Effects of Lipoprotein Lipase on Uptake and Transcytosis of Low Density Lipoprotein (LDL) and LDL-associated \(\alpha\)-Tocopherol in a Porcine \textit{in Vitro} Blood-Brain Barrier Model*

Daniel Gotti‡§, Zoltan Balazs‡, Ute Panzenboeck‡, Andelko Hrzenjak‡, Helga Reicher‡, Elke Wagner‡, Rudolf Zechner‡, Ernst Malle‡, and Wolfgang Sattler**

From the ‡Institute of Medical Biochemistry and Molecular Biology, University Graz, Harrachgasse 21, Graz 8010 and the ¶Institute of Molecular Biology, Biochemistry and Microbiology, University Graz, Heinrichstrasse 31, Graz 8010, Austria

During the present study the contribution of lipoprotein lipase (LPL) to low density lipoprotein (LDL) holoparticle and LDL-lipid (\(\alpha\)-tocopherol (\(\alpha\)TocH)) turnover in primary porcine brain capillary endothelial cells (BCECs) was investigated. The addition of increasing LPL concentrations to BCECs resulted in up to 11-fold higher LDL holoparticle cell association. LPL contributed to LDL holoparticle turnover, an effect that was substantially increased in response to LDL-receptor up-regulation. The addition of LPL increased selective uptake of LDL-associated \(\alpha\)TocH in BCECs up to 5-fold. LPL-dependent selective \(\alpha\)TocH uptake was unaffected by the lipase inhibitor tetradecanoylphosphatidin but was substantially inhibited in cells where proteoglycan sulfation was inhibited by treatment with NaClO\(_3\). Thus, selective uptake of LDL-associated \(\alpha\)TocH requires interaction of LPL with heparan-sulfate proteoglycans. Although high level adenoviral overexpression of scavenger receptor BI (SR-BI) in BCECs resulted in a 2-fold increase of selective LDL-\(\alpha\)TocH uptake, SR-BI did not act in a cooperative manner with LPL. Although the addition of LPL to BCEC Transwell cultures significantly increased LDL holoparticle cell association and selective uptake of LDL-associated \(\alpha\)TocH, holoparticle transcytosis across this porcine blood-brain barrier (BBB) model was unaffected by the presence of LPL. An important observation during transcytosis experiments was a substantial \(\alpha\)TocH depletion of LDL particles that were resecreted into the basolateral compartment. The relevance of LPL-dependent \(\alpha\)TocH uptake across the BBB was confirmed in LPL-deficient mice. The absence of LPL resulted in significantly lower cerebral \(\alpha\)TocH concentrations than observed in control animals.

Lipoprotein lipase (LPL)\(^1\) plays a central role in lipoprotein metabolism. LPL is synthesized in most extrahepatic tissues where it is transported to the endothelium (1, 2), a process dependent on heparan sulfate proteoglycans (HSPG) and the very low density lipoprotein (VLDL) receptor (3). There, LPL hydrolyzes triglycerides (TGs) in chylomicrons and VLDL, thereby generating free fatty acids that enter either storage or oxidative pathways. LPL also contributes to the exchange of lipids and apoproteins between different lipoprotein classes, thus affecting size and composition not only of TG-rich lipoproteins but also of low and high density lipoproteins (LDL and HDL) (1, 4). In addition to its lipolytic function, it was demonstrated that proteoglycan-bound LPL directly interacts with lipoproteins, concentrating lipoprotein particles on the cell surface where they are internalized along with lipoprotein receptors (5–8) or in conjunction with proteoglycans (9). This “bridging function” is independent of the enzymatic activity of LPL. In addition to lipoprotein receptor- or proteoglycan-mediated internalization of lipoproteins, LPL can contribute to selective lipid uptake. During selective uptake, originally lipoprotein-associated lipids from the surface and core are internalized by cells without concomitant endocytosis of the lipid-depleted lipoprotein (holo)particle. This pathway was described for HDL- and LDL-associated lipids to occur via pathways mediated by scavenger receptor BI (SR-BI) (10–14) or LPL (15–17).

In contrast to other tissues, the functional significance of LPL expression in brain remains unclear. Yet there is ample evidence that LPL is functional in this organ: LPL expression was documented in brain microvessels (18) and in various brain regions (19) and is regulated by and responsive to starvation (20). Therefore, it was suggested that LPL at the blood-brain barrier (BBB) could supply the neonatal brain with unsaturated fatty acids during embryonic development (21). On the other hand, the bridging function of LPL was proposed to contribute to the transport of lipoprotein-associated vitamins and cholesterol esters across the BBB (2). This hypothesis is supported by the fact that tissue-specific overexpression of LPL in transgenic animals results in significantly increased \(\alpha\)-tocopherol (\(\alpha\)TocH) concentrations at the site of LPL expression (22).

During the present study we examined the pathways of LDL holoparticle and selective LDL-\(\alpha\)TocH uptake in primary porcine brain capillary endothelial cells (BCECs) and the modulation of these pathways by LPL. In addition, we have studied lipoprotein; LPDS, lipoprotein-deficient serum; \(\alpha\)TocH, \(\alpha\)-tocopherol; BBB, blood-brain barrier; BCECs, brain capillary endothelial cells; acLDL, acetylated LDL; FCS, fetal calf serum; HSPG, heparan sulfate proteoglycans; SR-BI, scavenger receptor class B, type I; TER, transendothelial electrical resistance; TGs, triglycerides; TTHL, tetrahydrolipstatin; TBS, Tris-buffered saline; m.o.i., multiplicity of infection.

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§ Present address: Dept. of Pediatric Infectious Diseases, Johns Hopkins School of Medicine, Baltimore, MD 21205.

¶ Both authors contributed equally to this work.

** To whom correspondence should be addressed. Tel.: 43-316-380-4188; Fax: 43-316-380-9615; E-mail: wolfgang.sattler@kfunigraz.ac.at.

1 The abbreviations used are: LPL, lipoprotein lipase; LDL, low density lipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein; LPDS, lipoprotein-deficient serum; \(\alpha\)TocH, \(\alpha\)-tocopherol; BBB, blood-brain barrier; BCECs, brain capillary endothelial cells; acLDL, acetylated LDL; FCS, fetal calf serum; HSPG, heparan sulfate proteoglycans; SR-BI, scavenger receptor class B, type I; TER, transendothelial electrical resistance; TGs, triglycerides; TTHL, tetrahydrolipstatin; TBS, Tris-buffered saline; m.o.i., multiplicity of infection.
whether LPL affects transcytosis rates of LDL particles across an in vitro model of the BBB and whether targeted knock-out of the LPL gene affects cerebral αTocH levels.

**EXPERIMENTAL PROCEDURES**

**Materials**

Earl's medium M199, Dulbecco's modified Eagle's medium/Ham's F-12 (1:1, v/v) medium, penicillin/streptomycin, gentamicin, l-glutamine, and trypsin were obtained from Biochrom (Berlin, Germany). Ox serum was from PAA Laboratories (Linz, Austria). Plastic ware for cell culture and Transwell inserts (polycarbonate membrane, 0.4-μm pore size) were from Costar (Vienna, Austria). Na125I (specific activity 629 GBq/mg) was from PerkinElmer Life Sciences (Vienna). [14C]Iodoacetic acid ([14C]IAA) was from Amersham Biosciences (Tokyo, Japan). PD-10 size-exclusion columns, heparin-Sepharose, dNTPs, RNAguard, and random hexamer primers were obtained from Amersham Biosciences. An RNeasy kit was from Qiagen (Vienna); poly(A) reverse transcriptase were from Invitrogen (Vienna). PCR buffer and Taq DNA polymerase were from Finnzymes Oy (Vienna). PCR primers were from MWG Biotech (Ebersberg, Germany).

**Methods**

Isolation and Cultivation of BCECs—Porcine BCECs were isolated by sequential enzymatic digestion and centrifugation steps according to Tewes et al. (23) and characterized as described previously (24). During the first 2 days in vitro, BCECs (from one brain) were cultured in eight 75-cm2 cell culture flasks containing 10 ml of unheated bovine milk (1 liter) exactly as described previously (23). LDL was recovered by direct precipitation of free iodine with AgNO3 (24). [14C]TocH-labeled LDL (26) was from Amersham Biosciences (Tokyo, Japan). LDL uptake is expressed in terms of [14C]TocH-modified LDL turnover at the BBB.
marker proteins is indicated. The molecular mass of the LPL antiserum (1:500), peroxidase-conjugated goat-anti mouse IgG (1:2000), and subsequent ECL development. The molecular mass of the marker proteins is indicated. Lane 1, LPL purified from bovine milk (1 μg of protein); lane 2, BCECs lysate (50 μg of protein). B, effect of LPL on LDL association to BCECs. BCECs were cultivated on 12-well trays and incubated with the indicated molar ratios of LPL:125I-LDL (0–120 μg/ml LPL and 50 μg/ml LDL protein) for 4 h at 37 °C. At the end of the experiments the cells were washed and 125I-LDL cell association was determined as described under “Experimental Procedures.” Results represent means ± S.D. from one representative experiment performed in triplicates.

Fig. 1. A, endogenous, immunoreactive LPL is detectable in BCEC lysates. Porcine BCECs were cultured on 75-cm² flasks as described under “Experimental Procedures.” Cells were then scraped and lysed by sonication in sample buffer, and proteins were separated by SDS-PAGE (reducing conditions, 8% gels) and transferred to nitrocellulose. Immunochemical detection of LPL was performed with polyclonal anti-human LPL antiserum (1:500), peroxidase-conjugated goat-anti mouse IgG (1:2000), and subsequent ECL development. The molecular mass of the marker proteins is indicated. Lane 1, LPL purified from bovine milk (1 μg of protein); lane 2, BCECs lysate (50 μg of protein). B, effect of LPL on LDL association to BCECs. BCECs were cultivated on 12-well trays and incubated with the indicated molar ratios of LPL:125I-LDL (0–120 μg/ml LPL and 50 μg/ml LDL protein) for 4 h at 37 °C. At the end of the experiments the cells were washed and 125I-LDL cell association was determined as described under “Experimental Procedures.” Results represent means ± S.D. from one representative experiment performed in triplicates.

Effect of Exogenous LPL on LDL Cell Association—To determine the effects of LPL on cell association of 125I-LDL to BCECs, cells were incubated at increasing LPL:LDL molar ratios (Fig. 1B). The addition of LDL and enzymatically active LPL at increasing molar ratios dose-dependently increased the total cell association of 125I-LDL, indicating that LPL increases binding and/or uptake of LDL. The most pronounced effect (−11-fold increase, 403 versus 4529 ng/mg of cell protein) was observed at a molar ratio of 100:1 (LPL:LDL, 120 μg/ml LPL and 50 μg/ml LDL). At the concentration ranges tested during these experiments the effect of LPL tended to level off but did not reach a real saturation plateau.

A

B

Fig. 1. A

To assess whether this increase in cell association is due to enzymatically active LPL, incubations were performed in the presence of tetrahydrolipstatin (THL), a potent inhibitor of LPL activity (Fig. 2A). In the absence of LPL, THL and heparin were without effect on LDL association. The addition of LPL to the medium (molar ratio of LPL:LDL = 100) resulted in a pronounced, 11-fold increase of cell-associated LDL that was independent of the presence of THL. This indicates that the observed LPL-mediated effect does not depend on its TG-hydrolase activity and thus results from the “bridging function” of the enzyme. More than 50% of 125I-LDL was not heparin-releasable, indicating efficient lipoprotein particle uptake (Fig. 2B).

As LPL was discussed to contribute to the supply of the central nervous system with lipophilic vitamins, the effect of LPL on the uptake of LDL-associated [14C]αTocH was investigated. In principle, uptake of LDL-associated αTocH can occur by holoparticle uptake, selective uptake, or a combination of both pathways. The effect of LPL on either of these pathways is shown in Table I. In line with our previous results (24), αTocH uptake exceeded holoparticle uptake (14C versus 125I), indicating selective uptake of αTocH. The addition of LPL to the incubation medium resulted in significantly enhanced 125I- and [14C]αTocH-LDL association (4- to 10-fold) in a time-dependent manner (1–5 h). The addition of LPL augmented selective αTocH uptake at 3 and 5 h (3- and 4.7-fold, respectively). These findings indicate that LPL is able to promote LDL holoparticle uptake and selective uptake of LDL-associated αTocH in endothelial cells constituting the BBB.

The LDL-receptor Contributes to LPL-mediated Holoparticle Binding and Turnover—The fact that BCECs express the LDL-receptor (32–34) prompted us to study the interaction of the LDL-receptor and LPL at the BBB. During these experiments (Fig. 3) LDL-receptor expression was either down-regulated (incubation in FCS, inset lane 1) or up-regulated (preincubation in LPDS, inset lane 2), and then the cells were incubated with
Effect of LPL on LDL particles, and selective \(^{14}C\)αTocH uptake in BCECs

Cells were incubated in the presence of 15 μg of \(^{125}\text{I}-\) or \(^{14}\text{C}\)αTocH-labeled LDL in the absence or presence of LPL (20 μg). At the indicated time points, the cells were washed and \(^{125}\text{I}-\) LDL uptake (cell association and degradation), and cell-associated \(^{14}\text{C}\)αTocH was analyzed. Uptake was calculated as described under “Experimental Procedures.” Results represent mean ± S.D. from one representative experiment performed in triplicates.

| Incubations | \(^{14}\text{C}\)αTocH-LDL | \(^{125}\text{I}\)-LDL | \(^{14}\text{C} - \(^{125}\text{I}\) | μg LDL/mg cell protein |
|-------------|----------------|----------------|----------------|-------------------|
| 1-h incubation | LDL | 1.5 ± 0.1 | 0.5 ± 0.1 | 1.0 |
|              | LDL + LPL | 5.4 ± 0.6 | 5.2 ± 0.1 | 0.2 |
| 3-h incubation | LDL | 3.6 ± 0.3 | 0.7 ± 0.7 | 2.9 |
|              | LDL + LPL | 16.0 ± 0.6 | 7.3 ± 0.1 | 8.7 |
| 5-h incubation | LDL | 5.2 ± 0.5 | 0.9 ± 0.1 | 4.3 |
|              | LDL + LPL | 29.1 ± 2.9 | 8.7 ± 0.2 | 20.4 |

**Fig. 3. Effect of LDL-receptor up-regulation on LPL-mediated LDL binding by BCECs.** Prior to the experiment, cells in 12-well trays were precultured (24 h) in medium containing 10% FCS (indicated by –) or LPDS (indicated by +) to regulate LDL-receptor expression. Cells then received cold medium (4 °C) containing LDL or acLDL (10 μg/ml) in the absence or presence of LPL (10 μg/ml). After a 1.5-h incubation at 4 °C the cells were washed and lysed, and the radioactivity and the cellular protein content were determined. Results represent means ± S.D. from one representative experiment performed in triplicates. The inset shows immunoblot analysis of LDL-receptor expression in BCEC homogenates. Prior to Western blot analysis with a monoclonal antibody, cells were kept in medium containing FCS (lane 1) or LPDS (lane 2). The LDL-receptor was detected at ~160 kDa (under reducing conditions).

\(^{125}\text{I}-\text{LDL}\) or \(^{125}\text{I}-\text{acLDL}\) in the absence or presence of LPL. A preincubation of BCECs in LPDS-containing medium in the absence of LPL led to a 2-fold increase of \(^{125}\text{I}-\text{LDL}\) binding (56 versus 30 ng/mg of cell protein). The addition of LPL to FCS-preincubated cells resulted in a 27-fold increase in \(^{125}\text{I}-\text{LDL}\) binding. The effect of LPL was even more pronounced in LPDS-preincubated cells where the addition of LPL resulted in a 44-fold increase in \(^{125}\text{I}-\text{LDL}\) binding. To exclude the possibility that the effects described in Fig. 3 are due to up-regulation of SR-BI, the same experiments were performed with acLDL, which is a ligand for SR-BI but not for the LDL-receptor (Fig. 3). As observed with native LDL, the binding of acLDL was increased 1.5-fold in response to LPDS. However, the effects of LPL were much weaker with acLDL and led to 4- and 8-fold increases (FCS and LPDS, respectively) of acLDL binding.

To further explore whether the LDL-receptor is involved in LPL-dependent LDL particle turnover at 37 °C, the next series of experiments was performed under FCS or LPDS conditions and LDL binding, internalization, and degradation were determined (Fig. 4). Under these experimental conditions, the addition of LPL led to a pronounced 3.3-fold increase of binding at the beginning of the experiment, an effect that was further increased by a preincubation in LPDS-containing medium (4.7-fold, Fig. 4A). At longer incubation times this effect was ablated, due to internalization of the originally surface-bound particles. Up-regulated LDL-receptor expression resulted in ~2-fold higher LDL internalization rates (Fig. 4B). The presence of LPL increased \(^{125}\text{I}-\text{LDL}\) internalization between 8- and 18-fold. In line with data shown in Fig. 4 (A and B), degradation of \(^{125}\text{I}-\text{LDL}\) (Fig. 4C) was considerably enhanced under LPDS conditions and the presence of exogenous LPL; when cells were preincubated in LPDS, a 1.2-fold increase in overall degradation was observed. In the presence of LPL the degradation rates were increased 3.7-fold (LPD and FCS) and 4.4-fold (LPD and LPDS), respectively. In summary, the data shown in Fig. 4 strengthen the concept that LPL-supported LDL particle turnover by BCECs is modulated at the level of LDL-receptor expression.

**Mechanisms Contributing to LPL-mediated Selective Uptake of LDL-associated αTocH**—The prime candidate receptor mediating selective uptake of lipoprotein-associated lipids is SR-BI. During preliminary experiments we have tested whether SR-BI contributes to selective uptake of LDL-associated αTocH in transiently SR-BI-transfected COS cells. These experiments revealed that high level SR-BI overexpression resulted in only slightly increased (1.5-fold) selective uptake of LDL-associated αTocH (data not shown). To further determine whether SR-BI and LPL could act synergistically on αTocH uptake, BCECs were transfected by an adenoviral approach (30). Along this line it is important to note that the virus concentrations needed to obtain feasible transfection rates (~70%) were 100 times higher for BCECs as compared with COS cells (m.o.i. 1000 versus 10 in COS cells). Transfection of BCECs with a control virus (containing the β-galactosidase reporter gene) was without effect on selective αTocH uptake (Fig. 5), whereas adenoviral transduction of SR-BI resulted in a 2.5-fold increase in selective LDL-αTocH uptake. Addition of LPL resulted in 5.5- and 5.9-fold higher selective LDL-αTocH uptake in wild-type and SR-BI-transfected cells, respectively. Thus, it appears that LPL-mediated induction of selective LDL-αTocH uptake is independent of SR-BI expression. When cells were treated with NaClO₃ (an inhibitor of proteoglycan sulfation) prior to the uptake experiments, selective uptake of αTocH was significantly attenuated (2-fold), indicating that HSPG mediates at least part of LPL-dependent selective uptake of LDL-
normal or up-regulated LDL-receptor expression. Prior to the experiment, BCECs were infected with adenovirus containing either the human SR-BI (m.o.i. = 1000) or a β-galactosidase reporter gene (m.o.i. = 500) transgene as outlined under “Experimental Procedures.” Separate wells of BCECs were incubated in the presence of NaClO₃ (30 mM) for 36 h at 37 °C to inhibit sulfation of proteoglycans. Wild-type cells (wt), SR-BI overexpressors, β-galactosidase reporter gene-infected (mock) cells, and ClO₃⁻-treated cells were then incubated in the presence of [¹²⁵I]αTocH-labeled LDL (15 μg/ml) in the absence or presence of LPL (20 μg/ml) for 6 h at 37 °C. After the incubations the cells were washed and lysed to determine the radioactivity and the cellular protein content. Data shown represent mean values from one representative experiment performed in triplicates.

To test whether LPL contributes to αTocH uptake across the BBB in vivo—To test whether LPL contributes to αTocH uptake across the BBB in vivo, brains of control (L2), heterozygous (L1), or homozygous (L0) LPL knock-out mice were analyzed (31). The cerebral αTocH content of L0 animals was significantly (p < 0.01) lower as compared with L1 and L2 animals (1.28 ± 0.28, 1.56 ± 0.21, and 1.61 ± 0.15 μg/g wet tissue, respectively). In contrast, the αTocH concentrations in brains of L2 and L1 animals were not significantly different (Fig. 7). These findings indicate that the absence of LPL affects cerebral αTocH concentrations.

Effects of LPL on LDL Transcytosis—BCECs are polarized cells that actively regulate the diffusion and transport of circulating metabolites or xenobiotics into the brain parenchyma and vice versa (35). To study the effects of exogenous LPL on lipoprotein transcytosis, cells were cultured on Transwell inserts as described under “Experimental Procedures.” To get an idea about the contribution of lysosomal degradation to LDL holoparticle turnover, degradation was assessed as outlined under “Experimental Procedures.” The amount of trichloroacetic acid-soluble radioactivity was dependent on the presence of LPL (1756 ± 2215 ng/mg of cell protein, Fig. 6), in line with data shown in Fig. 4C. Analysis of cell-associated tracers revealed that the uptake of LDL-associated [¹⁴C]αTocH exceeded [¹²⁵I]αTocH uptake. Prior to the uptake experiment, BCECs were incubated with NaClO₃ (30 mM) for 36 h at 37 °C to inhibit sulfation of proteoglycans. Wild-type cells (wt), SR-BI overexpressors, β-galactosidase reporter gene-infected (mock) cells, and ClO₃⁻-treated cells were then incubated in the presence of [¹²⁵I]αTocH-labeled LDL (15 μg/ml) in the absence or presence of LPL (20 μg/ml) for 6 h at 37 °C. After the incubations the cells were washed and lysed to determine the radioactivity and the cellular protein content. Data shown represent mean values from one representative experiment performed in triplicates.

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LPL-modulated LDL Turnover at the BBB

FIG. 6. Effect of LPL on cell association and transcytosis of \(^{125}\)I- and \(^{14}\)C]TocH-LDL. BCECs were cultured on collagen-coated Transwell inserts (0.4-μm pore size, 1.13-cm\(^2\) surface area), and TER values were measured as outlined under "Experimental Procedures." Only BCEC monolayers with a TER ≥ 300 ohm/cm\(^2\) were used. Mean sucrose permeability was 4.1 × 10\(^{-6}\) cm/s. Cells were incubated in the presence of \(^{125}\)I-LDL or \(^{14}\)C]TocH-LDL (40 μg per well in the upper, apical compartment) in the absence or presence of LPL (10 μg/well) for 3 h at 37°C. Thereafter the insert was removed, washed twice with cold TBS (containing bovine serum albumin) and twice with TBS, and cut out, and the cells were lysed in NaOH (0.3 M). Part of the lysate was used to determine the cellular protein content, and the remaining aliquot was used to measure the cell-associated radioactivity. The medium from the basolateral compartment was removed and counted. Data shown represent mean values from one representative experiment performed in quintuplicates.

FIG. 7. \(\alpha\)TocH content in brains of control, heterozygous, and homozygous LPL knock-out mice. Newborn animals (L2, \(n = 13\); L1, \(n = 29\); L0, \(n = 18\)) were killed between 0 and 4 h after birth. Genotyping was performed from tail tip DNA by PCR analysis. Brains were removed, snap-frozen, and homogenized in liquid nitrogen. Genotyping was performed from tail tip DNA by PCR analysis. Brains were removed, snap-frozen, and homogenized in liquid nitrogen. Folating Polich extraction, the lipid extracts were analyzed by HPLC with fluorescence detection. Statistical significance was analyzed by Student’s t test, and data are presented as Box Whiskers plots.

DISCUSSION

Although LPL mRNA is present in brain and LPL protein expression was demonstrated in brain microvessels (18, 19), the functional significance of LPL at the BBB is not clear. The present study aimed at characterizing LPL-modulated uptake and transcytosis routes for LDL and LDL-associated \(\alpha\)TocH. LPL is able to form a molecular bridge between proteoglycans and lipoproteins in vitro (reviewed in Refs. 1 and 2) and in vitro (36); therefore, it was suggested that LPL could facilitate transcytosis of lipoprotein-associated vitamins across the BBB (2). This could result either from enhanced LPL-mediated holoparticle transcytosis across the endothelial layer constituting the BBB, or by LPL-mediated selective uptake, as described for HDL- and LDL-associated cholesterol esters (15–17, 37).

During the present studies we have found that primary BCECs isolated from porcine brains contain endogenous LPL that was most probably transferred to BCECs from surrounding cells. The addition of exogenous LPL augmented both LDL holoparticle and selective \(\alpha\)TocH uptake in BCEC monolayers. The addition of LPL resulted in significantly increased LDL holoparticle cell association and turnover via mechanisms related to LDL-receptor expression. In the presence of LPL, selective \(\alpha\)TocH uptake from LDL increased up to 5-fold, and this was inhibited by adding NaClO\(_3\). Although adenosinoverexpression of SR-BI resulted in 2-fold increased selective \(\alpha\)TocH uptake, SR-BI overexpression was without effect on \(\alpha\)TocH uptake in the presence of LPL. Transwell experiments revealed that LDL holoparticle transcytosis was unaffected by LPL. However, an important observation was the significant \(\alpha\)TocH depletion of lipoprotein particles that were resorbed into the basolateral compartment.

As demonstrated for BCECs obtained from other species (32, 38, 39), expression of the LDL-receptor in porcine BCECs is responsive to the availability of cholesterol in the culture medium. This is important because increased binding of LDL to HSPG in the presence of LPL is followed by LDL-receptor-mediated uptake (40, 41). During the present study the effects of LPL-mediated increase in binding, holoparticle internalization, and degradation were regulated in a similar manner as LDL holoparticle turnover in the absence of LPL, indicative of an involvement of the LDL-receptor. In line with earlier results (42) binding experiments performed at 4°C strongly imply that the interaction of LDL/LPL complexes occurs via the LDL-receptor. When the LDL-receptors of BCECs were saturated with \(^{125}\)I-LDL at 4°C and then switched to 37°C, the majority of \(^{125}\)I-LDL was internalized at the earliest time point analyzed (0.5 h), which roughly corresponds to the recycling time of the LDL-receptor (up to 15 min (43)). An involvement of the LDL-receptor during endocytosis is further substantiated by experiments with \(^{125}\)I-acLDL. acLDL is a ligand for the scavenger receptors class A and B, both being expressed on porcine BCECs (30, 39, 44), but not for the LDL-receptor (27). In accordance, the effect of exogenous LPL on acLDL binding to BCECs was much lower as compared with native LDL (4- to 8-fold increase versus 27- to 45-fold enhancement for native LDL).

Dehouck and colleagues (32) provided evidence that the LDL-receptor facilitates transcytosis of LDL across a bovine in vitro model of the BBB. This was based on two major observations: (i) a monoclonal anti-LDL-receptor antibody that interacts with the binding domain of the receptor completely blocked transcytosis and (ii) transcytosis occurred via a non-degradative pathway, obviously different from the classic LDL-receptor pathway. In BCEC monolayers LDL enters a degradative pathway that is upregulated in response to cholesterol depletion. Moreover, in the Transwell system the vast majority of degraded LDL (%99) was detected in the apical compartment, with only 1–1.5% detectable in the basolateral compartment. Whether this indicates that only a subset of LDL-receptors undergoes transcytosis and bypasses the lysosomal compartment is currently not clear. However, from our findings it is evident that LDL has no effects on the net transcytosis rates of LDL holoparticles. These observations suggest that the majority of LDL/LPL complexes formed in the luminal compartment are directed to lysosomal degradation and, thus, are not available for LDL-receptor-mediated transcytosis.

The brain depends on a constant and adequate supply with \(\alpha\)TocH. This is underscored by severe and characteristic neuropathologies that develop in response to \(\alpha\)TocH deficiency, in
a manner reminiscent of Friedreich's ataxia (45, 46). In fasting humans circulating αTocH is roughly equally distributed between the LDL and HDL fractions (22). Therefore, LDL holoparticle uptake or selective uptake of LDL-αTocH at the BBB and subsequent transcytosis would be an attractive pathway to facilitate αTocH uptake into the brain. As demonstrated in one of our earlier reports (24), BCECs are capable of selective αTocH uptake. The present study revealed that the basal capability of BCECs for selective αTocH uptake is increased ~5-fold in the presence of exogenous LPL. As has been reported for LDL-associated cholesterol ether (17), and HDL-associated cholesterol esters (15, 16), LPL-dependent selective αTocH uptake was independent of LPL catalytic activity as determined in the presence of TTHL. Treatment of the cells with NaClO4 (30 mM, higher concentrations affected cell viability) led to a 50% reduction in LPL-mediated selective uptake, indicating that proteoglycans are involved in this process. These findings are in line with other reports: See et al. (17) have demonstrated that LPL-mediated selective uptake of LDL-cholesterol ether was significantly reduced in fibroblasts after blockage of proteoglycan sulfation and in proteoglycan-deficient Chinese hamster ovary cells. HSPG was also implicated in the uptake of VLDL by macrophages (9) and LPL-mediated LDL uptake by HepG2 cells and fibroblasts (40).

The present study revealed that αTocH uptake into brain is modulated by LPL in vivo. Brains obtained from newborn homozygous LPL knock-out mice (31) had a significantly lower absolute αTocH content as compared with heterozygous (L1) and control (L2) animals. At birth the lipoprotein profiles of L2, L1, and L0 mice are not significantly different. Immediately after birth TGs of L0 mice are mildly increased and HDL levels are not significantly different from controls (31). This ensures that the reduced αTocH concentration in brain of LPL knock-out animals observed during the present study are due to the absence of LPL and not a result of altered lipoprotein profiles. Previously, we have shown that selective uptake of LDL-associated αTocH is mediated via SR-BI (30) and most probably bypasses LPL-dependent selective uptake. Consistent with this finding, it was demonstrated that the cerebral αTocH content of SR-BI (but not LDL-receptor) knock-out mice is significantly reduced (by ~70% (47)).

The potential role of SR-BI on selective LDL-αTocH uptake was studied by transient adenoviral transduction of human SR-BI into BCECs. SR-BI is a receptor with multiligand specificity and mediates selective lipid uptake also from LDL (14). During the present study SR-BI transduction resulted in increased selective LDL-αTocH uptake, however, this was only 50% of the value obtained in the presence of LPL. Most importantly the effect of SR-BI overexpression was ablated when LPL was present during the incubation. Along this line it is interesting to note that HDL turnover by BCECs was much less affected by the presence of exogenous LPL. The presence of LPL in the apical compartment of Transwell inserts had no effect on LDL holoparticle transcytosis (see above) and, thus, no effect on αTocH accumulation in the basolateral compartment. Probably the most intriguing observation during the present study was a significant αTocH depletion (~50% relative to protein) of the LDL particles that were resealed into the basolateral compartment. This would indicate that part of αTocH (and probably other lipids) is shed from non-degraded LDL particles during transcytosis across the in vitro BBB. A similar phenomenon of selective lipid depletion that was reported for HDL undergoing retroendothelialization in hepatocytes (48, 49) could occur at the BBB. In this hepatic, SR-BI-depend-
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40. Mulder, M., Lombardi, P., Jansen, H., van Berkel, T. J., Frants, R. R., and Havekes, L. M. (1993) J. Biol. Chem. 268, 9369–9375
41. Eisenberg, S., Seluyok, E., Olivecrona, T., and Vlodavsky, I. (1992) J. Clin. Invest. 90, 2013–2021
42. Medh, J. D., Bowen, S. L., Fry, G. L., Ruben, S., Andracki, M., Inoue, I., Lalouel, J. M., Strickland, D. K., and Chappell, D. A. (1996) J. Biol. Chem. 271, 17073–17080
43. Basu, S. K., Goldstein, J. L., Anderson, R. G., and Brown, M. S. (1981) Cell 24, 493–502
44. de Vries, H. E., Kuiper, J., van Berkel, T. J. C., and Breimer, J. J. (1993) J. Neurochem. 61, 1813–1821
45. Muller, D. P. R. (1995) Redox Report 1, 239–245
46. Ouahchi, K., Arita, M., Kayden, H., Hentati, F., Ben Hamida, M., Sokol, R., Arai, H., Inoue, K., Mandell, J. L., and Koenig, M. (1995) Nat. Genet. 9, 141–145
47. Mardones, P., Strobel, P., Miranda, S., Leighton, F., Quinones, V., Amigo, L., Rozowski, J., Krieger, M., and Rigotti, A. (2002) J. Nutr. 132, 443–449
48. Silver, D. L., Wang, N., and Tall, A. R. (2000) J. Clin. Invest. 105, 151–159
49. Silver, D. L., Wang, N., Xiao, X., and Tall, A. R. (2001) J. Biol. Chem. 276, 25287–25293