Transcytosis of Lipoprotein Lipase across Cultured Endothelial Cells Requires Both Heparan Sulfate Proteoglycans and the Very Low Density Lipoprotein Receptor*

Received for publication, September 26, 2000, and in revised form, December 11, 2000
Published, JBC Papers in Press, December 19, 2000, DOI 10.1074/jbc.M008813200

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Lipoprotein lipase (LPL), the major enzyme responsible for the hydrolysis of circulating lipoprotein triglyceride molecules, is synthesized in myocytes and adipocytes but functions while bound to heparan sulfate proteoglycans (HSPGs) on the luminal surface of vascular endothelial cells. This requires transfer of LPL from the abluminal side to the luminal side of endothelial cells. Studies were performed to investigate the mechanisms of LPL transcytosis using cultured monolayers of bovine aortic endothelial cells. We tested whether HSPGs and members of the low density lipoprotein (LDL) receptor superfamily were involved in transfer of LPL from the basolateral to the apical side of cultured endothelial cells. Heparinase/heparinitase treatment of the basolateral cell surface or addition of heparin to the basolateral medium decreased the movement of LPL. This suggested a requirement for HSPGs. To assess the role of receptors, we used either receptor-associated protein (RAP), a 39-kDa inhibitor of ligand binding to the LDL receptor-related protein (VLDLr) (5) or specific receptor antibodies. Receptor-associated protein reduced 125I-LPL and LPL activity transfer across the monolayers. When the basolateral surface of the cells was treated with antibodies, only anti-VLDL receptor antibodies inhibited transcytosis. Moreover, overexpression of the VLDL receptor using adenoviral-mediated gene transfer increased LPL transcytosis. Thus, movement of active LPL across endothelial cells involves both HSPGs and VLDLr receptor.

Lipoprotein lipase (LPL) is a 120-kDa dimeric protein that associates with the luminal surface of endothelial cells in multiple organs but especially in cardiac and skeletal muscle and in adipose tissue (1). This enzyme hydrolyzes the triglyceride in circulating lipoproteins such as chylomicrons and VLDL and produces free fatty acids that are used for metabolic energy or for fat storage. Endothelial cells do not synthesize LPL; rather, myocytes and adipocytes produce it. Thus, it is a protein that requires transcytosis across the endothelial cell barrier, in this case from the interstitial fluid to the luminal side of the cells.

There are several possible ways that LPL could cross the endothelial barrier. Nonspecific transport of molecules across endothelial monolayers occurs either via paracellular routes between the cells or via vesicular transport through cells (2). Alternatively, a specific transcytosis pathway could exist which requires LPL to associate with a cell surface receptor and then transports LPL through the cells. This process would be analogous to that which transfers IgA across epithelial cells (3). The first step in a specific LPL transcytosis pathway would involve LPL interaction with the basolateral side of endothelial cells. LPL binds to a number of cell surface molecules including heparan sulfate proteoglycans (HSPGs) and members of the LDL receptor family (4). In bovine endothelial cells the most highly expressed of these receptors is the VLDL receptor (VLDLr) (5). A previous study suggested that HSPGs are required for LPL transcytosis (6). It is, however, unclear whether HSPGs are sufficient for transport or whether HSPGs must operate in concert with receptors. The binding of LPL to several members of the LDL receptor family leads to uptake and degradation of LPL by cells. There are no data on whether these receptors participate in transendothelial movement of LPL or other ligands.

In this report, we present data showing that LPL transcytosis across endothelial monolayers requires both HSPGs and the VLDLr. LPL transcytosis was diminished by removal of HSPGs and inhibition of receptors by RAP, a 39-kDa protein that was copurified with the LDL receptor-related protein (LRP) (7). This protein binds to members of the LDL receptor family and inhibits ligand binding and uptake by those receptors (8, 9). Furthermore, antibodies against the VLDLr blocked LPL translocation and increased expression of this receptor-increased transcytosis. Thus, LPL requires both HSPGs and receptors for translocation across endothelial cells.

EXPERIMENTAL PROCEDURES

Purification and Radioiodination of LPL—LPL was purified from unpasteurized bovine milk according to the method of Soccorro et al. (10)
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with modifications as described by Saxena et al. (11). 300–500 μg/ml purified enzyme was stored at −70 °C. Enzyme activity was assayed with a glicerol-containing triolein emulsion as described previously (11). The purified enzyme had a specific activity of 40–50 nmol of oleic acid released/h/mg of enzyme at 37 °C.

Lipidated LPL was enzymatically with glucose oxidase and lactoperoxidase (12). Radioiodinated LPL was purified by heparin-agarose (Bio-Rad) affinity chromatography and stored at −70 °C. Typical specific activity of the preparation was 1,000–2,000 cpm/nl, and >90% of the radioactivity was precipitated with trichloroacetic acid. 125I-LPL was purified by Sephadex G-25 gel filtration (PD-10, Amersham Pharmacia Biotech) prior to use to remove degradation products. Heat-inactivated LPL was prepared by heating LPL for 1 h at 52 °C.

Endothelial Cell Monolayers—Primary cultures of bovine aortic endothelial cells (BAECs) were established as reported (13) and were grown in DMEM containing 10% fetal bovine serum (Gemini Bio-products Inc., Calabasas, CA), 1% (v/v) penicillin and streptomycin solution, and 1% (v/v) glucose solution (both from Life Technologies, Inc.). Polarized BAEC monolayers were grown on gelatin and fibronectin-coated polyethylene terephthalate 10-mm filters (diameter, 3.0 μm) (Becton Dickinson Labware, Franklin Lakes, NJ). This allowed access to both the basolateral side of the cells adjacent to the lower chamber and the apical cell surface in contact with medium in the upper chamber (14). Approximately 4–5 × 104 cells were seeded onto 9-mm filters on nonviral infected cells were used after 5–7 days after seeding the endothelial cells. The media in the upper chamber (0.5 ml) and lower chamber (1 ml) were changed every other day. Movement of both [3H]dextran (70 kDa, American Radiolabeled Chemicals, St. Louis, MO) and LDL was routinely assessed to verify that the monolayer was intact.

Transport of 125I-LPL across Monolayers—RAP-sensitive transport of 125I-LPL across the monolayers was studied after adding 1 μg/ml radiolabeled LPL to DMEM and 1.5% BSA in the basolateral chambers. In some experiments, the basolateral side of the cells was incubated with 2.5 units/ml heparinase/heparitinase (Seikagaku America Inc., Bethesda, MD) or 5 μg/ml RAP-containing medium. After 1 h, the medium was removed, and the cells were washed prior to adding 125I-LPL. 125I-LPL that appeared in the upper chamber was monitored over time by removing 100 μl of medium from the apical side of the cells at 30, 60, 120, and 180 min. The chambers were not stirred to avoid disruption of the monolayers. All experiments were performed using triplicate chambers. At the conclusion of the experiments, the cells were washed, and 125I-LPL associated with luminal and basolateral surfaces was released by the addition of DMEM-BSA containing 100 μunits/ml heparin (Elkins Sinn, Cherry Hill, NJ) at 4 °C for 30 min at 100 μl in medium. In some experiments, 125I-LPL was then estimated by dissolving the heparin-treated cells in 0.1 N NaOH and by measuring the radioactivity. In other experiments, heparin was added to the basolateral chamber along with 125I-LPL, and the appearance of 125I-LPL in the upper chamber was determined. The radioactivity was routinely immunoprecipitated with 10% trichloroacetic acid; in all experiments less than 10% of the counts in the chambers were not precipitated with a glycerol-containing triolein emulsion as described previously (11).

Effects of RAP and Antibodies on LPL Transport—RAP was produced as a fusion protein with glutathione S-transferase in an expression vector pGEX (Pharmacia Biotech) prior to use to remove degradation products. Heat-inactivated LPL was prepared by heating LPL for 1 h at 52 °C. For expression of VLDLr in cells, BAECs were infected with adenovirus-containing human VLDL receptor (AdhVLDLr) and β-galactosidase-expressing adenovirus (AdLa2Z) when the cells were 80–90% confluent; experiments using these cells were conducted 24 h after infection. The barrier function of the endothelial cell monolayers was examined by using trypsin blue and dextran transport as described previously (6).

RESULTS

Transport of 125I-LPL across Endothelial Cell Monolayers—We first assessed how increasing amounts of LPL added to the basolateral side of BAECs affected the amount of LPL that crossed the monolayer. As shown in Fig. 1A, increasing 125I-LPL in the medium on the basolateral side of the cells led to more 125I-LPL in the upper chamber. However, when the 125I-LPL concentration exceeded 1 μg/ml, a lower percentage of the LPL was transported. For that reason, 1 μg/ml was used for subsequent experiments.

To determine the specificity of LPL transport, we used unlabeled LPL to inhibit 125I-LPL transport. The addition of 300 μg/ml LPL to the lower chamber in the presence of 1 μg/ml 125I-LPL decreased the appearance of 125I-LPL in the upper chamber to 46 ± 5% of control (Fig. 1B). The amounts of 125I-LPL within the cells and released from the apical surface are shown in Fig. 1, C and D. More 125I-LPL was within the cells than on the cell surface. Increasing amounts of 125I-LPL in the lower chamber led to more labeled LPL in the cells and on the apical surface. In the presence of an excess of unlabeled LPL, both the intracellular and cell surface-labeled LPL were decreased by >50%. Thus cellular uptake and transfer to the apical surface were inhibited, but not completely.

Effects of Modulation of HSPGs on LPL Transport—Although the role of HSPGs as members of the LDL receptor family and LPL-binding molecules is well documented, only limited studies (22, 23) have been performed with respect to their role as transcytosis molecules. Because members of the family of receptors often act in concert with HSPGs (22, 23), we tested whether LPL movement across monolayers requires association with HSPGs. 125I-LPL translocation across BAEC monolayers was studied in the presence of heparin. As shown...
in Fig. 2, heparin at 5 units/ml decreased $^{125}$I-LPL in the upper chamber after 3 h by >54%. Inhibition also was found with a higher dose of heparin (50 units/ml).

To test whether HSPG degradation affected LPL transfer, HSPGs on the basolateral side of the cells were removed by incubating the cells with HSPG-degrading enzymes. The results shown in Fig. 2, inset, demonstrate that heparinase/heparitinase treatment of the basolateral side of the cells reduced $^{125}$I-LPL movement across the monolayers, a 41% inhibition. Thus, optimal LPL movement across the monolayers required its association with proteoglycans on the basolateral side of the endothelial cells. However, a large amount of the radiolabeled LPL was not affected by unlabeled LPL, heparin, and heparitinase. This suggested that the radiolabeled LPL was tracing two different transport pathways only one of which was reduced by unlabeled LPL and inhibition of LPL-HSPG interaction.

Effects of RAP on LPL Transport across Endothelial Cell Monolayers—We next determined whether HSPGs are sufficient for LPL transcytosis or whether they serve as accessory molecules for transcytosis receptors. To test whether LRP, VLDLr, or other members of this family are involved in LPL transport, 1 VLDLr, or other members of this family are involved in LPL transcytosis or whether they serve as accessory molecules for transcytosis receptors. To test whether LRP, VLDLr, or other members of this family are involved in LPL transport, 1

Effects of RAP and Heparin on Cell Surface and Intracellular $^{125}$I-LPL—The amount of radioactive LPL within the cells and its movement over the ensuing 3 h was assessed. Dextran movement to the upper chamber was determined after a 3-h incubation (Fig. 3B); this was not affected by heparin or the addition of RAP.

Movement of LPL from the apical to the basolateral side of the monolayers was also studied in the presence and absence of RAP and using cells that were treated with heparinase/heparitinase. LPL movement was much greater in this direction, both RAP and removal of glycosaminoglycan chains from HSPGs decreased the amount of $^{125}$I-LPL appearing in the medium on the basolateral side of the cells.
FIG. 2. Role of LPL-HSPG interaction on LPL transport. Effects of adding heparin on $^{125}$I-LPL transport to the upper chamber. $1 \mu g/ml$ $^{125}$I-LPL was added to the lower chamber in the presence of heparin, and radioactivity appearing in the upper chamber was assessed. Both 5 and 50 units/ml heparin decreased the amount of LPL. Inset, the basolateral side of BAECs was treated with heparinase/heparinitase (5 units each) prior to the addition of $^{125}$I-LPL. Shown are the amounts of radioactive LPL appearing in the medium on the apical side of the monolayers after 3 h. The results are the averages ± S.D. of experiments performed in triplicate.

FIG. 3. Effects of RAP on LPL transport across endothelial cell monolayers. Panel A, effects of different concentrations of RAP. The basolateral side of the cultured monolayers was incubated with different concentrations of RAP (0.33–10 $\mu g/ml$) for 1 h at 37 °C. $1 \mu g/ml$ $^{125}$I-LPL was then added to the medium in the lower chamber. The cells were incubated at 37 °C, and $^{125}$I-LPL transported to the upper chamber was determined. Data are the means ± S.D. of experiments performed in triplicate; the control is without RAP. Panel B, the addition of heparin and unlabeled LPL to RAP treated cells. 5 $\mu g/ml$ RAP was added to cells, and $^{125}$I-LPL transport in the presence of 5 units/ml heparin or an excess of unlabeled LPL (300 $\mu g/ml$) was assessed. In this experiment, as in panel A, RAP decreased transport ~50%; neither heparin nor unlabeled LPL greatly increased the inhibition of $^{125}$I-LPL transport. Panel C, $[3H]$dextran movement across monolayers. 1 $\mu g/ml$ dextran was included in the lower chamber, and the amount of dextran in the upper chamber was monitored over time. Similar studies were performed using monolayers treated with RAP or heparin as described in panel B. Panel D, movement of $^{125}$I-LPL from the apical to basolateral side of endothelial monolayers. $1 \mu g/ml$ $^{125}$I-LPL was added to the upper chamber, and the transfer of radioactivity to the lower chamber was monitored over time. Shown is the amount of $^{125}$I-LPL transported across BAECs under control conditions and using cells in which the apical surface was treated with 5 $\mu g/ml$ RAP or heparinase/heparinitase.

$^{125}$I-LPL >50%. In the experiment illustrated in the figure that equaled a reduction from 1.05 ± 0.07 to 0.52 ± 0.12 ng/cell protein. Intracellular $^{125}$I-LPL was reduced from 1.68 ± 1.98 to 6.75 ± 0.99 ng/cell protein by RAP. Treating the cell monolayers with heparin alone, or heparin plus RAP, also decreased both cell surface and intracellular LPL. Heparin was more effective than RAP, possibly because it decreased both receptor and nonreceptor HSPG-mediated uptake of $^{125}$I-LPL. Adding RAP to heparin led to no further decrease in intracellular or cell surface $^{125}$I-LPL. Because RAP and heparin decreased both LPL in the upper chamber and in the cells, it suggested that the LPL movement was via an intracellular process.

**Effects of Heparin and RAP on LPL Activity Transport across Endothelial Cell Monolayers**—To investigate the effect of RAP and heparin on the appearance of LPL activity on the apical side of the endothelial cell monolayer, 100 $\mu g/ml$ purified LPL was added to the lower chamber in the absence or presence of 5 units/ml heparin, 5 $\mu g/ml$ RAP, or to monolayers in which the basolateral side of the cells was treated with 5 $\mu g/ml$ RAP. As shown in Fig. 5, treatment of the cells with RAP (denoted RAP treated) and addition of RAP along with LPL (denoted LPL+RAP) or adding heparin (denoted Heparin) reduced the amount of LPL activity appearing in the upper chamber. At 1 h, all of the treatments decreased LPL activity transport by >90% compared with control cells. A similar inhibition of LPL activity movement through the endothelial monolayer was found at 2 h.
The amounts of LPL in the cells and on the surface of monolayers treated as described in Figs. 1–3 were determined. After a 3-h incubation, media were removed, cells washed, and cell surface LPL was released by treating the apical surface with DMEM-BSA containing 100 units/ml heparin at 4 °C for 30 min. After heparin treatment of the basolateral side of the cells, the cells were washed and then dissolved in 0.1 M NaOH. Radioactivity in the NaOH fraction represents the intracellular LPL. Open bar, control; filled bar, treated with 5 μg/ml RAP; lined bar, treated with 5 units/ml heparin; and dotted bar, RAP + heparin. Values represent the average ± S.D. of experiment performed in triplicate.

Active LPL
Inactive LPL
Inactive LPL+RAP
Treated
Inactive LPL +RAP
Complex

FIG. 6. Transcytosis of heat-inactivated 125I-LPL. Control LPL (denoted Active LPL) or 1 μg/ml heat-inactivated 125I-LPL (denoted Inactive LPL) was added to the basolateral side of an endothelial cell monolayer, and the amount of 125I-LPL in the upper chamber was determined after a 3-h incubation. In some chambers the endothelial cell monolayers were first treated with 5 μg/ml RAP for 1 h; in other chambers the inactive LPL was incubated with RAP prior to its inclusion in the lower chamber (denoted Inactive LPL + RAP). Data are the means ± S.D. of experiments performed in triplicate; the amount of nonheated 125I-LPL found in the upper chamber is shown as 100%.

Effects of Antibodies to LPL and Receptors— Antibodies (IgG, 30 μg) against LPL or members of the LDL receptor family were added to the lower chamber prior to the addition of 125I-LPL. These amounts of antibodies were sufficient to inhibit the respective receptors (24) or to bind to each LPL molecule. As shown in Fig. 7A, anti-VLDLr and anti-LPL antibodies inhibited transcytosis by ~50%; these data are shown in the open squares and open inverted triangles, respectively. In contrast, antibodies to the LDL receptor (open circles) and LRP (filled triangles) had no effect. The same antibody treatment was used to assess the role of the VLDLr in transport of active LPL (Fig. 7B). Anti-VLDLr antibodies decreased LPL movement to the apical side of the cells by ~80%. This inhibition was comparable to that found with RAP, RAP and anti-VLDLr antibody did not have an additive effect on inhibition of transport. Therefore, inhibition of the VLDLr decreased LPL transport across the cultured endothelial cells.

VLDLr Overexpression Increases LPL Transport across Endothelial Cell Monolayers—We next tested whether more VLDLr expression increases LPL transcytosis. BAECs were infected with either AdhVLDLr or AdLacZ, and the expressed VLDLr was examined by RAP ligand blotting of membrane extracts (Fig. 8A). In control and infected cells a strong band for a protein of M₆~120,000 which corresponded to the VLDLr was found. The identity of this band was confirmed using anti-VLDLr antibodies. Only AdhVLDLr-infected cells had an intensely staining human VLDLr band, seen in Fig. 8A, lane 3. BAECs express a lower molecular weight form of the VLDLr than human cells (5). Two other less intense high molecular weight bands were observed. The second band reacted with anti-LRP antibodies; the highest molecular weight protein was, presumably, megalin.

As expected, VLDLr expression increased LPL transport. A time course of 125I-LPL transcytosis across control and infected BAECs is shown in Fig. 8B. Cells infected with AdhVLDLr are

(data not shown). Therefore, RAP and heparin markedly decreased the amount of LPL activity transferring from the lower to the upper chamber. This effect of RAP and heparin on LPL activity transcytosis was much greater than that on 125I-LPL transport.

Transcytosis of Heat-inactivated LPL—One possible reason for the greater effect of RAP on LPL activity than 125I-LPL was that inactive LPL protein was transported across the monolayers by a non-RAP-inhibited process. It should be noted that during these experiments, some iodinated LPL would have been converted to inactive monomer, and the data using this tracer would assess both active and inactive LPL. To determine whether inactive LPL was transported in a manner similar to that of active dimeric LPL, heat-inactivated 125I-LPL was studied. These preparations have been characterized previously and consist primarily of inactive LPL that elutes from heparin at a lower salt concentration and is thought to be monomeric (23). Our preparation was assessed by SDS-polyacrylamide gel electrophoresis and consisted primarily of an ~55-kDa protein; however, ~80% of this preparation eluted from heparin affinity gel with 0.5 M NaCl-containing buffer. This is in contrast to nonheated LPL, in which ~20% of the
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Panel A

The apical surface was approximately doubled, and that inside the cells was increased by RAP. Thus, VLDLr overexpression increased cellular uptake and transfer of LPL across the monolayer. Results indicate that overexpression of the VLDLr in endothelial cells increased LPL movement. Our data suggested that receptors, especially the VLDLr, and interaction with the VLDLr. Our previous studies showed that the IL-8 remained in the bloodstream via the postcapillary venules and then circulates in the blood. A histological study showed that injection of heparinase blocked IL-8 transcytosis, i.e., the IL-8 remained in the injected tissues presumably because it was unable to bind to proteoglycans, a step required for interaction with the IL-8 receptor. Our current studies suggest that a similar process is required for LPL. Although our observations in cultured BAECs implicate HSPGs and VLDLr as components of a LPL transcytosis system one must question whether these observations are of physiologic importance for LPL actions. Muscle and adipose tissue are the two most important sites of LPL-medi-
are the means ± S.D. of experiments performed in triplicate. Panels C and D, effects of VLDLr expression on apical cell surface (panel C) and intracellular (panel D) LPL. The amounts of intracellular and apical surface LPL recovered from control and infected cells ± RAP are shown. Data are the means ± S.D. of experiments performed in triplicate.

FIG. 8. VLDLr overexpression in endothelial cell monolayers. Panel A, RAP-binding proteins in control and virus-infected BAECs. Cell extracts prepared from control and virus-infected BAECs were used for SDS-polyacrylamide gel electrophoresis on 5% gels under nonreducing conditions and transferred to a nitrocellulose membrane. RAP-binding proteins were visualized as described under “Experimental Procedures.” Lane 1 shows control BAECs; lane 2 shows cells infected with AdLacZ (LacZ); lane 3 shows cells infected with AdhVLDLr (VLDLr). Panel B, transport of 125I-LPL across control and virus-infected BAECs. 1 µg/ml 125I-LPL was added to DMEM and 1.5% BSA on the basolateral medium of control or infected BAECs, and the amount transported to the upper chamber medium after a 3-h incubation at 37 °C is shown. AdhVLDLr (denoted VLDLr), but not AdLacZ (denoted LacZ), infected cells had more LPL transcytosis, and this increase was completely inhibited by RAP. Data are the means ± S.D. of experiments performed in triplicate.
REFERENCES

1. Zechner, R. (1997) Curr. Opin. Lipidol. 8, 77–88
2. Lum, H., and Malik, A. B. (1996) Can. J. Physiol. Pharmacol. 74, 787–800
3. Mostov, K. (1991) Semin. Cell Biol. 2, 411–418
4. Goldberg, I. J. (1996) J. Lipid Res. 37, 693–707
5. Magrane, J., Reina, M., Pagan, R., Luna, A., Casaroli-Marano, R. P., Angelin, B., Gafvels, M., and Vilaro, S. (1998) J. Lipid Res. 39, 2172–2181
6. Saxena, U., Klein, M. G., and Goldberg, I. J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 2554–2558
7. Williams, S. E., Kounnas, M. Z., Argraves, K. M., Argraves, W. S., and Strickland, D. K. (1994) Ann. N. Y. Acad. Sci. 737, 1–13
8. Williams, S. E., Ashcom, J. D., Argraves, W. S., and Strickland, D. K. (1992) J. Biol. Chem. 267, 9035–9040
9. Herz, J., Goldstein, J. L., Strickland, D. K., Ho, Y. K., and Brown, M. S. (1991) J. Lipid Res. 32, 1213–1221
10. Socorro, L., Green, C. C., and Jackson, R. L. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 645–649
11. Williams, S. E., Kounnas, M. Z., Argraves, K. M., Argraves, W. S., and Strickland, D. K. (1991) J. Biol. Chem. 266, 17073–17080
12. Pillarisetti, S. (1999) Methods Mol. Biol. 109, 267–278
13. Cornicelli, J. A., Witte, L. D., and Goodman, D. S. (1983) Arteriosclerosis 3, 560–567
14. Stiros, M. P., Nemani, P. V., Wass, C., and Kim, K. S. (1999) Infect. Immun. 67, 5522–5525
15. Hoequette, J. F., Graulet, B., and Olivecrona, T. (1998) Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 121, 201–212
16. Strickland, D. K., Ashcom, J. D., Williams, S., Burgess, W. H., Migliorini, M., and Argraves, W. S. (1990) J. Biol. Chem. 265, 17401–17404
17. Takahashi, S., Kawarabayashi, Y., Nakai, T., Sakai, J., and Yamamoto, T. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 9252–9256
18. Medh, J. D., Fry, G. L., Bowen, S. L., Pladet, M. W., Strickland, D. K., and Chappell, D. A. (1995) J. Biol. Chem. 270, 536–540
19. 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
20. Argraves, K. M., Battey, F. D., MacCalman, C. D., McCrane, K. R., Gafvels, M., Kozarsky, K. F., Chappell, D. A., Strauss, J. F., 3rd, and Strickland, D. K. (1995) J. Biol. Chem. 270, 26550–26557
21. van Vlijmen, B. J., Rohlmann, A., Page, S. T., Bensaadoun, A., Bos, I. S., van Berkel, T. J., Havekes, L. M., and Herz, J. (1999) J. Biol. Chem. 274, 35219–35226
22. Obunike, J. C., Sivaram, P., Paka, L., Low, M. G., and Goldberg, I. J. (1996) J. Lipid Res. 37, 2439–2449
23. Bengtsson-Olivecrona, G., and Olivecrona, T. (1985) Biochem. J. 226, 409–413
24. Chappell, D. A., Fry, G. L., Wakkazit, M. A., Muohon, L. E., Pladet, M. W., Iverius, P. H., and Strickland, D. K. (1993) J. Biol. Chem. 268, 14168–14175
25. Saxena, U., Klein, M. G., and Goldberg, I. J. (1990) J. Biol. Chem. 265, 12880–12886
26. Osborne, J. C., Jr., Bengtsson-Olivecrona, G., Lee, N. S., and Olivecrona, T. Biochem. 24, 5606–5611
27. Friedman, G., Chajek-Shaul, T., Olivecrona, T., Stein, O., and Stein, Y. (1982) Biochim. Biophys. Acta 711, 114–122
28. Fazio, S., Linton, M. P., Hasty, A. H., and Swift, L. L. (1999) J. Biol. Chem. 274, 8247–8253
29. Marino, M., Zheng, G., Chiovato, L., Pinchera, A., Brown, D., Andrews, D., and McCluskey, R. T. (2000) J. Biol. Chem. 275, 7125–7137
30. McFadden, G., and Kelvin, D. (1997) Biochem. Pharmacol. 54, 1271–1280
31. Witt, D. P., and Lander, A. D. (1994) Curr. Biol. 4, 394–400
32. Middleton, J., Neil, S., Wintle, J., Clark-Lewis, I., Moore, H., Lam, C., Auer, M., Hub, E., and Rot, A. (1997) Cell 81, 385–395
33. Wyne, K. L., Pathak, K., Seabra, M. C., and Hobbs, H. H. (1996) Arterioscler. Thromb. Vasc. Biol. 16, 407–415
34. Kwak, S., Singli-Biot, A., Naut, V., and Kraemer, F. B. (1997) Horm. Metab. Res. 29, 524–529
35. Willnow, T. E., Sheng, Z., Ishibashi, S., and Herz, J. (1994) Science 264, 1471–1474
36. Veniant, M. M., Zlot, C. H., Walzem, R. L., Pierotti, V., Driscoll, R., Dickeh, D., Herz, J., and Young, S. G. (1998) J. Clin. Invest. 102, 1559–1568
37. Frykman, P. K., Brown, M. S., Yamamoto, T., Goldstein, J. L., and Herz, J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 8453–8457
38. Weinstock, P. H., Levak-Frank, S., Hudgins, L. C., Radner, H., Friedman, J. M., Zechner, R., and Breslow, J. L. (1997). Proc. Natl. Acad. Sci. U. S. A. 94, 10261–10266
39. Kako, Y., Huang, L. S., Yang, J., Katopodis, T., Ramakrishnan, R., and Goldberg, I. J. (1999) J. Lipid Res. 40, 2185–2194
40. Tacken, P. J., Teusink, B., Jong, M. C., Havekes, L. M., Vennema van Dijk, K., and Hofker, M. H. (2000) J. Biol. Chem. 275, 2699–2705
41. Saxena, U., Klein, M. G., and Goldberg, I. J. (1991) J. Biol. Chem. 266, 17516–17521
42. Parthasarathy, N., Goldberg, I. J., Sivaram, P., Malloy, B., Flory, D. M., and Wagner, W. D. (1994) J. Biol. Chem. 269, 22391–22396
43. Sivaram, P., Klein, M. G., and Goldberg, I. J. (1992) J. Biol. Chem. 267, 16517–16522
44. Sivaram, P., Chi, S. Y., Curtiss, L. K., and Goldberg, I. J. (1994) J. Biol. Chem. 269, 9409–9412
45. Hussain, M. M., Obunike, J. C., Shaleness, A., Hussain, M. J., Sheldin, G. S., and Goldberg, I. J. (2000) J. Biol. Chem. 275, 29324–29330