500 μg LPL/l milk with activities between 420–680 μmol FFA/h per mg LPL protein.

Cell culture studies

Thioglycollate-elicited peritoneal mouse macrophages were plated on 6- or 12-well trays in DMEM. After 4 h under standard conditions (37°C, 5% CO_2, 95% humidity) cells were washed 3 times with PBS (pH 7.4) and kept in DMEM containing 10% FCS. Where indicated, cells were cultured in DMEM containing 10% LPDS 12 h prior to the experiments.

Lipid loading experiments were performed with ^{125}I-labeled-HDL_3 or [^{3}H]Ch18:2–HDL_3 in the presence or absence of a 10- or 40-fold excess of unlabeled HDL_3. After an incubation in the presence of the correspondingly labeled HDL_3 preparation, cells were washed twice in PBS containing BSA (5%, w/v) followed by three washes in PBS. To release LPL-bound HDL_3, cells were incubated in the presence of heparin (20 U/ml) at

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37°C for 10 min (37). The supernatant was collected and counted, and is referred to as LPL-bound HDL_3.

Binding studies in the presence and absence of LPL were performed at 4°C. Briefly, cells were incubated in the presence of 25 μg ^{125}I-labeled HDL_3 (±20 μg LPL) in the presence or absence of unlabeled HDL_3 for 4 h. Subsequently cells were washed as described above and the cells were lysed with 0.3 N NaOH to determine bound radioactivity and the cellular protein content. Specific binding was calculated as described below.

Time-dependent metabolism of ^{125}I-labeled HDL_3 and [^{3}H]Ch18:2–HDL_3 by control macrophages in the presence and absence of LPL was investigated at 37°C. Membrane-bound HDL_3 was released by trypsin treatment (0.05%, 37°C, 10 min) as described (31). The trypsin-releasable fraction is referred to as bound fraction. After trypsin treatment, cells were lysed in NaOH (1 ml, 0.3 N, 1 h at 25 °C) to determine the non-trypsin-releasable (internalized) fraction and the cellular protein content present in the lysate. Specific binding/internalization was calculated as the difference between total and nonspecific binding/internalization. Protein was measured according to the method of Lowry et al. (38), the average protein content on 6-well plates was 300–350 μg protein/well. Degradation of ^{125}I-labeled HDL_3 by mouse peritoneal macrophages was estimated by measuring the non-trichloroacetic acid (TCA)-precipitable radioactivity in the medium after precipitation of free iodine with $AgNO_3$. Briefly, 0.5 ml of medium was removed, mixed with 100 μl BSA (30 mg/ml) and 1 ml TCA (3 M, 4°C) and left at 4°C for 30 min. Subsequently 250 μl of $AgNO_3$ (0.7 M) was added, mixed, and the samples were centrifuged at 3,000 rpm at 4°C for 15 min. One ml of the supernatant was counted on a γ-counter (31).

Intracellular hydrolysis of [^{3}H]Ch18:2 was analyzed as follows. MPMs (cultured on 35-mm petri dishes as described above) were incubated with prewarmed medium containing 100 μg of [^{3}H]Ch18:2–HDL_3. At the indicated time points the medium was removed and the petri dishes were placed on ice immediately. Cells were washed twice with PBS containing BSA (5%, w/v) followed by two washes with PBS. Cellular lipids were then extracted with hexane–2-propanol 3:2 (v/v) and further processed for TLC analysis as described above.

To facilitate the comparison of results obtained with ^{125}I-labeled HDL_3 and [^{3}H]Ch18:2–HDL_3, selective HDL_3-CE uptake is expressed as apparent HDL_3 particle uptake as suggested by Pittman et al. (8). Apparent HDL_3 particle uptake is expressed in terms of HDL_3 protein (calculated on the basis of the specific activity of the corresponding [^{3}H]Ch18:2–HDL_3 preparations used) that would be necessary to deliver the observed amount of tracer. These calculations are performed to compare uptake of ^{125}I and ^{3}H tracers on the same basis. HDL_3 holoparticle uptake is characterized by equal uptake of both tracers.

RESULTS

Expression and activity of LPL in peritoneal macrophages obtained from control and transgenic knockout animals

Prior to LPL activity measurements, peritoneal macrophages were plated and cultured for 12 h in DMEM containing either 10% FCS or 10% LPDS. After this preincubation period, approximately 85% of the total LPL activity was found in the cellular supernatant of control macrophages **(Table 1).** Incubation of the cells with heparin (0.1 U/ml) led to a further release of 5% of total LPL activity while the remaining activity (10% of total) was found intracellularly. As a result of LPL gene knockout, LPL activities in macrophages obtained from transgenic knockout mice were undetectable (Table 1). To investigate whether the absence of LPL activity was directly related to LPL knockout on the DNA level, RNA was isolated from macrophages obtained from control and transgenic knockout mice. Northern blot analysis clearly demonstrated the presence of LPL-RNA in control macrophages while LPL-RNA was undetectable in macrophages obtained from transgenic knockout mice **(Fig. 1).**

Selective HDL-CE uptake by MPMs obtained from control and LPL gene knockout mice

Uptake experiments of apoE-free HDL_3 were performed with peritoneal macrophages obtained from control and transgenic knockout animals. Cells were seeded and incubated in the presence of ^{125}I-labeled HDL_3 and HDL_3 labeled with [^{3}H]Ch18:2 ([^{3}H]Ch18:2–HDL_3). In these experiments we have not distinguished between bound and internalized HDL_3 and therefore uptake refers to total cell association, i.e., the sum of bound and internalized HDL_3.

In the first set of experiments we compared HDL_3 holoparticle, total CE, and selective uptake by control and LPL-deficient macrophages. For these studies peritoneal macrophages were incubated in the presence of increasing amounts of the correspondingly labeled HDL_3 preparation either in the absence (total cell association) or in the presence (nonspecific cell association) of a 40-fold excess of unlabeled HDL_3. From the data shown in **Fig. 2A and B** it is evident that specific

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TABLE 1. LPL activities in control and LPL-deficient mouse peritoneal macrophages

	LPL Activity					
	Supernatant		Heparin-Releasable		Intracellular	
Macrophages	FCS	LPDS	FCS	LPDS	FCS	LPDS
	nmol FFA/min/mg cell protein					
Control	45 ± 1.8	40 ± 8.8	2.8 ± 0.4	4.5 ± 1.6	5.9 ± 0.3	3.7 ± 0.8
LPL-deficient	0.1 ± 0.1	0.1 ± 0.1	0.08 ± 0.2	0.03 ± 0.03	0.08 ± 0.02	0.02 ± 0.07

Thioglycollate-elicited macrophages obtained from control and transgenic knockout animals were seeded and incubated in DMEM (containing 10% FCS) for 12 h. After this preincubation, adherent cells were incubated for another 12 h in DMEM containing 10% FCS or 10% LPDS, respectively. Heparin-releasable, cell-associated, and LPL activities in the cellular supernatant were analyzed as described in Materials and Methods. Data represent mean ± SD (n = 8).

particle uptake of ^{125}I-labeled HDL_3 was almost identical for control (161 ± 17 HDL_3/mg cell protein) and LPL-deficient macrophages (187 ± 18 ng HDL_3/mg cell protein) at the highest ^{125}I-labeled HDL_3 concentration used (100 μg). Non-linear regression analysis revealed apparent B_{max} values of 386 and 376 ng HDL_3/mg cell protein (control and LPL-deficient macrophages, respectively).

In line with reports for permanent J774 macrophages (39), MPMs studied here displayed pronounced capacity for lipid tracer uptake in excess of particle association, exceeding holoparticle association 7.8-fold (control macrophages, 161 vs. 1251 ng/mg cell protein) and 7-fold (LPL-deficient macrophages, 187 vs. 1300 ng/mg cell protein) at the highest HDL_3 concentrations used (Fig. 2A and B). Independent of cell-associated LPL activities, selective uptake of HDL-CE was almost identical in both cell lines. Selective uptake exceeded particle uptake by a factor of 6.8-fold (control macrophages, 1090 vs. 161 ng/mg cell protein) and 6-fold (LPL-deficient macrophages, 1113 vs. 187 ng/mg cell protein). Apparent B_{max} values calculated for total CE uptake were 2272 and 2144 ng/mg cell protein (control and LPL-deficient macrophages, respectively). The data shown in Fig. 2A and 2B suggest that the capacity of MPMs for selective HDL_3-CE uptake is independent of LPL expression in these cells.

To further clarify the role of endogenous LPL during selective HDL-CE delivery, uptake studies were performed in the presence of heparin to release membrane-bound endogenous LPL. Macrophages were coincubated with ^{125}I-labeled HDL_3 or [^{3}H]Ch18:2–HDL_3 (100 μg, 6 h) in the absence or presence of 20 U/ml heparin. Results of these incubations are shown in **Table 2.** The values for specific holoparticle association were 158 ± 16 and 174 ± 21 ng HDL_3 protein/mg of cell protein for control and LPL-deficient macrophages, respectively. Particle association remained unaffected by the presence of heparin during the uptake experiments (180 and 177 ng HDL_3 protein/mg cell protein; control and LPL-deficient macrophages, respectively). In a parallel set of experiments, peritoneal macrophages were incubated with [^{3}H]Ch18:2–HDL_3 in the absence or presence of heparin. At the HDL_3 concentration used during these experiments, [^{3}H]Ch 18:2 uptake exceeded HDL_3 holoparticle uptake ≈8-fold, in line with data shown in Fig. 2. The values for specific [^{3}H]Ch18:2 cell association in the absence of heparin were 1312 ± 146 and 1328 ± 160 ng HDL_3 protein/mg cell protein (control and LPL-deficient macrophages,

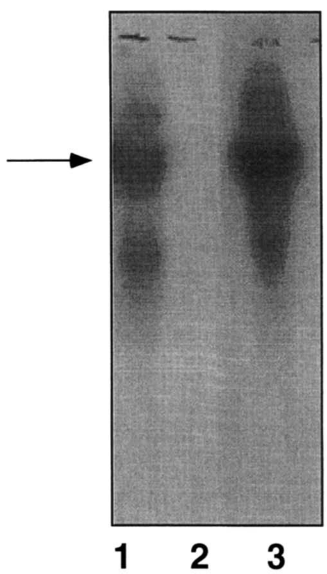

Fig. 1. Northern blot analysis of LPL in peritoneal macrophages obtained from control and transgenic knockout mice. Total RNA was isolated from control (lane 1) and LPL-deficient (lane 2) macrophages. RNA isolated from human adipose tissue (lane 3) served as control. RNA (10 μg) was separated on a 1.5% agarose gel. Hybridization was performed with mouse LPL-cDNA (lanes 1 and 2) and a human exon 10 LPL-probe (lane 3), respectively. The arrow indicates the position of LPL-mRNA.

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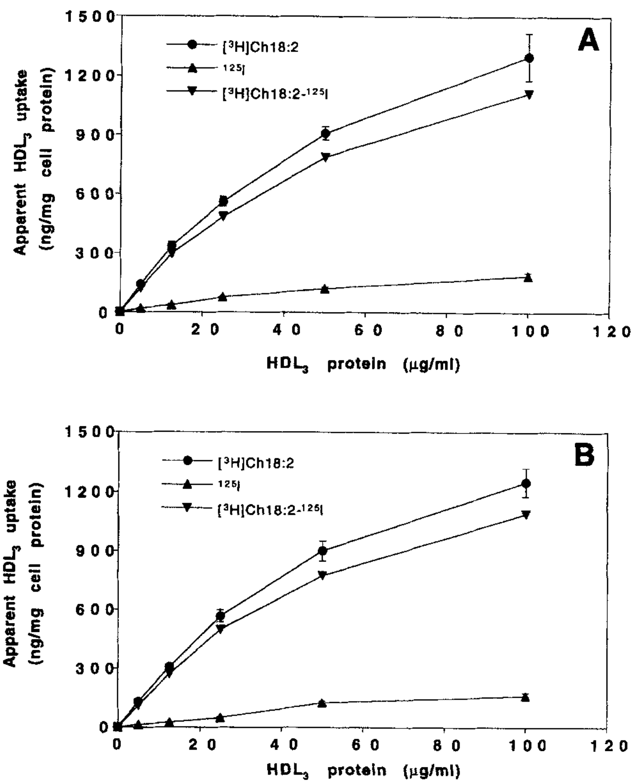

Fig. 2. HDL_3 holoparticle, total HDL_3-CE, and selective HDL_3-CE uptake by control (A) and LPL-deficient (B) macrophages. Macrophages were obtained from thioglycollate-elicited control and transgenic knockout mice as described in Materials and Methods. Cells were seeded in DMEM containing 10% FCS and cultivated for 4 h. Twelve h prior to the uptake experiments the medium was switched to DMEM containing 10% LPDS. Cells were then incubated in the presence of the indicated concentrations of ^{125}I- labeled and [^{3}H]Ch18:2–HDL_3 for 6 h at 37°C. Subsequently, the cells were washed and the total (i. e., bound and internalized) radioactivity was measured. ^{125}I uptake represents HDL_3 holoparticle cell association, while uptake of [^{3}H]Ch18:2 represents cell association of HDL_3-associated cholesteryl esters. Selective uptake was calculated as the difference between [^{3}H]Ch18:2 and ^{125}I-labeled HDL_3 cell association. To allow the comparison of cellular uptake of HDL_3 tracers, uptake is shown in terms of apparent HDL_3 particle uptake (expressed as HDL_3 protein that would be necessary to account for the observed tracer uptake; see Materials and Methods). Data represent mean ± SD from triplicate dishes from one representative experiment. Values shown represent specific cell association calculated as the difference of activities measured in the absence or presence of a 40-fold excess of unlabeled HDL_3.

respectively). The addition of heparin (20 U/ml) resulted in no significant changes of [^{3}H]Ch18:2 uptake when compared to results obtained in the absence of heparin (Table 2). Consequently, selective uptake of HDL_3-CEs also remained unaffected by the addition of heparin.

Previous studies have demonstrated that the capacity for selective HDL_3-CE uptake by fibroblasts and HepG2 cells is regulated by the cellular cholesterol content (40). We therefore studied whether cholesterol depletion of mouse peritoneal macrophages prior to uptake experiments affects selective uptake of HDL_3-CEs. For these experiments control and LPL-deficient macrophages were preincubated in DMEM containing either 10% FCS or 10% LPDS. A preincubation of control macrophages in DMEM containing 10% LPDS 12 h prior to the uptake experiments resulted in a significant decrease of cellular cholesterol levels as estimated by HPLC analysis. The cholesterol content of macrophages cultured in DMEM containing 10% FCS was 9.9 ± 0.43 µg/mg cell protein, while a 12-h preincubation in DMEM containing 10% LPDS decreased the cellular cholesterol content to 4.2 ± 0.25 µg/mg cell protein. In line with a regulatory effect of the intracellular cholesterol content on HDL_3-CE uptake, we have observed a 30% increase in [^{3}H]Ch18:2 association (**Fig. 3**) (1729 ± 164 vs. 1300 ± 57 ng/mg cell protein, LPDS vs. FCS incubation, respectively) when cells were depleted of cholesterol. HDL_3 holoparticle association remained unaffected (90 ± 9 vs. 97 ± 12 ng/mg cell protein, LPDS vs. FCS incubation, respectively). Accordingly, selective uptake of [^{3}H]Ch18:2 increased from 1164 to 1533 ng/mg cell protein (FCS vs. LPDS). The same effects were observed with LPL-deficient macrophages (data not shown). Collectively, our findings (Table 2, Fig. 2 and Fig. 3) suggest that endogenous LPL is of little (if any) importance for selective uptake. In contrast, the cellular cholesterol content appeared to be of importance for the capacity of selective [^{3}H]Ch18:2 uptake.

Intracellular hydrolysis of [^{3}H]Ch18:2–HDL_3 in MPMs

Prior to experiments aimed to determine intracellular hydrolysis rates of [^{3}H]Ch18:2, we examined the purity of tracers recovered from MPMs by HPLC with UV and subsequent radiometric detection. Therefore, the cells were incubated with [^{3}H]Ch18:2–HDL_3 for 1 h at 37°C. The cellular lipids (unlabeled and radioactive tracers) were extracted and separated by TLC; the bands comigrating with authentic (non-radioactive) cholesterol and cholesteryl linoleate standards were scraped off the plate; the TLC support was extracted twice with $CHCl_3$–MeOH 2:1 (v/v), dried under N_2, and analyzed by HPLC. The reproduction of a TLC separation of a standard mixture containing the indicated lipids is shown in **Figs. 4A** (inset) and **B** (inset). The area that was scraped off for further analysis by radio–UV–HPLC analysis is indicated. HPLC separation of the cellular lipids scraped off the TLC plate revealed the presence of [^{3}H]Ch (Fig. 4A) and [^{3}H]Ch18:2 (Fig. 4B) eluting as single peaks in the HPLC chromatogram coeluting with a standard mixture containing non-labeled Ch and Ch18:2 (Fig. 4C, 210 nm detection). It is impor-

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TABLE 2. Effect of heparin on tracer uptake by macrophages obtained from control and transgenic knockout animals

	Apparent HDL_3 Uptake *ng/mg cell protein*					
	^{125}I		$[^3H]Ch18:2$		$[^3H]Ch18:2 - {}^{125}I$	
Macrophages	−	+	−	+	−	+
Control	158 ± 16	180 ± 19	1312 ± 146	1451 ± 146	1154 ± 91	1271 ± 67
LPL-deficient	174 ± 21	177 ± 14	1328 ± 160	1232 ± 71	1154 ± 121	1055 ± 98

Macrophages were isolated and cultivated as described in Materials and Methods. Cells were incubated in the presence of ^{125}I-labeled HDL_3 or $[^3H]Ch18:2-HDL_3$ (100 μg protein, 6 h, 37°C) in the absence or presence of heparin (20 U/ml) to investigate the contribution of cell membrane-bound LPL during HDL_3 particle, total, and selective HDL_3 − CE uptake. Data shown represent mean ± SD from triplicate dishes from a typical experiment; (−) and (+) indicate the absence or presence of heparin. Values are expressed as (apparent) particle uptake (see Materials and Methods) in ng HDL_3 protein that would be necessary to account for the observed tracer uptake.

tant to note that UV detection at 210 nm provided sufficient sensitivity to detect endogenous Ch in the cellular lipid extracts (see above); however, no endogenous CEs were detectable. These analyses provided evidence that no other radioactive metabolites were comigrating with $[^3H]$Ch and $[^3H]$Ch18:2 under the chromatographic conditions used for TLC separation.

To assess the time-dependent intracellular hydrolysis of $[^3H]$Ch18:2 by control MPMs, the cellular lipid extracts obtained at the indicated time points were separated by TLC. The Ch and CE bands were cut out and counted on a β-counter. The total ($[^3H]$Ch plus $[^3H]$Ch18:2) and $[^3H]$Ch activity present in MPMs increased almost linearly up to 6 h **(Fig. 5).** Similarly, the radioactivity found in the $[^3H]$Ch18:2 fraction increased, but to a much lesser extent than observed for the activity found in the cholesterol fraction (72 to 570 vs. 45 to 5,560 cpm/mg cell protein, $[^3H]$Ch18:2 and $[^3H]$Ch activity, respectively). It is noteworthy that the radioactivity found in the $[^3H]$Ch18:2 band reached a plateau value at 30 min. The inset in Fig. 5 shows $[^3H]$Ch18:2 hydrolysis at incubation times from 2 to 30 min. As can be seen, $[^3H]$Ch formation started to increase between 2 and 5 min and exceeded $[^3H]$Ch18:2 activity within 15 min. The findings presented in Fig. 5 indicate that CEs, once internalized by macrophages, are hydrolyzed very rapidly. Intracellular hydrolysis was not affected by the addition of chloroquine (50 μM) indicating extralysosomal hydrolysis of HDL_3-CEs (data not shown).

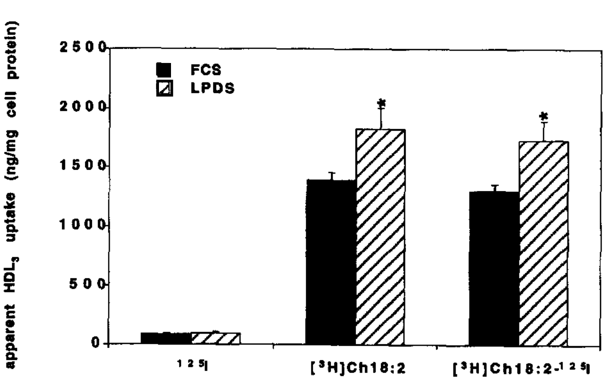

Fig. 3. Effect of cholesterol depletion on HDL_3-holoparticle, total CE, and selective CE uptake by control macrophages. Control macrophages were incubated for 12 h in DMEM containing 10% FCS or 10% LPDS. After this preincubation period, cells were incubated in the presence of 100 μg of ^{125}I-labeled HDL_3 or $[^3H]Ch18:2-HDL_3$ for 6 h at 37°C. Cells were washed and the total cell-associated radioactivity (i. e., bound and internalized) was measured. Data shown are mean ± SD (triplicates from one representative experiment) and represent specific cell association (20-fold excess of unlabeled HDL_3).

Contribution of exogenous LPL to HDL_3-holoparticle and selective uptake

Exogenous LPL was shown to enhance net transfer of cholesteryl esters and ethers from model lipid emulsion to fibroblasts and J774 macrophages (14). To test the hypothesis that exogenous LPL might enhance selective uptake of HDL_3-CEs, uptake experiments with control and LPL-deficient macrophages were performed in the presence of increasing LPL concentrations. Results of uptake experiments performed in the presence of exogenous LPL are shown in **Fig. 6 A–C.** Addition of increasing LPL concentrations led to a dose-dependent increase of ^{125}I-labeled HDL_3 and $[^3H]Ch18:2-HDL_3$ cell association. At a concentration of 40 μg/ml (corresponding to a molar ratio of LPL:HDL_3 = 0.5) particle association was enhanced from 145 to 293 and 155 to 290 ng HDL_3/mg cell protein for control and LPL-deficient MPMs, respectively (Fig. 6A). Total $[^3H]Ch18:2-HDL_3$ association increased from 1088 to 2581 and 1251 to 2522 ng HDL_3 protein/mg cell protein (control and LPL-deficient macrophages, respectively) (Fig. 6B). Consequently, selective uptake of HDL_3-associated $[^3H]$Ch18:2 was enhanced 2.4-fold (943 to 2288 ng HDL_3/mg cell protein, con-

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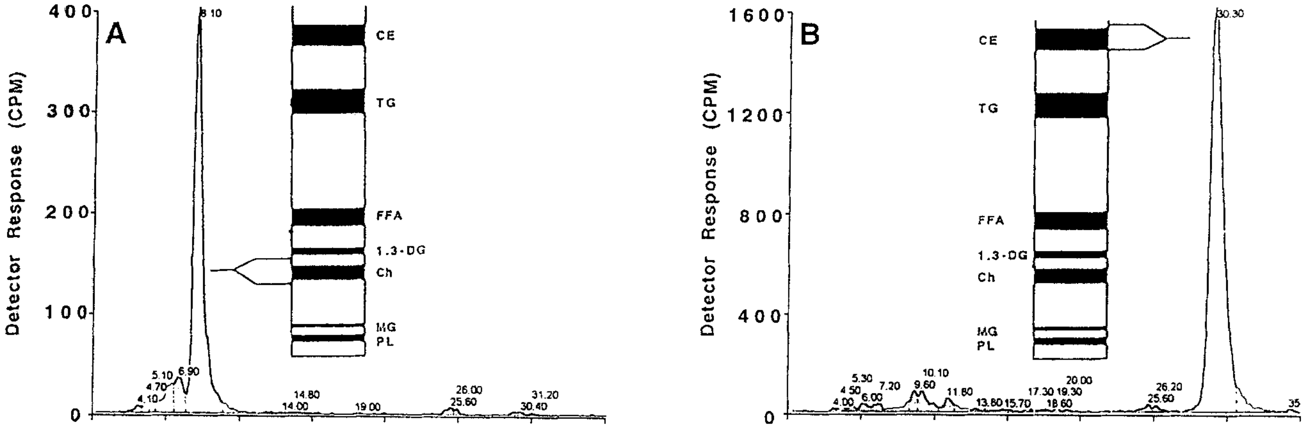

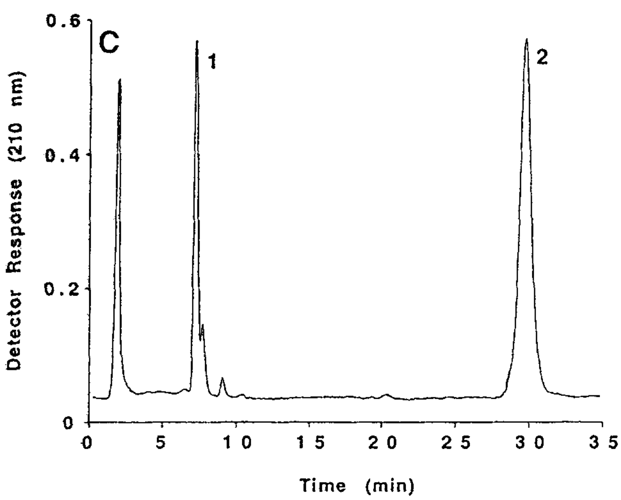

Fig. 4. UV-radiometric-HPLC analysis of macrophage cell-lipid extracts preseparated by TLC. Macrophages obtained from control mice were preincubated in DMEM containing 10% LPDS for 12 h prior to the experiment. Subsequently, cells were incubated in the presence of [^{3}H]Ch18:2-HDL$_3$ (100 μg, 1 h at 37°C) washed and extracted with hexane–2-propanol as described in Materials and Methods. The lipid extracts were dried under nitrogen and applied onto a silica 60 TLC plate. The bands comigrating with authentic cholesterol and cholesteryl linoleate standards were cut out, extracted twice with $CHCl_3$–MeOH, dried, redissolved in 100 μl of mobile phase, and subjected to HPLC analysis as described in Materials and Methods. The HPLC traces show the elution profile of radioactive material comigrating on TLC with cholesterol (A) and cholesteryl linoleate (B). Panel C shows the elution profile of a nonradioactive standard mixture containing cholesterol and cholesteryl linoleate. The insets shown in (A) and (B) represent a reproduction of a TLC separation of a unlabeled lipid standard mixture containing phosphatidylcholine (PL), monopalmitoyl-*rac*-glycerol (MG), cholesterol (Ch), 1,3 dipalmitoyl-glycerol (1,3-DG), linoleic acid (FFA), tripalmitoyl-glycerol (TG), and cholesteryl linoleate (CE) on silica gel 60 with hexane–diethylether–acetic acid 70:29:1 (v/v/v) as the mobile phase. The area scraped off and used for further analysis of cellular lipids by radio-UV-HPLC analysis is indicated. Peak assignment: 1-cholesterol; 2-cholesteryl linoleate (Ch18:2).

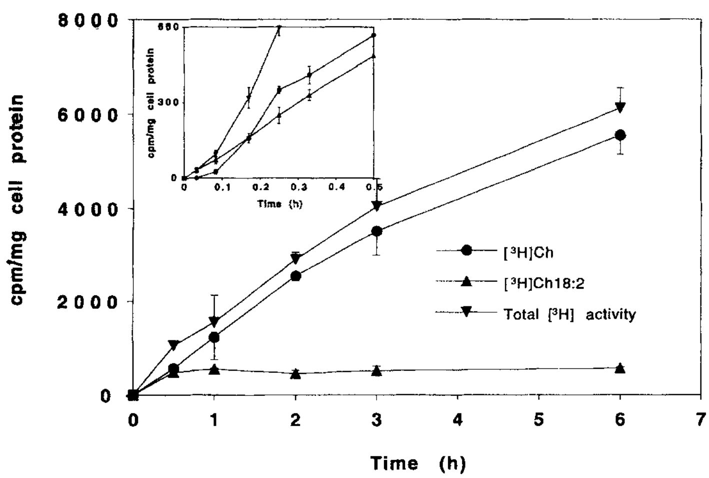

Fig. 5. Intracellular hydrolysis of HDL$_3$-associated [^{3}H]Ch18:2 in mouse peritoneal macrophages. Macrophages obtained from control mice were preincubated in DMEM containing 10% LPDS for 12 h prior to the experiments. Subsequently, 100 μg [^{3}H]Ch18:2-HDL$_3$ was added. At the indicated time points cells were washed and the cellular lipids were extracted twice with 1 ml hexane–2-propanol 3:2 (v/v). The lipid extracts were dried under nitrogen and separated on a silica 60 TLC plate as described in Materials and Methods. The bands comigrating with authentic cholesterol and cholesteryl linoleate standards were cut out and the radioactivity was counted. A shorter time course of intracellular [^{3}H]Ch18:2 hydrolysis is shown in the inset. Data represent mean ± SD of triplicate dishes from two representative experiments.

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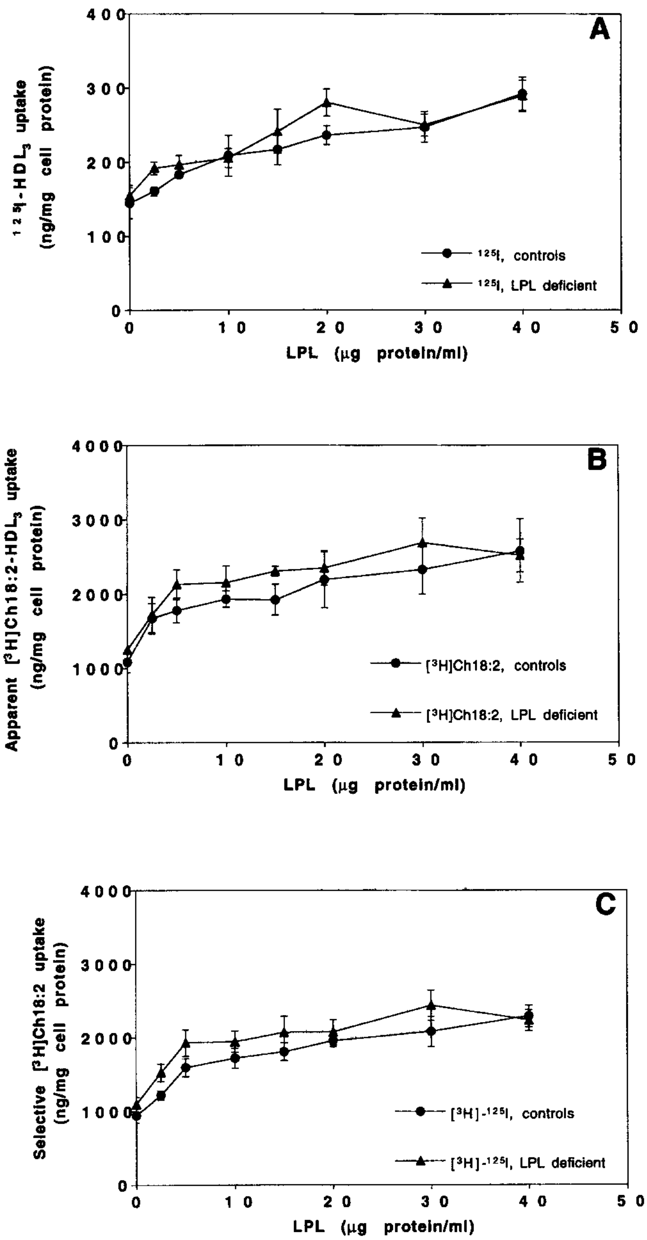

Fig. 6. Effect of exogenous LPL on HDL$_3$ holoparticle (A), total HDL$_3$-CE (B), and selective HDL$_3$-CE (C) uptake by control and LPL-deficient macrophages. Cells were preincubated in DMEM containing 10% LPDS (12 h) prior to the uptake experiments. The indicated concentrations of LPL were added together with 100 µg of ^{125}I-labeled HDL$_3$ or [^{3}H]Ch18:2-HDL$_3$. Cells were incubated for 6 h, washed, and the cell-associated radioactivity was determined. Data represent mean ± SD from two independent experiments, performed in triplicate.

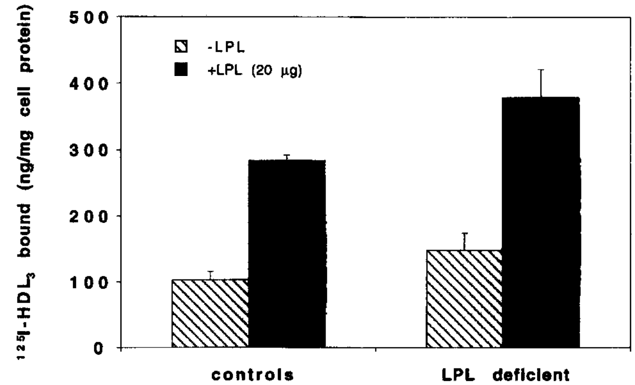

Fig. 7. Effect of exogenous LPL on HDL$_3$ holoparticle binding by control and LPL-deficient macrophages. Cells were preincubated in DMEM containing 10% LPDS (12 h) prior to the uptake experiments. Cells were then chilled on ice, washed, and incubated at 4°C in the presence of 25 µg ^{125}I-labeled HDL$_3$ in the presence and absence of 20 µg LPL (2 h). Cells were then washed and lysed in 0.3 N NaOH to determine the amount of bound label and the cellular protein content. Data shown represent mean ± SD from one experiment.

trols) and 2.1-fold (1096 vs. 2232 ng HDL$_3$/mg cell protein, LPL-deficient macrophages) (Fig. 6C). From these observations it appears that LPL concentrations have to be sufficiently high to enhance HDL$_3$ particle cell association and selective uptake of HDL$_3$ associated cholesteryl esters.

In the next set of experiments we attempted to identify the metabolic pathway responsible for LPL-enhanced HDL$_3$ uptake by MPMs. Therefore, binding, internalization, degradation, and selective uptake of ^{125}I-labeled HDL$_3$ and [^{3}H]Ch18:2–HDL$_3$ was investigated. The effect of exogenous LPL on HDL$_3$ binding (4°C) to control and LPL-deficient MPMs is shown in **Fig. 7.** The values observed for ^{125}I-labeled HDL$_3$ binding in the absence of exogenous LPL were 104 ± 13 versus 147 ± 27 ng/mg cell protein (control and LPL-deficient MPMs, respectively). In the presence of exogenous LPL ^{125}I-labeled HDL$_3$ binding was increased 2.7- and 2.6-fold (284 ± 8 vs. 378 ± 43 ng/mg cell protein; control and LPL-deficient macrophages, respectively). These data demonstrate that exogenous LPL significantly increased binding of ^{125}I-labeled HDL$_3$ to control and LPL-deficient MPMs.

To clarify whether LPL enhances (particle) binding and (CE) internalization to the same extent, uptake experiments were performed with MPMs in the presence of LPL, followed by heparin release of LPL-anchored HDL$_3$ particles. Results of these experiments are shown in **Fig. 8.** In line with results shown in Figs. 6 A–C, addition of LPL increased the amount of cell-associated tracer in a dose-dependent manner (Fig. 8A) and the amount of internalized label exceeded the amount of LPL bound (heparin-releasable label) at all LPL concentrations used during this experiment. However, the ratio of internalized:bound label decreased from 6.5 (no LPL) to 4 (11 µg LPL; molar ratio LPL:HDL$_3$ = 0.55). The effect of LPL on the percentage of heparin-releasable and non-releasable radioactivity is shown in Fig. 8B. Addition of increasing LPL concentrations led

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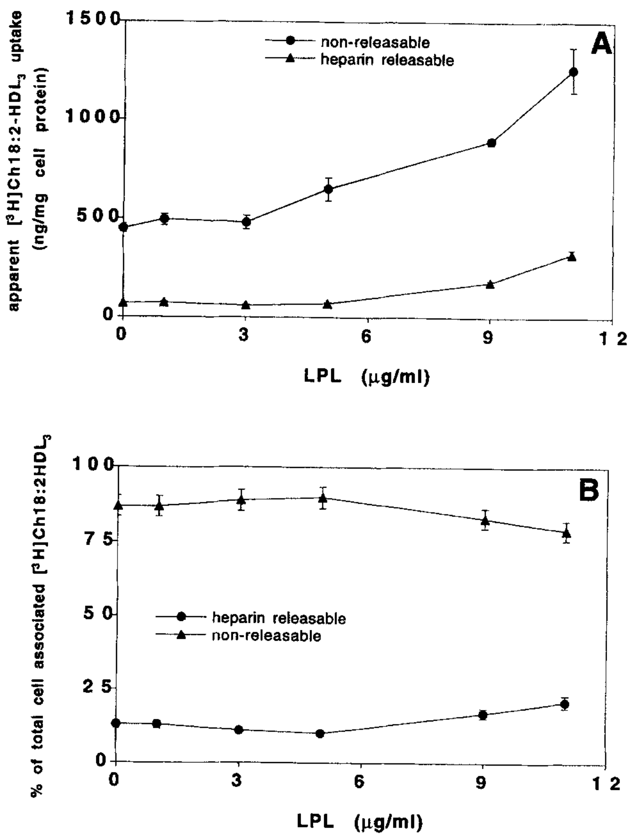

Fig. 8. Effect of exogenous LPL on [^{3}H]Ch18:2-HDL$_3$ binding and internalization by control macrophages. Cells were preincubated in DMEM containing 10% LPDS (12 h) prior to the uptake experiments. The indicated amounts of LPL were added together with 25 μg [^{3}H]Ch18:2-HDL$_3$ and incubated for 6 h (37°C). Cells were then washed at 4°C and incubated with heparin (50 U/ml, 15 min) to release LPL-bound [^{3}H]Ch18:2-HDL$_3$. The remaining cells were lysed with 0.3 N NaOH to estimate the amount of non-releasable [^{3}H]Ch18:2-HDL$_3$. Data shown represent absolute values (A) and relative data (B), expressed as releasable and non-releasable [^{3}H]Ch18:2-HDL$_3$ fraction of the total cell-associated radioactivity at the indicated time points. Data shown represent mean ± SD from triplicate dishes from one experiment.

to an increase in the heparin-releasable fraction (13 to 21%), while the non-releasable fraction decreased from 87 to 79%. These data could indicate that (beyond a certain molar ratio of LPL:HDL$_3$) HDL$_3$ internalization might become a rate-limiting step during (LPL supported) selective uptake.

A time course of ^{125}I-labeled HDL$_3$ holoparticle binding, internalization, and degradation by control macrophages in the absence and presence of exogenous LPL is shown in **Fig. 9.** As can be seen from Fig. 9A and B the amount of trypsin-releasable (bound) and non-releasable (internalized) ^{125}I-labeled HDL$_3$ reached a steady state plateau within 1 to 3 h. The addition of exogenous LPL significantly increased ^{125}I-labeled HDL$_3$ particle binding to MPMs (on average by 2-fold, except

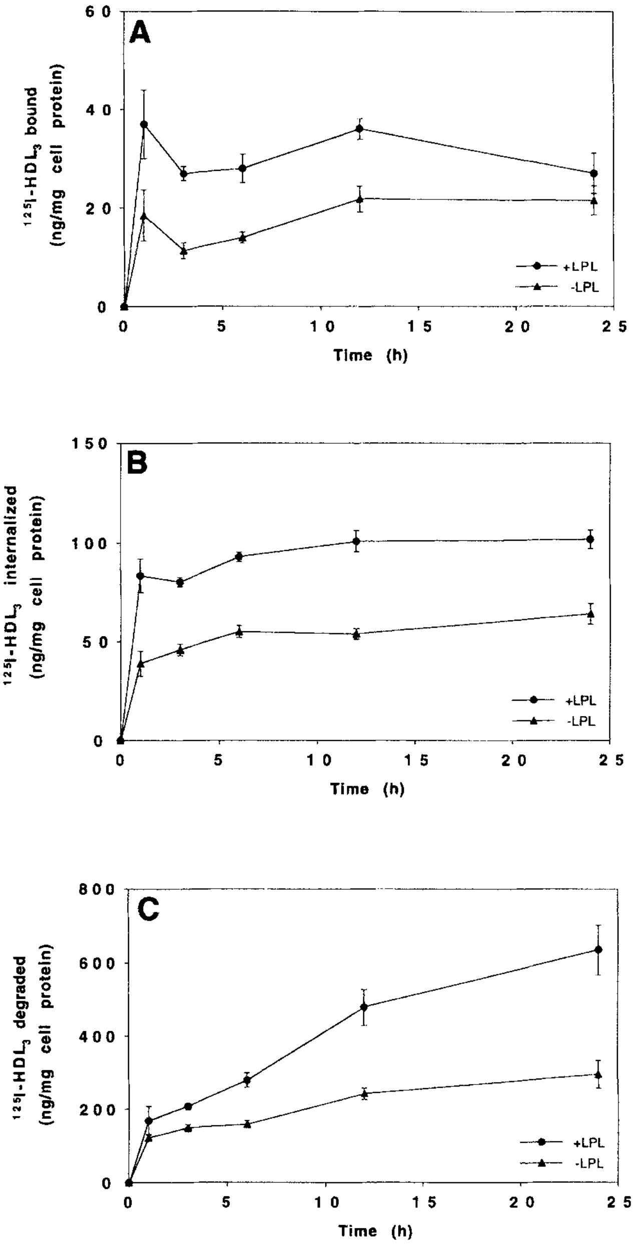

Fig. 9. Time-dependent effect of exogenous LPL on HDL$_3$ holoparticle binding (A), internalization (B), and degradation (C) of ^{125}I-labeled HDL$_3$ by control macrophages. Cells were preincubated in DMEM containing 10% LPDS (12 h) prior to the uptake experiments. Cells were incubated for the indicated times with 50 μg ^{125}I-labeled HDL$_3$ in the presence or absence of 50 μg exogenous LPL. At the indicated time points cells were washed, treated with trypsin (0.05%, 37°C, 10 min) to release bound HDL$_3$ holoparticles (A). The remaining cells were lysed in 0.3 N NaOH to determine the internalized fraction (B) and the cellular protein content. Non-TCA-precipitable degradation products were measured in the cellular supernatant as described in Materials and Methods (C). Data shown represent mean ± SD from triplicate dishes from one experiment.

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at the 24-h time point, where particle binding was not significantly different; Fig. 9A). In line with these observations, internalization of ^{125}I-labeled HDL_3 was significantly increased in the presence of LPL (2-fold increase at 24 h; 64 ± 5 vs. 120 ± 5 ng/mg cell protein, absence and presence of LPL, respectively; Fig. 9B). Also, degradation rates were significantly enhanced by the addition of exogenous LPL to MPMs (2.2-fold increase at 24 h; 635 vs. 295 ng/mg cell protein, respectively). However, in contrast to binding and internalization, degradation of HDL_3 did not reach a steady state plateau and was most pronounced at 24 h (Fig. 9C).

Next we studied the time-dependent effect of exogenous LPL on binding and internalization of [^{3}H]Ch18:2-HDL_3 and selective uptake of [^{3}H]Ch18:2 **(Fig. 10).** In the presence of exogenous LPL, binding of [^{3}H]Ch18:2-HDL_3 by MPMs was increased between 1.9 (3 h) and 2.3 (12 h)-fold when compared to results obtained in the absence of LPL (Fig. 10A). The absolute amount of bound [^{3}H]Ch18:2-HDL_3 was nearly identical to the amounts of ^{125}I-labeled HDL_3 released under the same conditions (Fig. 9A), indicating that trypsin releases HDL_3 particles from the cellular plasma membrane. The presence of exogenous LPL resulted in increased internalization of [^{3}H]Ch18:2 as is evident from Fig. 10B. After a 24-h incubation, the presence of LPL enhanced internalization 1.6-fold (1825 ± 255 vs. 3108 ± 226 ng/mg cell protein, respectively). However, internalization of [^{3}H]Ch18:2-HDL_3 increased continuously over the entire time frame investigated (Fig. 10B) and this is in contrast to the steady state situation observed with ^{125}I-labeled HDL_3 (Fig. 9B). Finally, selective uptake of [^{3}H]Ch18:2 by MPMs was also increased in a time-dependent fashion in the presence of LPL (Fig. 10C) resulting in 1.8-fold higher selective uptake as compared to results obtained in the absence of LPL. In line with results shown in Fig. 10B, selective uptake increased continuously during the 24-h incubation.

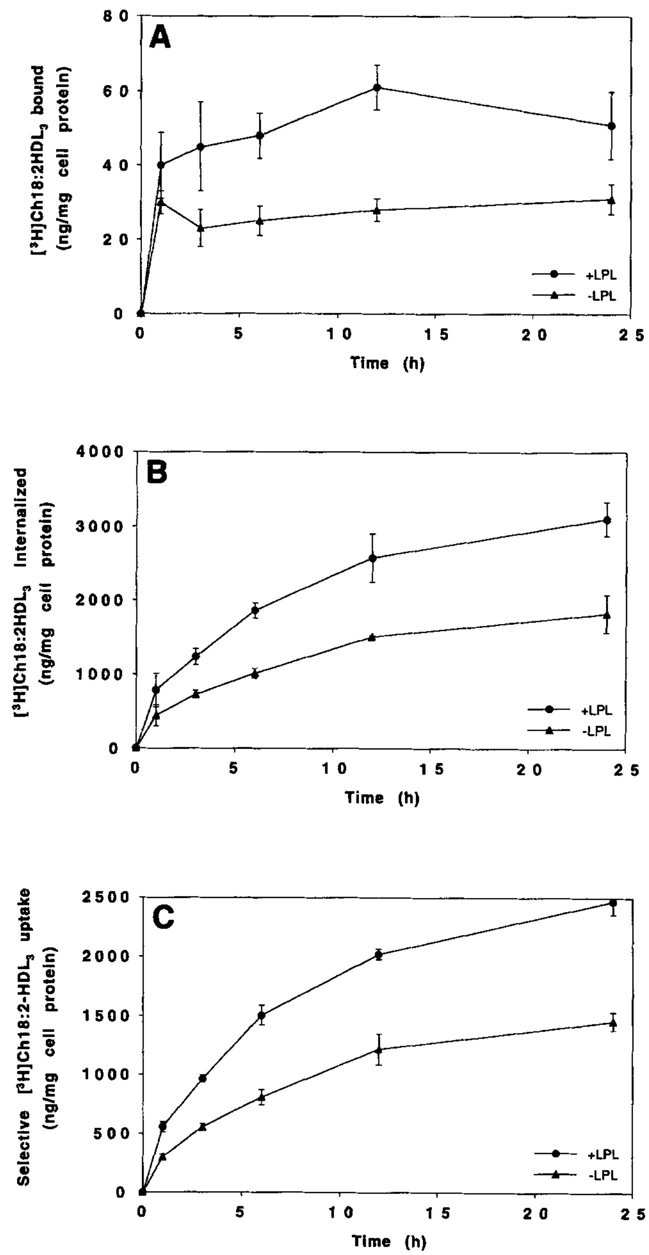

Fig. 10. Time-dependent effect of exogenous LPL on [^{3}H]Ch18:2-HDL_3 binding (A), internalization (B), and selective uptake of [^{3}H]Ch18:2 (C) by control macrophages. Cells were preincubated in DMEM containing 10% LPDS (12 h) prior to the uptake experiments. Cells were incubated for the indicated times with 50 μg [^{3}H]Ch18:2-HDL_3 in the presence or absence of 50 μg exogenous LPL. At the indicated time points cells were washed, treated with trypsin (0.05%, 37°C, 10 min) to release membrane bound HDL_3 holoparticles (A). The remaining cells were lysed in 0.3 N NaOH to determine the internalized fraction (B) and the cellular protein content. Selective uptake (C) was calculated by subtracting the values shown in Fig. 9 (bound + internalized + degraded) from the amount of bound and internalized [^{3}H]Ch18:2-HDL_3 shown here in (A) and (B).

DISCUSSION

In addition to its function as a TG-hydrolase, LPL was shown to enhance binding of essentially all lipoproteins to a variety of cells of different origin (17–25). Two functionally different domains in the LPL molecule, a heparin and a lipid binding site, were suggested to mediate enhanced lipoprotein binding to cell membranes (41). This mechanism is a fast, high capacity binding to heparan sulfate proteoglycans, concentrating lipoprotein particles on the cellular surface. In addition, LPL was shown to enhance transfer of CEs, cholesteryl ethers, and phospholipid-ether analogs from lipopro-

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tein-like lipid emulsions to cells (14). Traber, Olivecrona, and Kayden (42) have demonstrated enhanced transfer of tocopherols along with FFA from a TG model emulsion to fibroblasts upon addition of bovine LPL to the transfer reaction. In the present study we intended to differentiate between the role of endogenous and exogenous LPL during HDL_3-particle metabolism and selective uptake of HDL_3-CEs. To investigate the contribution of endogenous LPL, we performed uptake experiments with control macrophages (expressing functional LPL) and LPL-deficient macrophages obtained from transgenic knockout mice lacking LPL in macrophages (26).

We have hypothesized that endogenous LPL might contribute to selective uptake of HDL_3-CEs by the proposed bridging function of the enzyme. However, the results obtained during the present study argue against the participation of endogenous LPL during the uptake of CEs into a releasable plasma membrane pool (11). Two lines of evidence support these findings. First, selective uptake of HDL_3-CEs was almost identical in peritoneal macrophages obtained from control mice and transgenic LPL-knockout animals (Fig. 2). Second, the addition of heparin to control macrophages resulted only in a marginal, nonsignificant decrease of selective HDL_3-CE uptake and had no effect on HDL_3-cell association (Fig. 3). In line with these observations are findings that selective HDL_3-CE uptake by macrophages from transgenic mice specifically overexpressing LPL in these cells was almost identical as compared to controls (U. Panzenboeck, R. Zechner, and W. Sattler, unpublished observations). The present finding that HDL_3-CE uptake is insensitive to heparin is in accordance with data reported for permanent J774 macrophages (39) and human monocyte-derived macrophages (HMM; ref. 43). With respect to uptake mechanisms, MPMs and HMMs behave very similarly as both cell types display selective uptake of HDL_3-CEs over a wide range of HDL_3 concentrations (43). However, from our data it appears that MPMs have a much higher capacity for selective uptake than HMMs (6-fold vs. 2-fold in excess of HDL_3 particle uptake, ref. 43), while selective uptake by murine J774 macrophages was shown to level off at HDL_3 concentrations >25 μg/ml (39). The reasons for these differences are not entirely clear, but might reflect different CE acceptor capacity of the plasma membrane, probably as a result of different membrane lipid composition. CETP-mediated transfer of HDL-CEs to secreted lipoproteins that are subsequently subjected to reuptake via cellular lipoprotein receptors was demonstrated to contribute to selective CE uptake by HepG2 cells (39).

The fact that HDL-CEs are also selectively taken up by fibroblasts (40), cells that do not express LPL, supports our findings that the basal capacity for selective uptake is not necessarily dependent on the presence of endogenous LPL. One could envisage that HDL-binding proteins (44–46) could facilitate HDL_3-cell membrane association leading to selective HDL_3-CE uptake in further consequence. However, which of the macrophage HDL binding proteins is responsible for selective uptake remains to be elucidated.

It was also proposed that association of HDL_3 with endothelial cells, smooth muscle cells, and fibroblasts could be a receptor-independent process, regulated solely by the lipid content and/or lipid composition of the plasma membrane (47). The present results obtained during [^{3}H]Ch18:2–HDL_3 uptake experiments performed with cholesterol-deprived macrophages are in accordance with the proposed regulatory role of the membrane lipid content. Preincubation of MPMs (controls and LPL-deficient) in cholesterol-depleted medium increased selective uptake by 1.3-fold, while particle uptake remained unaffected by the cellular cholesterol status. This observation is in line with findings obtained with J774 macrophages (39), HMMs (43), and human fibroblasts (40). Incubation of J774 macrophages with acetylated LDL (increasing the intracellular cholesterol content) resulted in decreased selective HDL_3-CE uptake (39). The possibility that enhanced LDL-receptor expression might be responsible for increased selective HDL_3-CE uptake in cholesterol-deprived MPMs (Table 2) appears to be low because the HDL_3 preparations used were free of apoE, a ligand for the LDL-receptor. HDL_3-CEs selectively taken up by MPMs are subjected to rapid intracellular hydrolysis. Under our experimental conditions a delay of approximately 2–5 min prior to [^{3}H]Ch18:2 hydrolysis was observed and this is in line with findings published by Reaven, Tsai, and Azhar (48). In that study (48) rapid internalization of HDL_3-associated fluorescently labeled CEs was demonstrated in rat ovarian granulosa cells.

In contrast to endogenous LPL, a pronounced dose-dependent increase in HDL_3 particle, total HDL_3-CE, and selective HDL_3-CE cell association by increasing concentrations of exogenous LPL during uptake experiments was observed. In the presence of exogenous LPL, HDL_3 particle binding (4°C) was enhanced 2.7- and 2.6-fold (control and LPL-deficient macrophages, respectively). Experiments performed at 37°C revealed that above a certain threshold concentration (molar ratio of HDL_3: LPL = 0.5) the presence of LPL resulted in a gradual increase of the heparin-releasable (LPL-bound) fraction, while the internalized fraction was decreased. These findings suggest that the transfer of HDL-CEs into a non-releasable compartment (11) might be a rate-limiting step during selective uptake.

LPL appears to contribute to the observed increase

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of HDL_3-CE uptake by at least two different effects. First, addition of LPL resulted in increased HDL_3 particle binding to the plasma membrane and some of these HDL_3 particles are internalized by MPMs as indicated by increased ^{125}I-labeled HDL_3 internalization and degradation. However, increased HDL_3 holoparticle metabolism accounted for only 30–40% of the observed increase in selective HDL_3-CE uptake. Therefore, LPL appears to support the attachment of a second pool of HDL_3 particles to the cellular plasma membrane and these bound particles could deliver their CEs selectively to a non-releasable, intracellular compartment without parallel internalization of the donor particle. The question whether receptor-dependent (22) or receptor-independent endocytosis pathways (49) are responsible for the observed increase in HDL_3 holoparticle internalization and degradation remains to be elucidated.

Our results suggest that the concentration of exogenous LPL has to be sufficiently high to observe increased HDL_3-particle and -CE uptake. This is compatible with findings by Chajek-Shaul and coworkers (50) who have observed LPL-dependent uptake of chylomicron-associated cholesteryl ethers by lactating mammary glands in rats. However, in line with our observations, this dependency became apparent only when a certain threshold concentration of LPL activity was exceeded. The present and other findings (50) indicate that locally high tissue concentrations of LPL may promote selective uptake of cholesteryl esters. In addition, the effect of LPL on HDL_3 binding is dependent on the cellular system under investigation. Eisenberg et al. (21) have demonstrated that the addition of exogenous LPL to CHO cells, dermal fibroblasts, and smooth muscle cells enhanced HDL_3 binding by 20-, 16-, and 2.5-fold, respectively. In contrast, LPL was without effect on HDL_3 binding by HepG2 cells (21). The association of LPL with HDL in preheparin plasma was demonstrated by Villella et al. (51). Whether HDL-associated LPL itself could provide a signal for selective uptake of HDL-CEs remains to be elucidated.

The observed enhancement of selective HDL_3-CE uptake is partially compatible with a mechanism mediated by SR-BI; a murine class B scavenger receptor (12), identified as a docking receptor for HDL (13). SR-BI was shown to mediate HDL binding (but not internalization) and selective uptake of HDL-associated lipids by transfected CHO cells (13). In line with these observations SR-BI is most abundantly expressed in liver, ovaries, and adrenal glands, tissues where the majority of selective uptake occurs in vivo (13). It seems conceivable that the mechanism of LPL-mediated selective uptake could be quite similar. LPL appears to concentrate HDL_3 particles on the outer leaflet of the cell membrane thus increasing net transfer of CEs to a plasma membrane compartment. It is important to note, however, that (in contrast to SR-BI) LPL enhanced HDL_3 particle internalization and degradation.

It is generally assumed that selective uptake of HDL-CE has a beneficial effect, by preventing the accumulation of cholesterol in extrahepatic tissues (6). Accordingly, selective uptake was demonstrated in hepatic cells (52), perfused liver (6), and steroidogenic tissues (53, 54). Experiments performed in HepG2 cells, in situ perfused rat liver, and intact rats suggested that selective uptake of oxidized HDL_3-CE represents an efficient detoxification route for these potentially (cyto)toxic lipids (31, 55, 56). Taken together, all the effects mediated by selective HDL_3-CE uptake could be considered as potentially antiatherogenic. In contrast, LPL expression by foam cells in atherosclerotic plaques (57), LPL-dependent binding of unmodified and oxidized LDL by the endothelial cell matrix (58, 59), and LPL-mediated uptake of oxidized LDL by macrophages (60) suggest that LPL-mediated lipoprotein binding and uptake might contribute to atherogenesis. Whether selective uptake of HDL-CEs mediated by locally high tissue concentrations of LPL could contribute to cholesterol accumulation in macrophages in vivo remains to be elucidated.

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