Lipoprotein lipase in highly vascularized structures of the eye

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Abstract Ocular tissues are highly dependent on lipid turnover and metabolism, which requires an uptake mechanism for fatty acids from lipoproteins. We studied the activity and expression of lipoprotein lipase (LPL), which catalyzes the hydrolysis of plasma triglycerides, in different ocular regions. Human and bovine eyes were dissected and various specialized anatomical areas were assayed for LPL activity, mRNA, and immunoreactivity. Variable levels of LPL activity were detected in all structures in human and bovine eyes. LPL activity was much higher in vascularized structures, such as ciliary body, iris, and retina than in avascular eye structures, such as vitreous body, lens, and cornea. In both human and bovine eyes, ciliary body contained the highest LPL lipolytic activity. LPL mRNA was detected by reverse transcription followed by polymerase chain reaction (RT-PCR) in all regions of human eyes. By RT-PCR analysis it was shown that bovine eyes contained high levels of LPL mRNA in ciliary body and iris, lower levels were found in retina, optic nerve, and lens, whereas no LPL mRNA could be found in bovine cornea. RT-PCR data, obtained in bovine eyes, agree with the results obtained by Northern blot experiments, confirming the high levels of LPL mRNA in iris and ciliary body. Immunofluorescence experiments performed on human eye samples indicated that the LPL protein is mostly distributed on the choroides, the choriocapillaris, and on the vessels of ciliary body, iris, optic nerve, and retina. The present study demonstrates that active LPL protein is synthesized, secreted, and located among microvessels in several specialized regions of the eye, and suggests that LPL could be involved in the uptake of fatty acids by the ocular tissues.—**Casaroli-Marano, R. P., J. Peinado-Onsurbe, M. Reina, B. Staels, J. Auwerx, and S. Vilaró.** Lipoprotein lipase in highly vascularized structures of the eye. *J. Lipid Res.* 1996. **37:** 1037–1044.

Supplementary key words retina • iris • ciliary body • lipid metabolism • immunocytochemistry • very low density lipoprotein • chylomicrons • triglycerides

Ocular tissues do not normally store large reserves of fat. Most of their lipids are located in cell membranes and their organelles. For example, polyunsaturated fatty acids are abundant in retinal photoreceptor cells, which are crucial for visual transduction and neurotransmission (1–3). These processes require a high turnover of the lipid constituents, which implies an effective mechanism for their uptake and further processing. Lipoproteins, the major source of lipids for organs, are efficient carriers of energy-rich but insoluble plasma lipids from sites of synthesis (liver) to sites of storage or utilization (4). Several classes of lipoproteins can be distinguished on the basis of their lipid and protein composition. The major lipids transported within chylomicrons and very low density lipoprotein (VLDL) particles are triglycerides (5, 6). These triglyceride-rich lipoproteins bind transiently to endothelial sites, where their triglycerides are hydrolyzed by lipoprotein lipase (LPL), liberating fatty acids and monoglycerides, which are consequently taken up by cells and used in metabolic reactions (7). LPL is synthesized in parenchymal cells of many tissues, but its functional site is at the vascular endothelium, where it binds to heparan sulfate proteoglycans (7). The enzyme is located in the glycocalyx, where it can interact with lipoprotein particles. Previous studies (8–10) have shown that LPL is synthesized in a wide variety of cell types such as adipocytes, skeletal and smooth muscle cells, neurons, myocytes, mammary epithelial cells, and macrophages (see ref. 8 for review), and its distribution has been demonstrated in vessels of different tissues such as lung, spleen, brain, and liver (9–11).

Dyslipidemic conditions, such as those seen in LPL

Abbreviations: LPL, lipoprotein lipase; VLDL, very low density lipoprotein; LDL, low density lipoprotein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse transcription polymerase chain reaction.

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deficiencies, are associated with increased levels of chylomicrons transported in plasma. Mild or severe ocular manifestations such as lipemia retinalis, retinal degeneration, amongst others can be observed in such disorders (12–14). In view of the low lipid storage capacity of the eye, despite an extremely active fatty acid metabolism, and in view of the possible presence of ocular alterations observed in genetic deficiencies in LPL, we were interested in studying this enzyme in ocular tissues. Because in other tissues LPL is the functional enzyme responsible for the hydrolysis of plasma triglycerides and the uptake of fatty acids from triglyceride-rich lipoproteins, we hypothesized that LPL may be involved in the uptake of fatty acids by ocular tissues. For this purpose we analyzed LPL activity, mRNA expression, and LPL immunoreactivity by immunofluorescence studies in different areas of both human and bovine eyes. The results provide direct evidence in favor of the hypothesis that LPL is synthesized and active in the eye.

MATERIALS AND METHODS

Tissue samples

Normal human eyes (n = 13), donated for corneal transplant in accordance with the Standardized Rules for Development and Applications of Organ Transplants contemplated by Spanish law, were obtained from the Eye Bank of Barraquer Ophthalmological Center (Barcelona, Spain). Donors were aged between 3 and 85 yr (mean age = 60.3 yr) and the ocular samples were processed within 12–48 h post-mortem. Bovine eyes (n = 10) were obtained from young animals in a local slaughterhouse, and processed within 2–6 h of death. All specimens were carefully examined, dissected into pieces corresponding to the anatomical zones (cornea, iris, lens, ciliary body, retina, vitreous body, and optic nerve), weighed, rapidly frozen in liquid nitrogen, and maintained at -80°C, until preparation of tissue homogenates for LPL activity assays and RNA extraction. For immunofluorescence studies, a part of the fresh tissue was immediately fixed with 3% paraformaldehyde in 0.1 M PBS, pH 7.4, for at least 12 h at 4°C. Tissue was then rinsed in 0.1 M PBS and immersed in 2.1 M sucrose in PBS solution for 2 to 4 h. The samples were then embedded in OCT (Miles, Elkhart, IN), frozen in isopentane, and stored at -80°C for cryostat sections.

Chemicals and antibodies

Bovine serum albumin (essentially fatty acid-free), egg albumin, gelatin, dithiothreitol, trioleoylglycerol, and PIPES, were obtained from Sigma Chemical (St. Louis, MO). HEPES was from Boehringer-Mannheim (Mannheim, Germany). Tri[9,10-(n)-^{3}H]oleoylglycerol was obtained from Amersham (Bucks, U.K.). Affinity-purified chicken antibodies against bovine milk LPL (ChaLPL) and preimmune antibodies were generously given by Dr. Gunilla Olivecrona (University of Umeå, Umeå, Sweden). Rabbit antiserum against recombinant human LPL (RaLPL) and preimmune serum were produced in our laboratory. All other reagents were of the highest purity available.

Indirect immunofluorescence procedure

For immunofluorescence techniques, sections (7 μm to 12 μm) were obtained by cryostat (Frigocut 2800 E, Reichert-Jung, Wein, Austria), placed on 0.5% gelatin-coated slides, and kept in a humidified chamber at 4°C for immunocytochemistry. Indirect immunofluorescence was performed according to Camps and colleagues (10, 11). Primary antibodies against LPL were incubated for 2 h. The secondary conjugated antibodies, FITC-conjugated rabbit anti-chicken IgG (Sigma) for the chicken antibody, and FITC-conjugated goat anti-rabbit IgG F(ab′)$_2$ fragment (Boehringer-Mannhein) for the rabbit antiserum, were applied for 1 h in darkness. Negative control sections were prepared either by omission, by immunoabsorption of the first antibody with purified bovine LPL, or by substitution of the antiserum with preimmune serum. Immunostaining was visualized with an epifluorescence and interferential contrast microscope (Polyvar II, Reichert-Jung).

LPL activity assay

In order to determine LPL activity in different anatomical zones of human and bovine eyes, each sample was homogenized at 4°C with 10 vol buffer containing 1 mM dithiothreitol, 1 mM EDTA, 0.25 M sucrose, 10 mM HEPES, pH 7.5, in a Potter homogenizer and centrifuged at 1,000 *g* for 10 min. The supernatant was divided into aliquots and stored at -40°C. LPL activity was determined according to Ramírez and colleagues (15) with minor modifications. The assay mixture contained 0.6 mM tri[9,10-(n)-^{3}H]oleoylglycerol (12 Ci/mol), 50 mM $MgCl_2$, 0.05% albumin (fatty acid free), 3% serum (preheated 60 min at 50°C), 25 mM PIPES (pH 7.5), and 20 μl of sample in a final volume of 0.2 ml. Incubation was carried out for 30 min at 25°C. The reaction was terminated and the [^{3}H]oleate released was quantified as previously described (16). One unit of enzymatic activity is the amount of enzyme that catalyzes the release of 1 μmol of oleate/min. All experiments involving LPL activity determinations were performed in six different samples. Results are given as mean ± SE. In some experiments, samples (20 μl) of homogenates were incubated with different dilutions (1:1 up to 1:1024) of ChaLPL. Dilutions were incubated for 2 h at 4°C, and duplicate 20 μl samples were then taken to determine

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LPL activity (see above). Some of the samples were incubated in parallel with preimmune chicken antibodies as control.

Northern blotting, LPL hybridization, and reverse transcription-PCR (RT-PCR) procedures

Total cellular RNA was prepared by the acid guanidinium thiocyanate/phenol-chloroform method (17) from fresh human ocular tissues (between 12 and 48 h post-mortem), and from frozen bovine ocular tissues (2 at 6 h post-mortem). Northern and dot-blot hybridizations of total cellular RNA were performed as described previously (18). A 1.36 kb *Eco* RI fragment of the human LPL cDNA clone hLPL26 (19) was randomly primed, labeled, and used as a probe. Autoradiograms were analyzed by quantitative scanning densitometry (GS670 Densitometer, Bio-Rad, Richmond, CA).

For PCR amplification of human LPL, 1 μg of total RNA extracted from different human ocular tissues was reverse-transcribed using random primer hexamers. LPL, and GAPDH as internal control, were subsequently PCR-amplified (7 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 58°C, and 1 min at 72°C) simultaneously in the same tube using specific primers (LPL sense primer: GCA AGC TTG GTA CCA ATG GAG AGC AAA GCC CTG; LPL antisense primer: TAC ATT CCT GTT ACC GTC CAG CCA TGG ATC; GAPDH sense primer: TGA TGA CAT CAA GAA GCT GGT GAA G; and GAPDH antisense primer: TCC TTG GAG GCC ATG TGG GCC AT). Expected PCR fragments, 277 bp long for LPL, corresponding to bases 175–437 (20), and 239 bp long for GAPDH, were separated by 2% agarose gel electrophoresis. As controls we used RT-PCR from COS-1 cell RNA isolated either from non-transfected cells or from cells transfected with hLPL29 (21). The LPL PCR fragment (277 bp) was cloned in pGEM-T® vector (Promega, Madison, WI) and the insert was sequenced by the dideoxy termination method (22) to confirm the presence of the LPL sequence.

From bovine eyes, 133 ng of total RNA extracted from different ocular tissues was reverse-transcribed using random primer hexamers. LPL was subsequently PCR-amplified (40 cycles of 1 min at 94°C, 1 min at 57°C, and 30 sec at 72°C) using specific primers (LPL sense primer: GTG GTG GAC TGG CTG TCA CGG GC; and LPL antisense primer: CTG TTG ACC TTC TTA TTG GTC AGA CTT CCT GCA AT), which correspond to bases 266–288 (sense primer) and 437–471 (anti sense primer) of the bovine LPL cDNA sequence (23), and recognize both human and bovine LPL. The resulting 206 bp PCR fragment was separated by 2% agarose gel electrophoresis. RNA extracted from THP-I cells, which express LPL mRNA after differentiation by treatment with phorbol esters (19), was used as a control for LPL expression. GAPDH specific primers were used as internal controls (24).

RESULTS

LPL activity in human and bovine ocular regions

In order to determine whether LPL activity is present in human and bovine eyes, post-mortem specimens were carefully dissected into pieces corresponding to the major anatomical regions of the eye (cornea, iris, lens, ciliary body, retina, vitreous body, and optic nerve) and LPL hydrolytic activity was assayed. The results indicate that LPL activity is present in all of the ocular regions in both human and bovine eyes (**Fig. 1**). However, the levels of LPL activity found in the various regions were different. Comparing the levels of LPL activity in different areas of human and bovine eyes we detected a similar pattern, although lipolytic activity tended to be slightly higher in human eyes. In both cases, it was possible to distinguish three different types of areas according to the degree of tissue vascularization and LPL lipolytic activity: *a)* avascular regions, vitreous body, lens, and cornea, which presented low levels of LPL activity, in the range of 0.1 to 1.0 mU/g of tissue; *b)* vascularized regions, which presented intermediate LPL activity (in human samples these correspond to the optic nerve (1.6 ± 0.68), and in bovine samples to the optic nerve (1.08 ± 0.29), the iris (1.6 ± 0.9), and the retina

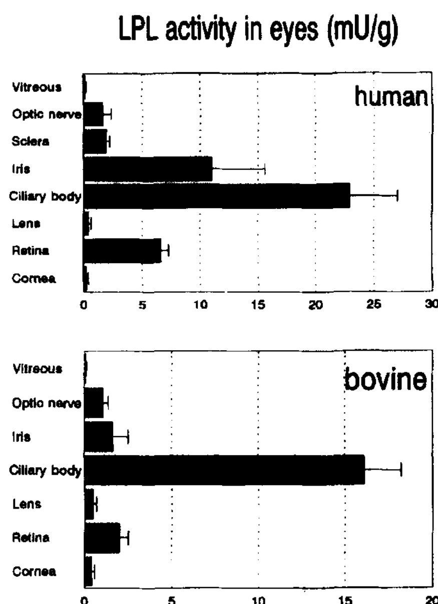

Fig. 1. LPL activity in human and bovine eyes. LPL activity was determined in human (n = 13) and bovine (n = 10) eyes as described in Material and Methods. Lipolytic activity in human and bovine ocular tissues was determined in 20 μl of homogenate of each indicated anatomical region. Results are given as mean ± SE.

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(2.0 ± 0.5)); and *c)* highly vascularized regions, which presented higher LPL lipolytic activity, such as the iris (10.97 ± 4.65), the retina (6.59 ± 0.68), and the ciliary body (22.9 ± 4.1) in human samples, and the ciliary body (16.0 ± 2.1) in bovine eyes. Values obtained in highly vascularized structures were considerably high when compared to the activity detected in rat adipose tissue (78 ± 5.0) using the same assay (25). The differences in activity found between iris and retina in human and bovine samples may be explained by the more developed vascularization in human than in bovine structures (26). In contrast, in both human and bovine eye, the ciliary body, a smooth muscle structure, contained the highest LPL lipolytic activity levels. To confirm that the lypolytic activity measured was due to the hydrolytic action of LPL, we performed immunoinhibition experiments, in which human ciliary body homogenates were incubated with different dilutions of specific anti-LPL antibody (ChaLPL) for 2 h at 4°C and centrifuged, and the LPL activity was assayed in the supernatants. The results indicate that lipolytic activity detected in ciliary body was inhibited between 50% and 70% when compared with control samples incubated with corresponding preimmune antibodies (not shown). This incomplete inhibition of the LPL lipolytic activity may be due to moderate specificity of the ChaLPL when used against human tissue homogenates. Parallel experiments performed to test for the presence of hepatic lipase in eye tissue did not detect lipolytic activity. However, the possibility that there could be other lipolytic activity in the eye, not correlated with LPL, was not ruled out.

LPL mRNA in human and bovine ocular tissues

To determine whether the LPL gene is expressed in human eyes, the presence of LPL mRNA was assayed by

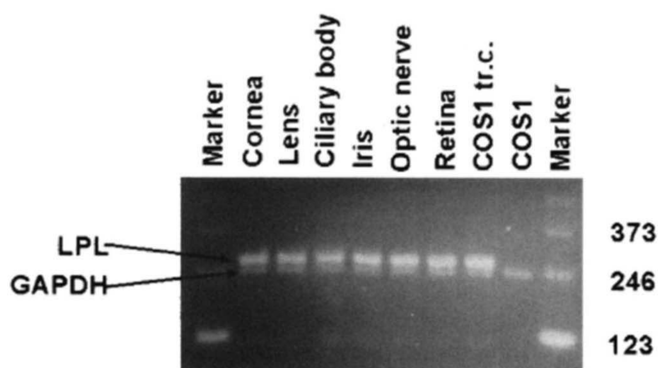

Fig. 2. Detection of human LPL mRNA in the indicated regions of human eye. RT-PCR of LPL and GAPDH as internal control was performed with 1 μg of RNA isolated from each ocular region. Expected sizes of RT-PCR fragments were 277 bp for LPL and 239 bp for GAPDH. As positive control, RNA isolated from 48 h COS-1 transfected cells (COS1 tr.c.) with human LPL was used. Non-transfected COS-1 cells (COS1) were used as negative control.

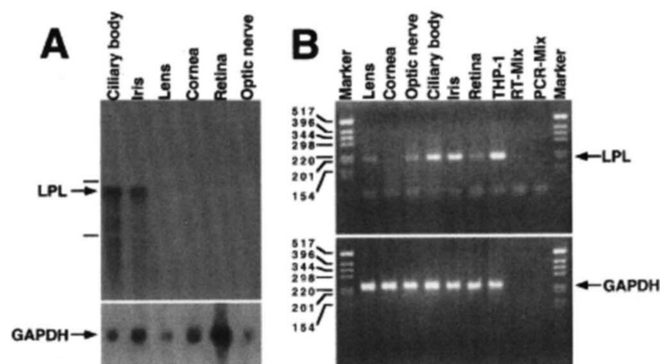

Fig. 3. Detection of bovine LPL mRNA in different areas of bovine eye by Northern blot and RT-PCR. Northern blot analysis (A) was performed as described in Material and Methods with human LPL cDNA as a probe. RT-PCR (B) was carried out with 133 ng of RNA isolated from each of the indicated ocular areas. As positive control RNA from THP-1 cells induced with phorbol esters was used. RT-Mix and PCR-Mix were used as negative controls.

RT-PCR on total RNA isolated from the same human ocular areas in which LPL activity was previously detected. RNA could not be isolated from vitreous body, so this structure was not analyzed. Using this approach LPL mRNA was detected in all ocular regions assayed: cornea, lens, ciliary body, iris, optic nerve, and retina (**Fig. 2**). As internal control we performed simultaneous GAPDH RT-PCR amplification. To confirm the specificity of the amplification, a PCR fragment of 277 bp from human retina was cloned in pGEM-T® (Promega) and the DNA sequence of the insert was then determined. As expected, the sequence corresponded to bases 175 to 437 of human LPL. Quantification of human LPL mRNA in the different areas by either Northern or slot-blot hybridization methods was unsuccessful. This could be attributed to partial mRNA degradation due to the long time that passed between the death of the donor and RNA extraction (12–48 h).

In contrast to the human eye samples, the sampling conditions of bovine ocular tissues were easier to control experimentally, and hence it was possible to detect the presence of bovine LPL mRNA by Northern blot hybridization in ciliary body and iris (**Fig. 3A**). Using RT-PCR, LPL mRNA was also detected in iris, ciliary body, retina, optic nerve, and lens. When LPL mRNA levels were quantified by dot-blot in quantitative scanning densitometry, relative levels of 100% ± 10% were arbitrarily assigned to ciliary body and 89% ± 19% was found in iris, whereas LPL mRNA could not be detected in other eye areas by this technique. In comparison, relative levels of LPL mRNA in rat heart muscle were 1200% ± 40% of the mRNA levels in ciliary body.

Immunolocalization of LPL in human ocular tissues

The pattern of distribution of LPL protein in vivo was established by indirect immunofluorescence in cryostat

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sections from different human ocular regions in which LPL activity and mRNA were detected. As expected, immunolabeling for LPL was found in highly vascularized structures, which corroborates our previous results obtained in the experiments on activity and mRNA expression. However, the intensity of fluorescent label-

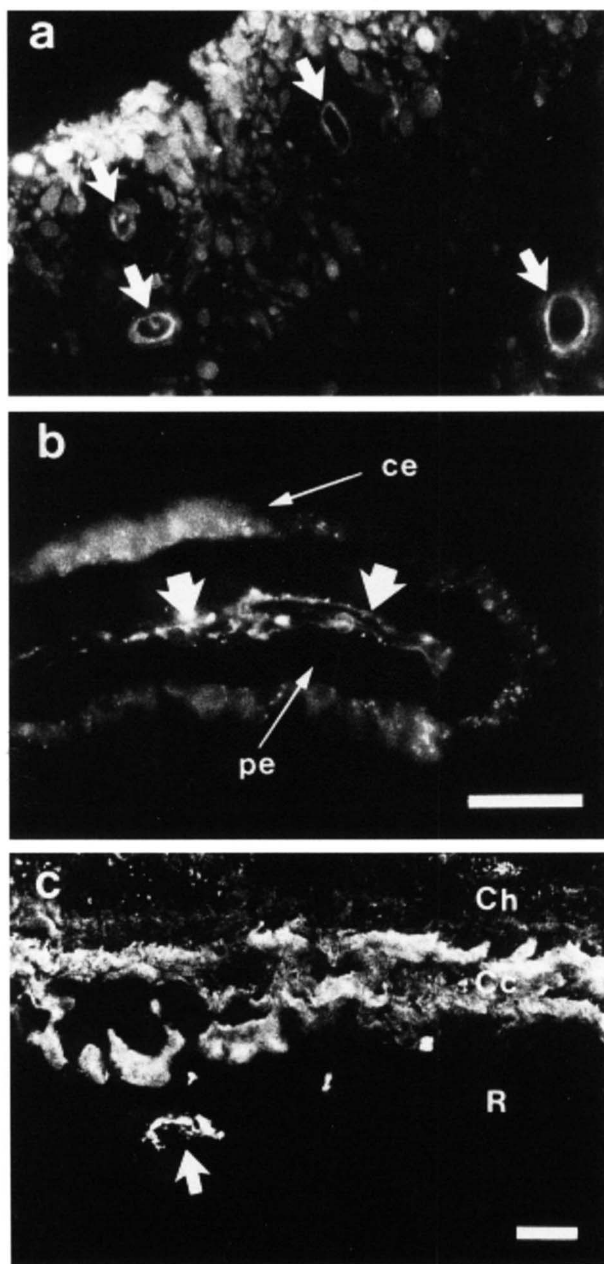

Fig. 4. Immunolocalization of LPL in human ocular tissues. Cryostat sections were incubated with specific anti-human LPL serum (RaLPL) and stained with FITC-conjugated IgG F(ab′)$_2$ fragment. a: Iris capillaries (arrows) and iris superficial stroma presented a mild and faint immunostain, respectively. b: Capillaries of the ciliary processes (arrows) and non-pigmented epithelium (ce) of this structure presented a mild stain; pigmented epithelium (pe) of ciliary process was negative. c: LPL immunostaining in choroides (Ch), choriocapillaris (Cc) and capillar (arrow) of the retina (R) were strongly positive for anti-LPL serum. a and b (× 500); c (× 260); bar = 40 μm.

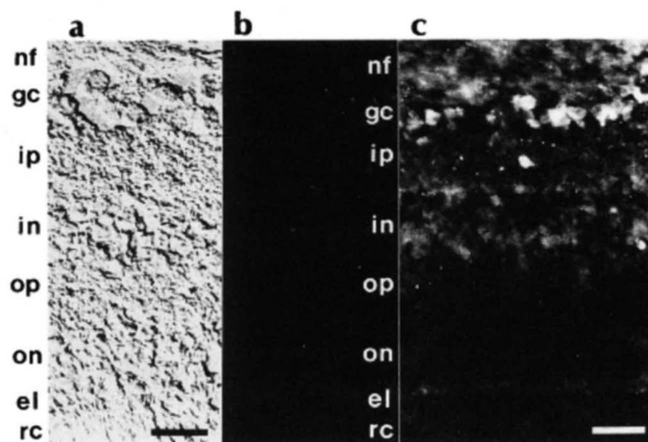

Fig. 5. Immunolocalization of LPL in human retina. Interferential contrast microscopy (a) reveals the layers of the retina. Indirect immunofluorescence for LPL staining on consecutive cryosections of the retina was performed with RaLPL (c) and by immunoabsorption of the primary antiserum with purified bovine LPL (b), as control. LPL labeling (c) was mainly seen in internal layers of the retina (nf, nerve fibers layer; gc, ganglion cell layer; ip, inner plexiform layer; in, inner nuclear layer), whereas poor or no labeling was observed in most external layers (op, outer plexiform layer; on, outer nuclear layer; el, external limiting membrane; and rc, photoreceptor layer). a (× 950); b and c (× 900); bar = 20 μm.

ing was mild in most cases. LPL was observed mainly in vascularized regions such as the choroides, the choriocapillaris, and in the capillaries of ciliary body, iris, and retina (**Fig. 4** and **Fig. 5**), and also in the central retinal vein in the optic nerve (not shown). Ocular tissues such as the interstitial stroma of the ciliary body and the iris, loose connective tissues, showed diffuse labeling (not shown). LPL immunostaining was usually found in the capillaries of the iris (Fig. 4a) and ciliary processes (Fig. 4b), where little stromal tissue surrounds capillaries, whereas the smooth muscle that conforms the iris-ciliary body complex exhibited poor labeling. Immunoreactivity for this enzyme was also found on non-pigmented ciliary epithelium (Fig. 4a). Highly vascularized choriocapillaris and choroides showed intense staining (Fig. 4c). Consecutive cryostat sections used as control were found negative for LPL labeling (not shown). Bruch's membrane, the border between ciliary stroma and ciliary epithelium, presented a characteristic autofluorescence when excited by ultraviolet light, which made it difficult to discern the presence of the enzyme in this structure (not shown). Internal layers of the retina showed a moderate labeling intensity, whereas little staining was observed in the external layers (Fig. 5).

DISCUSSION

The pathways by which fatty acids and phospholipids are metabolized in the eye are similar to those in other tissues (1–3). It has been shown that a number of

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radioactive lipid precursors are rapidly incorporated into phospholipids in the eye tissues (27). A very high lipid turnover is observed as the ocular tissues do not store lipids, and a mechanism of immediate uptake from the bloodstream is therefore needed (28). In other tissues, LPL is responsible for the generation of fatty acids from plasma lipoproteins, which can be taken up consecutively by the cells (7). For this reason we analyzed whether LPL is present in specialized regions of both human and bovine eyes. The results provide the first direct evidence that LPL is synthesized, secreted, and distributed among microvessels in several highly specialized ocular regions. Thus, LPL could, in part, be responsible for controlling the lipid uptake in ocular tissues.

In the present work, we found a positive correlation between the degree of vascularization in the ocular tissues and the presence of LPL activity. Avascular structures in human and bovine eyes, such as the cornea, the lens, and the vitreous body, showed low levels of LPL activity. Results from RT-PCR mRNA determinations in bovine eyes corroborated the findings of LPL activity assays and indicated rather low levels of LPL mRNA in these avascular structures. The optic nerve contained intermediate levels of LPL activity and mRNA whereas both human and bovine iris and ciliary body presented the highest LPL activity and LPL mRNA expression. The iris and the ciliary body are dynamic muscular structures capable of precise and rapid changes in response to light stimuli and to specific pharmacological agents. In addition, they are key components of the blood-aqueous barrier, which regulates both the composition and the ionic environment of ocular tissues (28). These tissues, which have a specialized metabolic function, are highly vascularized structures. In view of their dynamic muscular function, iris and ciliary body utilize large amounts of energy for which the preferred substrates are fatty acids (1-3, 27, 28). This fact requires the presence of high levels of LPL mRNA, immunoreactive mass, and lipolytic activity, as was detected by our assays. By immunolabeling we observed the LPL enzyme on the capillary endothelium, where it can exert its action on circulating lipoproteins. Unfortunately, due to the low level of LPL mRNA, it was not possible, by conventional in situ hybridization procedures, to localize the site of production of LPL within these tissues. Previous experiments (10, 11) indicate that in the major LPL-producing tissues, the enzyme is predominantly produced by the parenchymal cells or by tissue macrophages. The production and turnover of LPL is regulated in relation to substrate needs (8). Furthermore, cultured endothelial cells are unable to produce LPL (29), which implies that the LPL immunolocalized to the endothelium originates in parenchymal cells. Both iris and ciliary body are smooth muscle tissues (27, 28), and as smooth muscle cells have been reported to synthesize LPL (30), it is likely that the LPL found on the endothelium in these ocular regions is produced by the parenchymal muscle cells. Further, macrophage-like characteristics of trabecular meshwork cells in the iris-ciliary body complex have been reported (31). However, the precise cell type in which LPL is synthesized remains to be determined using more sensitive methods.

The presence of substantial amounts of LPL mRNA and activity in both human and bovine retina is particulary interesting. Previous studies (32, 33) have established that the development of the retina is highly dependent on the supply of polyunsaturated fatty acids synthesized from essential fatty acids in the liver. These are incorporated into triglycerides and then secreted as VLDL particles in the blood. VLDL triglycerides are excellent substrates for LPL action (7, 8) and, as the result of its lipolytic activity, the fatty acids incorporated into triglycerides are released in appropriate tissues. Polyunsaturated fatty acids represent the major components of plasma membranes in the retina (1, 2). Therefore, LPL action is crucial in liberating these fatty acids (essential or nonessential) again at the site of final utilization. The finding that dietary deficiency of n-3 fatty acids during development causes severe damage to the retina (32, 34) further underscores the importance of dietary n-3 fatty acids and of LPL for the continuous supply of this class of fatty acids. As these fatty acids are indispensable for normal eye development, the ocular tissues require a very efficient system for their uptake. The outer segments of photoreceptors in vertebrate retinas contain the highest concentration in the eye of docosahexaenoic acid (DHA 22:6n-3), an essential polyunsaturated fatty acid, which comprises up to one half of the fatty acids in the disc phospholipids (35). Recent studies (33, 36) show that DHA 22:6n-3 is transported within triglycerides in the circulation by the conventional lipoprotein pathways. This fact indicates the continuous need for this fatty acid in relevant events in eye metabolism (33-37). Our immunofluorescence data showed that more LPL was present in internal than in the external layers of the retina, which showed poor or no LPL labeling. As the choriocapillaris system provides the vascular irrigation of external retinal layers, and retinal capillaries are only observed just up to the half internal part of retina, but may aid to nourish the inner parts of the photoreceptors (38), it was as expected that the immunolabeling for LPL localized on the internal retina, which corresponds to the vascularized region. At this site, the enzyme would be optimal to facilitate the uptake of polyunsaturated fatty acids for the retinal cells. Thus, our present findings regarding LPL activity, immunoreactivity, and LPL mRNA in both human and bovine retina strongly suggest that LPL could contribute

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in a major fashion to the efficient uptake of fatty acids in the retina.

The presence of LPL in specialized regions of the human eye could also be relevant for further investigations about ocular alterations observed in some diseases. Disorders of lipid metabolism, such as hyperlipidemia or chylomicronemia, are associated with ocular and/or systemic manifestations (13). Chylomicrons have been shown to accumulate in plasma, in the inherited disorder familial LPL deficiency. Similar accumulation of triglyceride-rich lipoproteins can be observed in more common forms of familial hypertriglyceridemia or in patients with secondary forms of hyperlipidemia, such as seen in uncontrolled diabetes mellitus, during estrogen or antihypertensive drug therapy, or in alcohol abuse (12, 13). In all these disorders, eruptive xanthomata, pancreatitis, and lipemia retinalis, which consist in alterations of retinal arterioles and venules, can be observed (13). However, the precise role of eye LPL in such ocular alterations remains to be investigated.

In summary, the results observed in the present work demonstrate that LPL is expressed in highly specialized structures of human and bovine eyes, being highest in vascularized and lowest in avascular anatomical ocular areas. Therefore, LPL could play a crucial role in providing the eye with essential fatty acids.

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