Sphingomyelin Suppresses the Binding and Utilization of Low Density Lipoproteins by Skin Fibroblasts*

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Shimon Gatt3 and Edwin L. Biermans

From the Division of Metabolism and Endocrinology, Department of Medicine, University of Washington, Seattle, Washington 98195

Cultured human skin fibroblasts incubated at 37°C with sonically dispersed, positively charged liposomes containing sphingomyelin internalized and metabolized the phospholipid. Sphingomyelin incorporation into the cells produced a reduction in low density lipoprotein binding and degradation. Lecithin-containing liposomes were much less effective. In addition, incubation with sphingomyelin resulted in a marked increase in acetate incorporation into sterol. These results suggest that sphingomyelin, which is required by cells for membrane synthesis, can influence the regulation of the cell surface low density lipoprotein receptor and intracellular cholesterol balance.

Human nonhepatic cells appear to utilize a high affinity, specific cell surface receptor for the binding and internalization of the cholesterol-rich lipoprotein in plasma (low density lipoprotein), thus making extracellular cholesterol available for membrane synthesis (1). After binding to its receptor, LDL is internalized within endocytotic vesicles which fuse with lysosomes. Its protein and cholesterol esters are then hydrolyzed by lysosomal enzymes, liberating free cholesterol for use in membrane synthesis. Studies with cultured human skin fibroblasts and arterial smooth muscle cells indicate that the availability of extracellular cholesterol regulates the activity of the LDL receptor. Manipulations that increase cellular free cholesterol concentration inhibit cell surface LDL receptor activity, presumably by suppressing its synthesis. Restriction of available extracellular cholesterol or enhancement of cholesterol efflux from cells leads to both an increase in endogenous cholesterol synthesis (via regulation of the activity of \(\beta\)-hydroxy-\(\beta\)-methylglutaryl-CoA reductase) and an increase in the number of LDL receptors. This regulation of LDL utilization assures non-hepatic cells of an adequate supply of cholesterol for membrane synthesis and protects them from cholesterol overload.

Phospholipids, particularly lecithin and sphingomyelin, must also be continually available for membrane synthesis.

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3 Visiting Scientist of the Center for Advanced Studies of the University of Washington School of Medicine. Present address, Laboratory of Neurochemistry, Department of Biochemistry, Hebrew University-Hadassah Medical School, Jerusalem, Israel.

4 To whom reprint requests should be addressed.

Sphingomyelin, labeled with tritium in the choline moiety, was prepared according to Stoffel (6). Nonradioactive sphingomyelin of bovine brain was purchased from Makor Chemicals, P.O. Box 6570, Jerusalem. Lecithin was purchased from Sigma and octadecylamine (stearylamine) or dicetyl phosphate from Aldrich; \([1\text{-}^{14}\text{C}]\)palmitate and \([1\text{-}^{3}\text{H}]\)concavalsin A were purchased from New England Nuclear Corp. Phosphate-buffered saline, trypsin/EDTA solution, Heps buffer, and fetal calf serum were obtained from Grand Island Biological Co. Bovine albumin was purchased from Sigma Chemical Co. Dextran sulfate was obtained from Pharmacia Labs.

**Description of Cells—Skin fibroblasts were cultured from punch biopsies of human skin taken from the thighs of normal volunteers. Cells were grown in an atmosphere of humidified 95% air, 5% CO\(_2\) at 37°C and transferred after trypsinization as previously described (7) using modified Dulbecco-Vogt medium (glucose concentration, 3 mg/ ml) containing 10% fetal calf serum. Normal skin fibroblasts were used after 4 to 6 passages. Skin fibroblasts from a patient with receptor-negative familial hypercholesterolemia (GM 488) were obtained from the Human Genetic Mutant Cell Repository (Camden,
Preparation of Lipoproteins and Lipoprotein-deficient Serum—LDL were isolated from plasma obtained from normal donors by sequential preparative ultracentrifugation in a Beckman L2-65B ultracentrifuge as previously described (8). Iodination with \(^{125}\)I was performed by the monochloride technique as modified for lipoproteins (9). Following extensive dialysis to remove excess monochloride, more than 97% of the radioactivity was insoluble in chloroform/methanol. The specific activity of LDL approximated 2 \( \times 10^6 \) cpm/\( \mu \)g of LDL protein. Freshly prepared radioactive lipoprotein was sterilized by Milipore filtration. The lipoprotein-deficient serum was prepared by centrifugation at \( d = 1.25 \) as previously described (10) and reconstituted under identical conditions.

Preparation of Liposomes—Chloroform/methanol solutions of phospholipid and octadecylamine or dicetyl phosphate were mixed and the solvent evaporated under nitrogen. Saline was added and the dispersion was subjected to sonic irradiation using a Branson Sonifier model W185 equipped with a microtip. Each sample was exposed to two 1-min irradiations with a setting of 2 on the control knob. Sphingomyelin with or without additives was irradiated at 45°C and left in saline with lipoamine at 20°C. The dispersions were centrifuged for 10 min at 2 to 5000 rpm and the supernatant was carefully removed and retained.

Incubation of Cells in the Presence of Liposomes—Skin fibroblasts (1 \( \times 10^5 \) cells) were plated into 90-mm diameter plastic Petri dishes containing 4 ml of medium 6 to 14 days prior to each experiment to allow the monolayer to become confluent. Medium was changed every 15 min for the first 2 days. Immediately prior to the addition of the liposomes the medium was removed, the cell layer washed once with PBS buffer containing 0.2% bovine albumin, and replaced by 3.6 ml of fresh medium devoid of serum. Liposomes (0.02 to 0.8 ml) were added and the cells were incubated for 1 h at 37°C. Then 0.4 ml of 100% lipoprotein-deficient serum was added and the cells were incubated further at 37°C for the period indicated. Parallel control dishes contained only lipoprotein-deficient serum. For incubations at 4°C (in room air), Hepes buffer was substituted for bicarbonate buffer in the medium.

Measurement of Internalization of \[^{3}H\]Choline Sphingomyelin—The cell layer was washed 5 times with PBS-albumin buffer followed by gentle trypsinization (0.05% trypsin in EDTA) to release cells from the dish and remove material adsorbed to cell surfaces. A portion of the cell suspension was removed for measurement of cell number in a hemocytometer and the residue was sedimented by centrifugation. The cells were resuspended in buffer and resedimented twice. The cell pellet was then removed and the cell layer washed 4 times with 2 ml of PBS-albumin buffer, followed by 2 washes with 4 ml of buffer, each in contact with the cell layer for 10 min. Airdried films were washed 2 times with 2 ml of 0.1 N NaOH (to remove nonradioactive contaminant) followed by a wash with 2 ml of PBS buffer containing no albumin. Then 4 ml of dextran sulfate (4 mg/ml in 10 mm Hepes buffer) were added and the dishes incubated for 1 h at 4°C. Radioactivity released into the dextran solution was measured as an index of cell surface-bound LDL. After removal of the dextran solution, 1 ml of 0.1 N NaOH was added to each dish, the dishes were warmed to room temperature, and allowed to stand for 90 min with occasional mixing. An aliquot was removed for determination of cell protein by the Lowry method (13). LDL binding also was determined at 37°C, following replacement of medium containing the liposomes with fresh LDS and 4-h incubation with \(^{125}\)I-LDL, as the lipoprotein acid-soluble, non-lipid-soluble radioactivity releasable from cells by gentle trypsinization, as previously described (7). Uptake of LDL protein was determined by measurement of trichloroacetic acid-soluble protein breakdown products in the incubation medium (7) after 24 h of incubation with \(^{125}\)I-LDL. Non-lipid-soluble radioactivity, not releasable from cells by trypsin, was considered to represent LDL protein uptake.

The binding of \[^{1}H\]concanavalin A also was measured at 37°C. After incubation for 1 h in fresh medium containing tracer amounts of concanavalin A, the cell layer was washed 5 times with PBS and extracted for 1 h in 2 ml of 0.1 N NaOH with shaking. Aliquots were taken for measurement of cell protein content and radioactivity.

Utilization of \[^{14}C\]Palmitate—\[^{14}C\]Palmitate was dispersed in water, neutralized with 1 eq of aqueous NaOH, heated to about 80-90°C, cooled to about 30°C, and added to a solution of bovine serum albumin (fatty acid free, 10 mg/ml) in Krebs-Ringer phosphate medium maintained at 40°C. After a 15-min preincubation period (2 \( \times 10^6 \) cpm/\( \mu \)mol/30 ml of albumin) was added to the growth medium and the mixture was filtered through a 0.45 mm Milipore filter. The filtrate was added to cells previously incubated for 24 h at 37°C with either medium containing LDS alone or LDS plus nonradioactive sphingomyelin liposomes and incubated for 5 h. The determination of radioactivity in the various cell lipids was very similar to that described for the radioactive liposomes except that the cells were scraped off the dish using a Teflon policeman and a portion was taken for determination of protein content.

Incorporation of \[^{14}C\]Acetate into Sterol—Sodium \[^{14}C\]acetate (1.8 mCi/mmol) was added to the medium in each dish and incubated for 2 h at 37°C. The dishes were chilled, medium removed, washed once with cold PBS, and frozen. Extraction of lipids was performed by a modification of the method of Stein et al. (14). The frozen cells were scraped from the dishes with a Teflon policeman into 1.0 ml of cold methanol/water (2:1). The dishes were washed once with the same solvent and then twice with 1.0 ml of 100% methanol. The washes were combined with 3.0 ml of chloroform and the sterol was extracted at 4°C overnight. Protein content was determined in the pellet obtained after centrifugation. The extract was dried at 60°C under nitrogen (10) and, after addition of 70% methanol and 1 mol of NaOH (1 N), the sterol was extracted with hexane and developed by thin layer chromatography using a solvent system of hexane/ether/methanol/acetic acid (78:17:3:2). The free sterol spot was scraped into a counting vial and its radioactivity measured.

**RESULTS**

Internalization of Positively Charged Sphingomyelin Liposomes—Positively charged liposomes of sphingomyelin and octadecylamine were prepared by ultrasonic irradiation and added to the growth medium of cultured skin fibroblasts. Internalization of sphingomyelin into cultured fibroblasts was a function of the percentage of octadecylamine incorporated into the liposomal dispersion (Fig. 1A). The radioactivity recovered in the trypsin-treated cells was essentially linear up to 15 mol % of the amine; 30% octadecylamine resulted in cell death within 24 h, while 15% amine had no effect on cell number or protein content and the cells appeared healthy. Incubation of cells for periods of more than 48 h in the presence of positively charged sphingomyelin liposomes resulted in considerable cell death. When cells were exposed to liposomes containing 0.8 \( \mu \)mol of sphingomyelin and 0.12 \( \mu \)mol of amine for 24 h and were extracted with chloroform/methanol (2:1), about 2 to 3% of the radioactivity added to the medium was recovered in the phospholipid spots on thin layer
plates, mainly in sphingomyelin. Since the cells were treated with trypsin prior to extraction it was likely that most of the counts recovered from lipid that had been internalized (15, 16). When liposomes of sphingomyelin without any addition or with 2 to 30 mol % dicetyl phosphate to provide a negative charge were used, virtually no radioactivity was associated with lipids in the cell pellet.

Incorporation of phospholipid radioactivity using sphingomyelin with 15 mol % of octadecylamine was practically linear for 24 h (Fig. 1B). In this experiment, the culture dishes contained more cells (7.7 x 10^6, compared to 1.5 to 2.0 x 10^6 cells/dish in the experiment of Fig. 1A) and the recovery of radioactivity from the phospholipid spots on the silica gel plates was more than 5% of that added to the medium. The ratio of radioactivity in sphingomyelin to lecithin ranged between 5 and 10 in the two experiments, suggesting that some transfer or incorporation of the choline moiety of sphingomyelin to phosphatidylcholine occurred. Considerable intracellular granulation was observed in cells incubated for 24 h with positively charged sphingomyelin liposomes (Fig. 2).

To test whether a reversal of binding and/or internalization occurs, cells were incubated for 24 h in the presence of liposomes of sphingomyelin containing 15% octadecylamine. The cells were then washed 7 times with PBS-albumin buffer. After it was ascertained that the 7th wash had no radioactivity, fresh medium (containing 10% pooled and unfractionated human serum) was added. The medium was removed and fresh medium was added sequentially during the next 24 h. Radioactivity appeared in the medium at an initially rapid rate which gradually declined. An average total of 13% of the initial radioactivity in cells was recovered in the medium in contact with the fibroblasts during the 24 h. Virtually all the radioactivity in the phospholipids released into the medium was in the sphingomyelin fraction. These results suggest that the radioactivity released into the medium could have resulted from detachment of surface-bound liposomes. In a separate experiment, 14% of the total cell-associated radioactivity after 24 h of incubation with positive sphingomyelin liposomes at 37°C was releasable by trypsin confirming that most of the residual cell-associated sphingomyelin liposomes were internalized under these conditions. Approximately 97% of the radioactivity remaining within the cell migrated with sphingomyelin on thin layer chromatograms, while virtually none migrated with sphingosine. No water-soluble radioactivity (i.e., choline) was detected in either cells or medium.

**Binding of Low Density Lipoprotein**—The effect of incubation of cells in the presence of sphingomyelin liposomes was tested after normal fibroblasts were grown in a medium containing lipoprotein-deficient serum for 24 h. LDL binding was markedly reduced as a function of increasing concentration of positively charged liposomes (Fig. 3). When cells were incubated in the presence of liposomes containing 0.6 to 1.2 μmol of sphingomyelin and 0.09 to 0.18 μmol of octadecylamine, LDL binding decreased by 75 to 90%. When cholesterol was added to the medium containing lipoprotein-deficient serum, LDL binding was only 36% of that of the cells grown in the lipoprotein-free medium (Fig. 3). In addition, other cell lines showed similar decreases of LDL binding as a function of increased concentration of sphingomyelin liposomes. Experiments using liposomes of sphingomyelin without any additive or with 15 mol % of dicetyl phosphate (negatively charged) showed no reduction of LDL binding whatsoever.

**Mechanism of Effect of Sphingomyelin on LDL Binding**—Experiments were performed to test whether suppression of LDL binding by positively charged sphingomyelin liposomes involved intracellular mechanisms ultimately affecting the LDL receptor or, alternatively, a nonspecific blocking of surface receptors. Incubation of cells with positively charged sphingomyelin liposomes (1.0 μmol/dish) at 4°C for 4 h to minimize internalization failed to suppress LDL binding (+8%) in contrast to the marked reduction of LDL binding in parallel dishes incubated at 37°C (~76%). Since only ½ as much [3H]-labeled sphingomyelin was incorporated into cells at 4°C compared to that at 37°C, these results suggest that surface binding of sphingomyelin liposomes alone will not suppress LDL binding, but that internalization is required.

Suppression of LDL binding appears to depend on the length of time of incubation with the positively charged sphingomyelin liposomes since a maximal effect was not immediate. After 30 min at 37°C there was only about a 30% reduction in LDL binding, while after 4 h the decrease was more than 80%, reaching a maximum suppression of 90% by 10 h (Fig. 4).
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FIG. 2. Phase microscopy of cultured skin fibroblasts. Cultured skin fibroblasts were grown for 10 days in medium containing 10% fetal calf serum followed by 24 h in medium containing (A) LDS alone or (B) LDS with positively charged sphingomyelin liposomes containing 15% octadecylamine (1.0 μmol/dish). Arrows indicate large round refractile cellular inclusions in cells incubated with sphingomyelin liposomes. Magnification, × 2000.

FIG. 3. The effect of preincubation with positively charged sphingomyelin liposomes on \(^{125}\)I-LDL binding by cultured skin fibroblasts. Subconfluent monolayers of fibroblasts (4.8 × 10⁶ cells/dish) from a normal donor were incubated for 24 h at 37°C in medium containing 10% LDS and sphingomyelin liposomes containing 15% octadecylamine (0.1 to 3.0 μmol of sphingomyelin/dish). After extensive washing, \(^{125}\)I-LDL binding (2 h) was tested at 4°C by the dextran method. Control dishes were incubated with LDS alone or LDS plus cholesterol (10 ng/ml of medium) dissolved in a small amount of ethanol (marked X).

Exposure of normal cells, previously incubated with \(^{125}\)I-LDL for 1 h at 4°C to sphingomyelin liposomes for another hour at 4°C failed to displace bound LDL, further indicating that a nonspecific surface effect is unlikely. Positively charged sphingomyelin liposomes did not have any effect on LDL binding to receptor-negative cells from a patient with homozygous familial hypercholesterolemia.

Reversibility of the sphingomyelin-induced suppression of LDL binding was tested in two separate experiments by incubation of normal fibroblasts with sphingomyelin liposomes (1.0 or 1.5 μmol/dish) for 24 h, followed by a 24-h incubation with fresh LDS, which was changed once after 2 h. Cells incubated with the sphingomyelin liposomes in parallel for the full 48-h period produced a 76 to 84% reduction in LDL binding at 4°C compared to cells exposed for 48 h to LDS alone. The cells that had been exposed to sphingomyelin liposomes and then incubated with fresh LDS showed full recovery of LDL binding activity (105% of control).

To test if the effect on LDL binding is specific for sphingomyelin, liposomes of lecithin with 15% octadecylamine were prepared and added to medium containing LDS. Concentrations greater than 1.2 pmol/dish proved toxic to the cells. Therefore, comparison was made using 0.6 to 1.2 pmol of the two phospholipids. While LDL binding decreased 82 ± 9% (n = 4) after incubation with sphingomyelin, the comparable decrease produced by lecithin was only 43 ± 9%. In the one experiment in which 3 μmol of lecithin did not kill the cells, the reduction in LDL binding was 90% and 37% after preincubation with 3 μmol of sphingomyelin and lecithin, respectively. As with sphingomyelin, liposomes of lecithin with no additive or with 15 mol % dicetyl phosphate (negatively charged) had no effect on LDL binding. The reduction of LDL binding produced by several concentrations of mixed lipo-

FIG. 4. The effect of time of preincubation with sphingomyelin liposomes on LDL binding by cultured skin fibroblasts. Cells from a normal donor (6.8 × 10⁶ cells/dish) were incubated for 24 h in medium containing LDS. On the morning of the experiment, the medium was changed to 10% LDS alone or LDS plus sphingomyelin liposomes containing 15% octadecylamine (1.0 μmol of sphingomyelin/dish). After the indicated times of incubation at 37°C, the cell layer was washed extensively and \(^{125}\)I-LDL binding (2 h) was tested at 4°C by the dextran method. Results for LDL binding after preincubation with sphingomyelin are expressed as percentage of control LDL binding after incubation with LDS alone for the same period of time.
TABLE I
Incorporation of [1-14C]acetate into sterol

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Sphingomyelin</th>
<th>Liposomes</th>
<th>Time of preincubation</th>
<th>Sterol radioactivity</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol</td>
<td>h</td>
<td>cpm/mg cell protein</td>
<td>%</td>
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</tr>
<tr>
<td>1</td>
<td>0</td>
<td>24</td>
<td>2,340</td>
<td></td>
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<tr>
<td></td>
<td>1.5</td>
<td>24</td>
<td>7,710</td>
<td>+229%</td>
<td></td>
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<tr>
<td>2</td>
<td>0</td>
<td>48</td>
<td>5,570</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>48</td>
<td>50,840</td>
<td>+813%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0; 0</td>
<td>24; 24</td>
<td>18,360</td>
<td>-64%</td>
<td></td>
</tr>
</tbody>
</table>

Some cells containing equimolar amounts of sphingomyelin and lecithin with 15% octadecylamine was similar to that produced by liposomes containing the same amount of sphingomyelin as in the mixed liposomes (data not shown).

The specificity of the effect of sphingomyelin on LDL binding was tested using 13H-labeled concanavalin A. A reduction in concanavalin A binding at 37°C after 24-h incubation of normal cells in the presence of 2 µmol of positively charged sphingomyelin liposomes was negligible (11%).

To test the effect of added sphingomyelin on the cellular LDL pathway, cells were grown for 24 h at 37°C in the presence of 2 µmol of positively charged sphingomyelin liposomes. 125I-LDL in fresh LDS was then added after the incubation period had been washed several times with buffer and cells were further incubated at 37°C for 4 or 24 h. Relative to cells grown in LDS in the absence of sphingomyelin at 37°C, LDL binding at 4 h (trypsin-released radioactivity) decreased 48% and 53% in two cell lines, and 24-h LDL degradation decreased 50% and 59%. However, residual cell LDL protein radioactivity (for definition see “Materials and Methods”) at 24 h increased 100% and 140%. A similar experiment using receptor-negative cells showed no discernable effect of sphingomyelin liposomes on already minimal LDL binding, uptake, and degradation.

To ascertain that the positively charged sphingomyelin liposomes were not toxic to the cells, the fibroblasts were grown in the presence of the liposomes and albumin-bound [14C]palmitic acid was added. After 5 h cell lipids were extracted, fractionated, and separated on thin layer chromatography. Incorporation of 14C into the phospholipids (lecithin or sphingomyelin) was not decreased; in fact a 2-fold increase was observed in the presence of sphingomyelin in concentrations up to 3 µmol/dish. Furthermore, cells continued to proliferate at 37°C in contact with sphingomyelin liposomes (1.2 µmol) for 48 h. In separate experiments, marked enhancement of incorporation of 14C into cholesterol (3- to 9-fold increases) was observed after 24 to 48 h of incubation with positively charged sphingomyelin liposomes. This enhanced acetate incorporation into sterol was partially reversible after removal of sphingomyelin and 24-h incubation with fresh LDS (Table I).

**DISCUSSION**

Results in the present study suggest that introduction of sphingomyelin into cultured fibroblasts by incubation with sonically dispersed, positively charged sphingomyelin liposomes inhibit LDL receptors. It has been previously shown by several investigators that positively charged liposomes of lecithin (15, 17), sphingomyelin (18), or cholesterol ester (19) are taken up by a variety of cell types, while negatively charged or neutral liposomes are not. In the present study most of the sphingomyelin associated with cells after incubation at 37°C with positively charged liposomes appeared to be internalized as evident from the observed intracellular granulation and the fact that the majority of the radioactivity was in the cell pellet after trypsin treatment. A similar finding was obtained by Huang et al. (16) in studies of release of cell-associated lecithin vesicles from thymocytes by trypsinization.

In this study, intracellular localization of incorporated liposomes was not established, neither was the kind of uptake which could have occurred by membrane fusion, endocytosis, or other mechanisms (20). Incorporation of sphingomyelin into fibroblasts was time- and temperature-dependent and resulted in some conversion of the choline moiety to lecithin. Release of cell-associated sphingomyelin did occur suggesting either detachment of bound liposomes or reverse endocytosis, as has been observed previously with lipoproteins (7).

The accumulated intracellular sphingomyelin did not prove toxic to the cells provided that the octadecylamine concentration was limited to 15 mol % relative to the sphingomyelin and incubation periods with liposomes kept to less than 48 h. Under these conditions, the cultured fibroblasts continued to proliferate and metabolic integrity was maintained as evidenced by enhanced incorporation of fatty acid into phospholipids and acetate into sterols. For reasons not readily apparent positively charged lecithin liposomes appeared to damage cells at concentrations that were nontoxic with sphingomyelin.

Intracellular accumulation of sphingomyelin appears to suppress the binding of LDL to its cell surface receptor. When positively charged sphingomyelin liposomes were added to the medium, a rapid time-dependent fall in LDL receptor activity was observed in normal cells, but not in cells missing the LDL receptor. The possibility that residual surface-bound sphingomyelin (despite extensive washing of the cells) might compete with LDL and thereby inhibit its binding to the receptor is excluded by the experiments that show that (a) the effect was not observed in the cold when sphingomyelin was bound but poorly internalized, (b) suppression depended on time of exposure to sphingomyelin, (c) suppression was reversed in sphingomyelin-free medium, and (d) sphingomyelin failed to displace LDL once bound to the receptor. Although certain positively charged proteins can inhibit LDL binding, apparently by blocking negative charges on the receptor (21), it is doubtful that cell surface masking of receptors by sphingomyelin is responsible for the present observations. For example, protamine inhibits LDL binding when incubated together with LDL, but not if cells are exposed to LDL after protamine is washed off (21). In the present experiments, extensive washing was employed to remove unbound sphingomyelin liposomes prior to subsequent addition of 125I-LDL.

It is possible that an intracellular degradation product of sphingomyelin, rather than sphingomyelin itself, might be responsible for the observed effect on cellular LDL binding. However, the absence of detectable water-soluble radioactivity (which should contain any labeled choline freed from sphingomyelin), the retention of 97% of the cellular radioactivity as sphingomyelin, and the inability of albumin-bound long chain fatty acid to decrease cellular LDL binding (22) makes this possibility much less likely.

The effect appears to be relatively specific for both the type of phospholipid and the LDL receptor since lecithin was less effective in inhibiting LDL binding and concanavalin A cell surface binding was not affected by preincubation with sphingomyelin liposomes. Inhibition of LDL receptor binding there-
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fore appears to be a consequence of internalization and intracellular accumulation of sphingomyelin. This inhibition is readily reversing when sphingomyelin-loaded cells are incubated further in lipoprotein-deficient medium, additional evidence that a generalized toxic effect is not involved. LDL degradation is reduced in parallel with binding; however, increased cell accumulation of LDL protein was observed after incubation with sphingomyelin. Possibly lysosomal accumulation of sphingomyelin might have interfered with incorporation of LDL protein into lysosomes and their subsequent degradation.

By means of the cellular LDL pathway delineated by Brown and Goldstein (1), following binding of LDL to its specific high affinity cell surface receptor, LDL is internalized by endocytosis and, after being transported to the lysosome, undergoes hydrolysis of both lipid and protein moieties. The free cholesterol thus liberated from the intake of LDL regulates endogenous cellular cholesterol synthesis, cholesterol esterification, and synthesis of the LDL receptor itself. Thus an accumulation of free cholesterol in a putative intracellular regulatory pool results in a decrease in both cell cholesterol synthesis and LDL receptor activity. Intracellular accumulation of sphingomyelin could decrease LDL receptor activity by influencing this “regulatory pool” of free cholesterol. An association between sphingomyelin and cholesterol in normal cells is especially pronounced (2). Incubation of cells with sphingomyelin liposomes might be expected to remove some free cholesterol from cells. Furthermore, internalization of exogenous sphingomyelin might bind free cholesterol, thereby releasing the inhibitory regulation of cholesterol on its own biosynthesis. Indeed, after addition of positively charged sphingomyelin liposomes, cholesterol synthesis, as reflected by acetate incorporation into sterol, was greatly enhanced. Thus, it is possible that ingested sphingomyelin, by trapping intracellular cholesterol, changes the intracellular distribution of cholesterol, thereby independently releasing inhibition of the cholesterol synthetic pathway. However, despite the enhancement of cholesterol synthesis after incubation of cells with sphingomyelin, the activity of LDL receptors is reduced, which must be occurring by another mechanism. Additional experiments are necessary to define this mechanism in greater detail.

The precise role of sphingomyelin in the physiological regulation of the LDL receptor remains unclear. However, in view of the necessary role of sphingomyelin in membrane synthesis, and the potential ability of LDL to deliver sphingomyelin into the cell, these results suggest the possibility that this phospholipid participates in the mechanisms regulating both cholesterol biosynthesis and LDL receptors.

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REFERENCES