Supporting Information

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SI Materials and Methods

We obtained unlabeled cholesterol and 25-hydroxycholesterol (25-HC) from Steraloids; methyl-β-cyclodextrin (MCD) and 2-hydroxypropyl-β-cyclodextrin (HPCD) from Cyclodextrin Technologies Development; thioglycollate from BD Biosciences; *N*-acetyl-leucinal-leucinal-norleucinal (ALLN) from A.G. Scientific; *Staphylococcus aureus* sphingomyelinase, Optiprep Density Gradient Medium (60% iodixanol), and Sandoz 58-035 from Sigma; [26,27-^{3}H]25-HC (75–80 Ci/mmol), [1,2,6,7-^{3}H]cholesterol (60 Ci/mmol), [^{3}H]acetate (150 mCi/mmol), and [1-^{14}C]oleic acid (54.6 mCi/mmol) from American Radiolabeled Chemicals (1 Ci = 37 GBq); and Hybond C nitrocellulose filters from GE Healthcare Biosciences. Human LDL (d = 1.019–1.063 g/mL) (1), rabbit β-migrating very low density lipoprotein ($d <$ 1.006) (2), and human and newborn calf lipoprotein-deficient serum ($d <$ 1.215 g/mL) (1) were prepared by ultracentrifugation as described in the indicated reference. Complexes of cholesterol with MCD were prepared at a stock concentration of 2.5 mM (3). Solutions of compactin and sodium mevalonate were prepared as described in ref. 4.

Radiolabeling of Cultured Cells. Monolayers of macrophages or fibroblasts were radiolabeled with either [^{3}H]cholesterol or [^{3}H]acetate, respectively, as described in the figure legends. The radiolabeled lipids were extracted from cells and separated by TLC, and the bands corresponding to [^{3}H]cholesterol and [^{3}H]cholesteryl ester were quantified by scintillation counting (4).

Acyl-CoA Acyltransferase Assay. The rate of incorporation of sodium [^{14}C]oleate-albumin into cholesteryl [^{14}C]oleate by monolayers of fibroblasts was measured as described in ref. 1.

SREBP-2 Processing in Cultured Cells. Monolayers of fibroblasts were tested for proteolytic processing of SREBP-2 as described in ref. 5.

Preparation of Reconstituted LDL. Low-density lipoprotein was extracted with heptane and reconstituted with 25-HC oleate as described in ref. 6. 25-Hydroxycholesterol oleate was synthesized from *cis*-9-octadecenoic acid (Grace Davison Discovery Sciences) and 25-HC in the laboratory of Jef De Brabander (University of Texas Southwestern Medical Center, Dallas, TX). The product was isolated on a silica gel column, and its purity was verified by TLC.

Isolation of Purified Endoplasmic Reticulum Membranes. Purified endoplasmic reticulum (ER) membranes were isolated from human fibroblasts by a fractionation scheme described for CHO cells by Radhakrishnan et al. (7). Briefly, cells were disrupted by using a ball-bearing homogenizer and centrifuged at 3,000 $\times$ g. The supernatant was loaded at the top of a discontinuous sucrose gradient and centrifuged at 100,000 $\times$ g at 4 °C for 1 h, yielding two distinct membrane layers. The heavy membrane fraction was loaded below a continuous 19–25% iodixanol gradient and centrifuged for 2 h at 110,000 $\times$ g at 4 °C, after which fractions were collected from the bottom.

Enzyme Assays. The enzyme assay for acid phosphatase (lysosomal marker) was carried out by using an enzyme assay kit (Sigma) according to the manufacturer's instructions. Glucose-6-phosphatase activity (ER marker) was measured as described in ref. 8.

Immunoblot Analysis. Nuclear extract and membrane fractions from cultured cells were subjected to 8% SDS/PAGE, after which the proteins were transferred to nitrocellulose filters. The immunoblots were performed at room temperature by using the following primary antibodies: 5 μg/mL rabbit polyclonal antibody (IgG-1819) directed against amino acids 1–100 of human SREBP-2 (9); 1 μg/mL rabbit polyclonal antibody against human NPC1 (Novus Biologicals); 8 μg/mL rabbit polyclonal antibody (IgG-R139) directed against hamster Scap (10); 1 μg/mL rabbit polyclonal antibody directed against Sec61α (Millipore); 0.5 μg/mL mouse monoclonal antibody directed against transferrin receptor (Invitrogen); and 0.2 μg/mL mouse monoclonal antibody directed against Lamp-1 (Affinity BioReagents). Bound antibodies were visualized by chemiluminescence (SuperSignal West Pico Chemiluminescent Substrate, Thermo Scientific) using a 1:5,000 dilution of anti-mouse IgG (Jackson ImmunoResearch) or a 1:2,000 dilution of anti-rabbit IgG conjugated to HRP (Amersham Biosciences). The filters were exposed to Kodak X-Omat Blue XB-1 film at room temperature for 1–60 s.

Protein Measurements. Protein content for immunoblot analysis was measured with a bicinchoninic acid kit (Pierce). Other protein-content measurements were made by the method of Lowry et al. (11).

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Table S1. Cholesteryl ester formation in control and NPC1 mutant human fibroblasts: Inhibition by acyl-CoA acyltransferase (ACAT) inhibitor Sandoz 58-035

	Cholesteryl [^{14}C]oleate formed (nmol/h per milligram of protein)	
Cell line	−58-035	+58-035
Control	4.8	0.09
NPC1	22.1	0.10

On day 0, nontransformed control and NPC1 mutant fibroblasts were set up in medium A with 10% FCS and 2×10^4 cells per 60-mm dish. On day 3, cells were refed with the same medium. On day 6, cells were incubated with medium A with 10% FCS and 20 μg of protein per milliliter of LDL. After 16 h, each dish received 1.5 mL of medium A containing 10% FCS in the absence or presence of 20 μM ACAT inhibitor Sandoz 58-035. After 30 min at 37 °C, each dish received a direct addition of 0.5 mL of medium A containing 10% FCS and 0.1% 2-hydroxypropyl-β-cyclodextrin. After incubation for 4 h, each monolayer was pulse-labeled for 2 h with 0.2 mM sodium [^{14}C]oleate-albumin (7,668 dpm/nmol). The cells were then harvested for measurement of cholesteryl [^{14}C]oleate. Each value is the average of duplicate incubations.