Regulation of Glycosphingolipid Glycosyltransferase by Low Density Lipoprotein Receptors in Cultured Human Proximal Tubular Cells*

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We have shown previously that low density lipoproteins (LDL) suppressed the synthesis of lactosylceramide in normal human proximal tubular cells, but stimulated such synthesis in proximal tubular cells from LDL receptor negative subjects (Chatterjee, S., Clarke, K., and Kwiterovich, P. O., Jr. (1986) J. Biol. Chem. 261, 13474–13479). To understand the mechanism(s) of this effect of LDL, we have studied here the effects of LDL on the activity of UDP-Gal:Cer;β-galactosyltransferase (GalT-2).

Maximum suppression (70–80%) of the activity of GalT-2 in normal proximal tubular cells at 37 °C occurred at a LDL concentration of 25 μg/ml medium. Such suppression was not observed either when the cells were incubated with LDL at 4 °C, or when the cells were preincubated with leupeptin, followed by incubation with LDL at 37 °C. High density lipoproteins and fetuin did not suppress the activity of GalT-2 in normal proximal tubular cells. In contrast LDL modified by reductive methylation (M-LDL, 100 μg/ml) stimulated the activity of GalT-2, approximately 3-fold. The effects of LDL and M-LDL were not related to their glycosphingolipid content. Much less suppression and stimulation of the activity of GalT-2 in proximal tubular cells by LDL and M-LDL, respectively, was found in normal human skin fibroblasts, Chinese hamster ovary cells, and bovine smooth muscle cells, suggesting that the LDL-mediated effect may be tissue-specific. In cells grown to very high density, the activity of the LDL receptor is decreased, and there was less suppression of GalT-2 activity by LDL.

In normal proximal tubular cells, LDL stimulated the activity of UDP-Gal:LacCer, α-galactosyltransferase activity, UDP-Gal:LcOse&er, β-galactosyltransferase, and CMP-NeuAc:LacCer, α-sialyltransferase activity but did not alter the activity of sulfotransferase.

In conclusion, LDL that entered the normal proximal tubular cells via the LDL receptor-mediated pathway decreased GalT-2 activity, an effect that was dependent upon the binding, internalization, and degradation of receptor-bound LDL. In contrast LDL that entered normal or LDL receptor-negative proximal tubular cells via an LDL receptor-independent pathway failed to suppress GalT-2 activity, and led to a stimulation of LacCer synthesis.

The biosynthesis of glycosphingolipids has been studied in a variety of cultured cells and tissues (1–4). Briefly, serine is transferred to a fatty acyl-coenzyme A with decarboxylation, and long chain bases such as sphingosine (4-sphingosine) are formed (5). The aminog group of sphingosine is then acylated by long chain fatty acyl-coenzyme A to yield ceramide (6). Next, a stepwise transfer of monosaccharide units from nucleotide sugars to ceramide via specific glycosyltransferases occurs (7). Thus, the transfer of glucose and galactose from UDP-glucose and UDP-galactose to ceramide and glucosylceramide, respectively, results in the synthesis of GlcCer and LacCer.

Lactosylceramide plays a pivotal role in the biosynthesis of complex GSL including GbOse3Cer, GM3, and sulfatides (2, 3). While these steps in the biosynthesis of GSL have been elucidated, less information is available regarding the mechanism(s) involved in regulating GSL metabolism.

In cultured normal human proximal tubular cells, human plasma low density lipoproteins (LDL) inhibit the incorporation of radiolabeled serine, glucose, and galactose into LacCer (7). Such inhibition is dependent on the LDL receptor pathway, since proximal tubular cells from LDL receptor-negative familial hypercholesterolemic homozygotes did not exhibit such an effect. The lack of inhibition of GSL synthesis in familial hypercholesterolemic homozygous receptor-negative cells is consistent with our previous report that the levels of LacCer are elevated 15-fold in the urinary sediment of patients with homozygous familial hypercholesterolemia (8).

In this report, we studied the in vitro biosynthesis of LacCer via UDP-galactose:GlcCer, galactosyltransferase (GalT-2), and its regulation by exogenous LDL. The effect of LDL on other glycosyltransferases involved in the in vitro synthesis of sulfatide, GM3, LeOse&er and GbOse&er was also investigated. Our data indicate that LDL suppresses the synthesis of LacCer by decreasing the activity of GalT-2 in normal cells whereas, in familial hypercholesterolemic homozygous cells, LDL stimulates the synthesis of LacCer.

MATERIALS AND METHODS

Isotopes and Chemicals—[14C]UDP-galactose (specific activity, 327 mCi/mmol) and [14C]CMP-sialic acid (specific activity, 200 mCi/mmol) were purchased from Amersham Corp. Phosphodenosine 5-phosphosulfate tetrasodium salt (specific activity, 3.7 Ci/mmol) was purchased from Du Pont-New England Nuclear. The final specific

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‡ The abbreviations used are: GlcCer, glucosylceramide; GSL, glycosphingolipid; LacCer, lactosylceramide; GbOse3Cer, globotriosylceramide (trihexosylceramide); LDL, low density lipoproteins; M-LDL, methyl-LDL; HDL, high density lipoproteins; LPDS, lipoprotein-deficient serum; GalT-2, UDPGal:GlcCer, β-1,4-galactosyltransferase. GalT-4, UDPGal:LeOse&er, α-1,4-galactosyltransferase, SAT-1, CMP-NeuAc:LacCer sialyltransferase; LeOse&er, lactotriglycosylceramide.
activity of these compounds was adjusted with unlabeled substrates, as described below under glycosyltransferase assays. Glucosylceramide was prepared from a Gaucher's spleen. LacCer prepared from human granulocytes was a gift of Dr. Bruce Macher, University of San Francisco, and was further purified by high performance thin layer chromatography and quantified by high performance liquid chromatography (9). One Gaucher spleen and LacCer were grown in medium containing 10% fetal calf serum and no antibiotics of growth, medium was removed and fresh medium containing LDL (0-25 pg/ml) for 2 h at 4 °C. Subsequently, medium was removed and fresh medium containing LPDS and LDL (0-200 pg/ml) was added, and the incubation continued for 4 h at 37 °C. Subsequently, cells were harvested, and the products separated by agarose-gel electrophoresis and immunoelectrophoresis (11). Protein content was measured according to Lowry et al. (12), using crystalline bovine serum albumin as a standard.

Reductive Methylation of LDL — The lysine residues of apolipoprotein B of LDL were blocked by reductive methylation of LDL after Wasserman et al. (13). LDL and characterized as described previously (11).

Cell Culture — Cultured normal human proximal tubular cells were prepared from kidney (obtained at autopsy) (15). Normal human fibroblasts (from a male subject, GM 5659, and a female subject, GM 0038A) were purchased from the Mutant Cell Repository (Candcn, NJ) and characterized as described previously (14).

Incubation of Cells in Plasma Lipoproteins — Unless otherwise specified, cells (1 x 10⁶) were seeded in plastic Petri dishes (60 x 15 mm) in medium containing 10% fetal calf serum. On day 1 of cell growth, medium was removed, and fresh medium was added. On day 6 of cell growth, medium was removed and fresh medium containing LPDS (1 mg of protein/ml) was added, and the incubation continued for 24 h. Unless otherwise specified all the experiments described below were performed on confluent cell cultures (7 days after cell growth).

Incubation of Cells with Plasma Lipoproteins — Unless otherwise specified, following 24 h incubation with medium containing LPDS, LDL, or HDL (0-500 µg/ml) was added, and the incubation continued for 4 h at 37 °C. In another set of experiments, cells were incubated with LDL (0-25 µg/ml) for 2 h at 4 °C. Subsequently, medium was removed and cells were harvested and stained in 1% formaldehyde (pH 6.5), 0.2% sucrose, 0.1% 2-mercaptoethanol, 1 mM EDTA, 0.1% polyethylene glycol (M, 1000) centrifuged, washed, and saved at -20 °C.

Incubation of Cells with Leupeptin — On day 6 of cell growth, medium was removed and fresh medium containing LPDS and leupeptin (0.1 mM) was added to the culture dishes. Following incubation for 24 h at 37 °C, the medium was removed and fresh medium containing LPDS and LDL (0-200 µg/ml) was added and the incubation continued for 4 h at 37 °C. Subsequently, cells were harvested, and the activity of GaT-2 in cells incubated with and without leupeptin was measured.

Glycosyltransferase Assays — The activity of GaT-2 is cells was measured as described previously (17). Briefly, the GaT-2 assay mixture contained: 20 nmol of cacodylate buffer (pH 6.8), 1.0 mM Mn²⁺ (1:1), 0.2 mg/ml Triton X-100, cutinase (1:2 v/v), 3 µmol of glucosylceramide, and 0.1 mmol UDP-galactose. The activity of UDP-galactose, LacCer (α1-4-galactosyltransferase (GaT-6), UDP-galactose: LacCer, β-1-4-galactosyltransferase (GaT-4), CMP-NeuAc: LacCer, α-sialyltransferase (SAT-1), and PAPS:LacCer, sulfotransferase in proximal tubular cell homogenates was measured using the above assay conditions in the presence of appropriate GSL acceptors, and radioactive nucleotide sugar donors. Following incubation for 2 h at 37 °C, the assay mixtures were terminated, and the products separated by paper chromatography on SG-81-Whatman paper using chloroform/methanol/water (60:17:2 v/v) as solvent (3). Authentic GSL standards were cochromatographed simultaneously. Chromatogram areas corresponding with the migration of authentic GSL were cut and radioactivity measured in a Beckman LS-3000 scintillation spectrometer. Values without authentic GSL acceptor were measured as blanks and were subtracted from all respective data points.

Measurement of β-Galactosidase Activity — The activity of β-galactosidase was measured in proximal tubular cells, incubated with and without LDL, and M-LDL, employing [βH]LacCer as follows. [βH]LacCer was prepared as described previously (13). The assay mixture contained the following: 100 µg of homogenate protein, 0.4 ml of 1 x 10⁻² M sodium citrate-phosphate buffer (pH 4.0), 0.5 µmol of LacCer ([βH]LacCer, 300,000 cpm); 10 µmol of taurocholate; 10 µl of a mixture of dibromide X-100 (100 µg). Incubation was carried out at 37 °C for 16 h. The reaction was terminated with chloroform/methanol (2:1 v/v). Next, 100 µg of unlabeled galactose and 0.5 M KCl was added to the reaction mixture, and the contents centrifuged for 10 min at 500 x g at 10 °C. The upper phase was collected and washed with theoretical upper phase, centrifuged as above, and radioactivity measured in the upper phase in "Aquasol" (Dupont-New England Nuclear).

RESULTS

Effects of LDL, Fetuin, and Methyl-LDL on GaT-2 Activity in Normal Human Proximal Tubular Cells — In normal proximal tubular cells, there was a LDL concentration-dependent inhibition of GaT-2 activity (Fig. 1). Maximum inhibition (70-75%) occurred at a LDL concentration in the medium of 25 µg/ml, an effect that was observed is from four separate batches of proximal tubular cells analyzed in duplicate. Addition of higher amounts of LDL up to (500 µg/ml) to the normal proximal tubular cells did not increase further the inhibition of GaT-2 activity. When cells were incubated with fetuin (Fig. 1), or HDL (data not shown), there was no effect on GaT-2 activity.

Effect of Time of Incubation with LDL on GaT-2 Activity in Proximal Tubular Cells — In these series of experiments, proximal tubular cells grown for 6 days were primed with medium containing 1 mg/ml LPDS for 24 h. Subsequently, fresh medium containing 25 µg/ml LDL was added, and cells incubated for 1, 2, 4, 8, and 24 h. At the indicated time intervals, cells were harvested and GaT-2 activity measured. LDL exerted a time-dependent suppression of GaT-2 activity in proximal tubular cells (Fig. 2). GaT-2 activity decreased linearly up to 4 h of incubation with LDL. Increasing the time of incubation with LDL further did not decrease GaT-2 activity in proximal tubular cells (Fig. 2).

Reductive methylation of LDL (M-LDL) results in modification of the lysine residues. M-LDL are not recognized by the LDL receptor and are taken up by fibroblasts and proximal tubular cells via a LDL receptor-independent mechanism.
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Effect of time of incubation with LDL on GalT-2 activity in proximal tubular cells. Cells were grown as described in the legend for Fig. 1. Following priming the cells for 24 h in LPDS medium, fresh medium and 25 μg/ml LDL was added. At 0, 30 min, 1-, 2-, 4-, 8-, and 24-h time intervals, cells were harvested and GalT-2 activity was measured.

M-LDL does not suppress the incorporation of radioactive serine and glucose into LacCer in proximal tubular cells (6). We found that M-LDL, when present in the medium up to a concentration of 500 pg/ml, stimulated the activity of GalT-2 (Fig. 1). Maximum stimulation (300%) of GalT-2 occurred when the methyl-LDL concentration in the medium was 100 μg/ml (Fig. 1). These data suggest that receptor-mediated endocytosis of LDL in normal proximal tubular cells suppresses the activity of GalT-2. In contrast methyl-LDL, which enters these cells via a LDL receptor-independent mechanism, stimulated GalT-2 activity.

Effects of Incubation at 4 °C on GalT-2 Activity in Normal Human Proximal Tubular Cells—The activity of GalT-2 in proximal tubular cells, incubated without LDL at 4 °C for 2 h, was 9.58 nmol/h/mg protein. The addition of LDL (5–200 μg of protein) did not alter the activity of GalT-2 from that observed in cells incubated without LDL (data not shown). Since the internalization and degradation of LDL does not occur in proximal tubular cells at 4 °C, these data indicate that both internalization and degradation of receptor-bound LDL is necessary to suppress GalT-2 activity in normal proximal tubular cells.

Effects of Leupeptin on GalT-2 Activity in Normal Human Proximal Tubular Cells—To test further whether the degradation of receptor-bound LDL is essential to suppress GalT-2 activity in normal proximal tubular cells, we preincubated cells with leupeptin (a lysosomal serine protease inhibitor) for 24 h. Next, medium was removed and the cells were incubated with and without LDL (0–200 μg of protein/ml). After incubation for 4 h at 37 °C, cells were harvested and GalT-2 activity measured. The activity of GalT-2 in cells incubated with leupeptin (12.4 nmol/2 h/mg of protein) was identical to that of control (12.4 nmol/2 h/mg of protein). Thus, these findings suggest that impairment of the degradation of LDL is accompanied by the inability of LDL to suppress the activity of GalT-2 in normal proximal tubular cells.

Effect of Cell Density on GalT-2 Activity in Human Fibroblasts and Human Proximal Tubular Cells—Cells (1 x 10⁸) were grown in 60 x 15-mm Petri dishes for 3 days (low cell density) and 21 days with medium change once a week (high cell density). At the indicated time medium was removed and cells incubated with fresh medium containing 1 μg/ml LPDS for 24 h. Medium was removed and cells were incubated for 4 h with 25 μg/ml LDL and 100 μg/ml LDL. Subsequently, cells were harvested and GalT-2 activity was measured. We found that fibroblasts grown at low cell density had a GalT-2 activity of 0.45 nmol/2 h/mg of protein. Inclusion of LDL 25 and 100 μg/ml decreased the activity of GalT-2 approximately to 0.36 nmol/2 h/mg of protein and 0.27 nmol/2 h/mg of protein, respectively. This corresponds to a 19 and 40% decrease in GalT-2 activity as compared with control cells (average values obtained from two separate human fibroblasts cell lines). In contrast LDL exerted no effects on GalT-2 activity when the fibroblasts were grown at high cell density (data not shown).

At low cell density the activity of GalT-2 in proximal tubular cells was 15 nmol/2 h/mg of protein. LDL (25–100 μg/ml) decreased GalT-2 activity in the order of 3 nmol/2 h/mg of protein and 2.25 nmol/h/mg of protein, respectively. This corresponds to an 80–85% decrease in GalT-2 activity in proximal tubular cells. In highly confluent proximal tubular cell cultures, the activity of GalT-2 was 3.5 nmol/2 h/mg of protein. When such cells were incubated with 25 and 100 μg LDL/ml medium GalT-2 activity decreased to 3.3 nmol/2 h/mg of protein and 3.0 nmol/2 h/mg of protein, respectively. Thus, at high cell density of proximal tubular cells, LDL decreased GalT-2 activity in the order of 6 and 15% with 25 and 100 μg LDL/ml medium.

Effects of LDL from a Normal Subject, a Familial Hypercholesterolemic Homozygous Subject, and Methyl-LDL on GalT-2 Activity in a Variety of Cells—The effects of incubating various types of confluent cell cultures (7 days after cell growth) with normal LDL, methyl-LDL, and LDL obtained from a homozygous familial hypercholesterolemic subject is shown in Table I. Normal fibroblasts, CHO cells, and smooth muscle cells, which have been shown to have a functional LDL receptor pathway, exhibited a 33% decrease in GalT-2 activity. Such inhibition occurred when cells were incubated with 100 μg/ml LDL medium. However, at low concentrations of LDL (25 μg/ml medium) the decrease in GalT-2 activity was 0% in fibroblasts, 12% in Chinese hamster ovary cells, and 25% in smooth muscle cells. In contrast, LDL (25 μg/ml medium and 100 μg/ml medium) stimulated the activity of GalT-2 in the order of 11 and 13%, respectively, in familial hypercholesterolemic homozygous fibroblasts. These data indicate that LDL is not as effective in decreasing GalT-2 activity as compared with control cells (average values obtained from two separate human fibroblasts cell lines). In contrast LDL exerted no effects on GalT-2 activity when the fibroblasts were grown at high cell density (data not shown).

<table>
<thead>
<tr>
<th>Lipoprotein-deficient serum (control)</th>
<th>Normal human fibroblasts</th>
<th>Homozygous FH fibroblasts</th>
<th>Chinese hamster ovary cells</th>
<th>Smooth muscle cells</th>
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<td>GalT-2 activity (nmol/mg protein/2 h)</td>
<td>0.30</td>
<td>0.32</td>
<td>1.12</td>
<td>0.134</td>
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<tr>
<td>Normal LDL</td>
<td>25 μg/ml</td>
<td>100 μg/ml</td>
<td>0.20</td>
<td>0.40</td>
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<tr>
<td>FH-LDL</td>
<td>25 μg/ml</td>
<td>100 μg/ml</td>
<td>0.20</td>
<td>0.42</td>
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<tr>
<td>Methyl-LDL</td>
<td>25 μg/ml</td>
<td>100 μg/ml</td>
<td>0.35</td>
<td>ND</td>
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<tr>
<td>100 μg/ml</td>
<td>0.40</td>
<td>ND</td>
<td>1.80</td>
<td>0.150</td>
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* ND, not determined.
activity in these cells as compared to proximal tubular cells, suggesting, that this effect of LDL may be specific for proximal tubular cells.

LDL from a homozygous familial hypercholesterolemic subject exerted a similar decrease in GalT-2 activity in normal cells, and stimulated GalT-2 activity in homozygous familial hypercholesterolemic cells as found with LDL from a normal subject (Table I). In contrast methyl-LDL exerted a concentration-dependent stimulation of GalT-2 activity in all the normal cell lines. In normal human fibroblasts, M-LDL, 25 μg/ml and 100 μg/ml, stimulated GalT-2 activity in the order of 19 and 33%, respectively. Chinese hamster ovary cells incubated with 25 μg/ml M-LDL and 100 μg/ml LDL contained 34% and 60% higher GalT-2 activity as compared to control cells. In case of smooth muscle cells, M-LDL 25 μg/ml and 100 μg/ml stimulated the activity of GalT-2 in the order of 4 and 12%, respectively. These data suggest that M-LDL is also not as effective as stimulating GalT-2 activity in these cells as compared to proximal tubular cells.

Our preliminary studies with urinary proximal tubular cells from three familial hypercholesterolemic homozygotes (BA, TB, and KB) have revealed that the activity of GalT-2, in such cells grown for 24 h in LPDS medium, was 5.46 ± 0.5 nmol/2 h/mg of protein. Incubation of such cells with LPDS plus LDL (100 μg of protein/ml medium) for 24 h increased the activity of GalT-2 to 18.05 ± 1.0 nmol/2 h/mg of protein. Thus, LDL stimulated GalT-2 activity in familial hypercholesterolemic-proximal tubular cells in the order of 3-fold as compared to cells incubated without LDL.

Effect of LDL on Other GSL Glycosyltransferases—Next, the effects of LDL on the activity of GaIT-4, GaIT-6, GM3 synthetase, and FAPS: LacCer sulfotransferase (sulfatide synthetase) were measured. As shown in Fig. 3, LDL exerted a concentration-dependent stimulation of α-galactosyltransferase activity in proximal tubular cells. For instance in cells incubated with 25 μg/ml LDL and 100 μg/ml LDL, the activity of α-galactosyltransferase was increased approximately 12- and 40-fold, respectively, as compared to control. The activity of α-galactosyltransferase in cells incubated with 500 μg of LDL/ml medium was increased 25-fold. The activity of GaIT-4 in proximal tubular cells, incubated without LDL, was 74.27 nmol/2 h/mg of protein. When the cells were incubated with 20 and 200 μg LDL/ml medium for 4 h at 37 °C, the activity of GaIT-4 in such cells was 83.4 nmol/2 h/mg of protein and 107.2 nmol, respectively. Thus, LDL also stimulated GaIT-4 activity in proximal tubular cells. Similarly, LDL, 10 and 25 μg/ml, stimulated the activity of sialyltransferase in the order of 4- and 7.5-fold, respectively, as compared to control cells (Fig. 3). However, when cells were incubated with 100 μg LDL/ml of medium, there was no significant effect on the activity of sialyltransferase (Fig. 3). When cells were incubated with 500 μg LDL/ml of LDL it completely inhibited the activity of sialyltransferase. Incubation of cells with 0–500 μg LDL/ml medium had no effects on sulfotransferase activity (data not shown).

Effect of LDL on α-Galactosidase Activity in Proximal Tubular Cells—The activity of α-galactosidase in proximal tubular cells was measured employing [3H]LacCer as substrate. The activities of α-galactosidase (nmol of LacCer hydrolyzed/h/mg of protein) in proximal tubular cells incubated without and with LDL (25, 100, and 500 μg/ml medium) were 1.49, 1.46, and 1.33, respectively. Thus, LDL did not alter the activity of LacCer, α-galactosidase significantly in proximal tubular cells. Moreover, LDL itself did not have a α-galactosidase activity. These findings rule out the possibility that alterations in α-galactosidase activity in cells incubated with LDL may have contributed to the observed suppression of GaIT-2 activity, by catabolizing newly synthesized LacCer in vitro in proximal tubular cells.

Effect of LDL on GaIT-2 Activity in Proximal Tubular Cell Homogenates—In this experiment, proximal tubular cells, incubated for 24 h in medium containing LPDS, were harvested in buffer and homogenized. To suitable aliquots of cell homogenate, 0–500 μg LDL/ml assay mixture was added and GaIT-2 activity was measured as described above. The activity of GaIT-2 in proximal tubular cell homogenates incubated with 500 μg LDL/ml assay mixture and without LDL were 10.8 nmol/2 h/mg of protein and 10 nmol/2 h/mg of protein, respectively. Thus, when cell homogenates were incubated directly with LDL, it had no effect on GaIT-2 activity. These findings suggest that only LDL taken up via the intact cells containing functional LDL receptors affects GalT-2 activity.

**FIG. 3. Effect of LDL on the activity of UDPGal:LacCer, α-1-4-galactosyltransferase and CMP-NeuAc:LacCer, α-sialyltransferase in cultured human proximal tubular cells.** The protocol of this experiment was identical to that described in Fig. 1. The assay procedure is described in the text, except that LacCer served as the substrate. The results are average values of a single experiment analyzed in duplicate. A, effect of LDL on the activity of GaIT-6, effect of LDL on the activity of SAT-1 activity.

**DISCUSSION**

To understand better the mechanism(s) of LDL-mediated suppression of LacCer synthesis, and the involvement of LDL receptors in this phenomenon in normal cells, we measured the activity of GaIT-2, an enzyme known to be involved in the synthesis of LacCer (1–3). We found that LDL exerted a time and concentration-dependent suppression of GaIT-2 activity in normal proximal tubular cells. In contrast as judged by the activity of LacCer-β-galactosidase, LDL did not alter the catabolism of newly synthesized LacCer in these cells. Moreover, when GaIT-2 activity was measured in the presence and absence of 0.025 nmol of 5 AMP (an inhibitor of nucleotide pyrophosphatase activity), cells incubated with LDL showed a decreased activity of GaIT-2 compared with control cells. Thus, the LDL-mediated decrease in GaIT-2 activity in normal proximal tubular cells was not due to abnormal degradation of UDP-galactose.

Maximum suppression of GaIT-2 activity occurred when cells were incubated with 25 μg/ml medium of LDL, a finding in agreement with our previous incorporation studies, where we found that maximum inhibition of [3H]serine and [3H]glucose into LacCer occurred at a concentration of 25 μg of LDL/ml medium. Moreover, this concentration of LDL saturates the LDL uptake occurring via the high affinity receptors (13, 18), thereby, further implicating the involvement of LDL receptors in the suppression of GaIT-2 activity in normal proximal tubular cells.
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When freshly obtained proximal tubular cell homogenates were prepared and incubated with LDL (0–500) µg/ml, the activity of GaIT-2 was not suppressed. This finding supports the tenet that intact cells, having functional LDL receptor pathway, are required for LDL-mediated suppression of GaIT-2 activity. Since LacCer plays a pivotal role in the synthesis of complex GSL, some synthesis of GM₃, ganglioside, Gb₃Cer, LacCer, and sulfatides must occur all the time. This hypothesis is supported by our finding that LDL stimulated the activity of glycosyltransferases involved in the synthesis of GM₃, LacCer, and Gb₃Cer.

In cultured normal human fibroblasts, and normal proximal tubular cells, M-LDL impaired the high-affinity binding of LDL (12, 13). This was accompanied by lack of suppression of the incorporation of [³H]serine into LacCer by M-LDL (6). Here, we found that M-LDL stimulated GaIT-2 activity in normal proximal tubular cells, a finding consistent with our previous hypothesis that LDL entering the cells via an LDL receptor-independent mechanism does not suppress LacCer biosynthesis, whereas LDL entering the cells via the LDL receptor pathway suppresses LacCer biosynthesis. This hypothesis was supported further by the following findings. First, similar albeit less striking observations were made in other types of cells known to have functional LDL receptors. These include human skin fibroblasts, Chinese hamster ovary cells, and bovine smooth muscle cells. Since LDL was not as effective in decreasing GaIT-2 activity in these cells compared with normal proximal tubular cells suggests that the LDL-mediated effect may be specific for renal proximal tubular cells. Second, in fibroblasts obtained from a homozygous familial hypercholesterolemic subject that lacked LDL receptors, LDL did not suppress the activity of GaIT-2, and in fact, stimulated GaIT-2 activity to a small degree in such cells (11–13%). Our recent studies in proximal tubular cells from familial hypercholesterolemic homozygotes revealed that the stimulation of GaIT-2 activity is in the order of 3-fold.

The notion that the apolipoprotein B moiety of LDL is involved in the recognition of LDL on the LDL receptor, permitting LDL-receptor-mediated regulation of cholesterol metabolism is well-established (12). Thus, our finding that both modification of the apolipoprotein B moiety of LDL by methylation of lysine residues, and HDL which lacks apolipoprotein B failed to suppress GaIT-2 activity, are in accord with the previous findings in fibroblasts in which neither methyl-LDL nor HDL suppressed hydroxymethylglutaryl-CoA reductase activity (12).

Normal human LDL contains approximately 4.6 and 3 nmol of GlcCer and LacCer/mg of LDL cholesterol, respectively (21). The levels of GlcCer and LacCer in the LDL in familial hypercholesterolemic homozygotes contained 21.5 nmol and 6.3 nmol/mg of LDL cholesterol, respectively (21). When cells were incubated with LDL derived from a homozygous familial hypercholesterolemic subject (Table I), the suppression of GaIT-2 activity was similar to that found with normal LDL. Since LDL derived from the familial hypercholesterolemic subject had five times as much GlcCer and two times as much LacCer/mg of cholesterol as normal LDL, this observation suggests that GlcCer and LacCer on LDL are not involved in suppressing the activity of GaIT-2 in these cells. Since the glycosphingolipid composition of M-LDL was identical to that of unmodified normal LDL, this also argues in support of the conclusion that the GSL moiety of LDL is not involved in suppressing GaIT-2 activity in proximal tubular cells. HDL₂ contains an average of 1 mol of phosphatidylethanolamine, 21 mol of sphingomyelin, and 97 mol of phosphatidylcholine/mol (19). GSL represent approximately 0.5 to 1 mol/mol of HDL particle (20). Neither the phospholipid or GSL moieties of HDL altered GaIT-2 activity.

The activity of GaIT-2 was identical in cells incubated with and without cholesterol (0–200 µg/ml) for 4 h at 37 °C (data not shown). Thus cholesterol, a major component of LDL does not appear to suppress directly GaIT-2 activity in proximal tubular cells. Studies will be required to assess further the role of the lipid moiety of LDL in suppressing GaIT-2 activity in proximal tubular cells.

Previous studies have shown that normal human fibroblasts grown at low cell density have a greater number of LDL receptors than highly confluent fibroblasts (22). We found that LDL suppressed the activity of GaIT-2 in sparse cultures of both fibroblasts and proximal tubular cells more profoundly than in superconfluent cultures. Thus, the LDL-mediated regulation of GaIT-2 activity in cultured cells reported in our studies is dependent on the availability of the LDL receptors. LDL also decreases the incorporation of [³H]galactose into LacCer in freshly isolated normal human leukocytes (23). Our findings suggest that one mechanism by which LDL may exert this effect, namely the suppression of GaIT-2 activity in proximal tubular cells. Similarly, the decreased level of LacCer in normal proximal tubular cells incubated with LDL reported previously by us (13) may also be due to the suppression of GaIT-2 activity.

In summary LDL regulates LacCer metabolism in normal cells by suppressing the activity of GaIT-2. Our studies reveal that LDL mediates the regulation of GSL metabolism in normal human proximal tubular cells in a fashion similar to the down regulation of cholesterol biosynthesis reported previously in skin fibroblasts (18). Whether LDL also regulates GaIT-2 activity in normal proximal tubular cells by decreasing the mass of GaIT-2 is currently under investigation. Recent work with porcine submaxillary glands have revealed that UDP-galactosylceramide, glucosyltransferase, involved in the synthesis of GlcCer, is localized on the cytoplasmic face of the Golgi apparatus (22). Various GSL glucosyltransferases are concentrated in Golgi apparatus of rat liver (25) and rat kidney (26), but GaIT-2 has not been localized as yet to the cytoplasmic face of the Golgi apparatus in normal human proximal tubular cells. However, the product of the enzymatic reaction, i.e. LacCer, is cytoplasmic in origin (27, 28). We speculate that LDL taken up via LDL receptor may gain access to cytoplasmic GlcCer and inhibit the activity of this enzyme in proximal tubular cells. In contrast, LDL not taken up via the LDL receptor pathway may not have access to GaIT-2, thus allowing continued synthesis of LacCer.

REFERENCES
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