Supplementary Materials and Methods

Lipid and lipoprotein analysis. Fasting lipid profiles were measured by routine commercial assays with the LDL-C calculated using the Friedewald equation (1). Approximately one ml of plasma was frozen at -80^{o}C and then sent on dry ice to Liposcience (Raleigh, NC) for lipid profile and subfraction analysis. Plasma apolipoproteins (apoA-I, apoA-II, apoB, apoC-I, apoC-II, apoC-III, and apoE) were measured by immunoaffinity chromatography (Oklahoma Medical Research Foundation, OK). Hepatic lipase (HL), endothelial lipase (EL), lipoprotein lipase (LPL) phospholipase activities were measured from post heparin plasma according to the methods of McCoy et al. (2). EL, HL and LPL triglyceride lipase activity was measured using a glycerol-stabilized emulsion of triolein and egg phosphatidylcholine containing glycerol-tri[9,10(n)-^{3}H]oleate. Cholesteryl ester transfer protein (CETP) and lecithin cholesteryl acyltransferase (LCAT) activities were measured by an enzymatic method as described previously (3).

Cell Culture. *Human monocyte-derived macrophages*: peripheral blood mononuclear cells were isolated from buffy coats by Ficoll density gradient (4). After centrifugation at 2000*g* for 20 minutes at 10^{o}C, the lymphocytes were harvested from the Ficoll-plasma interface. Cells were then plated in RPMI medium containing 20% human serum and monocyte-derived macrophages (MDMs) were cultured by adherence to the tissue culture plates. After one day, nonadherent cells were removed and the monolayer washed four times with 10% human serum. Cells were then incubated with fresh 20% human serum for a total of 10-14 days. We previously showed that SR-BI protein expression is low in human monocytes but levels increase substantially by day 4 of culture and remain stable past day 14 of culture (5). *Establishment of murine RAW264.7 macrophage cell lines stably expressing human wild-type SR-BI and G2S variant:* RAW264.7 macrophages were transfected with pCDNA3.1-wild-type SR-BI or the rs4238001 [G2S] variant using FuGENE reagent (Roche) according to manufacturer instructions. Prior to transfection, plasmids were linearized at a unique *Mfe*I restriction site and purified using a QIAquick gel extraction kit (Qiagen). Selection in geneticin and isolation of individual cell lines was done according to manufacturer instructions for use of pCDNA3.1 (Invitrogen). For each clone stably expressing SR-BI, we performed western blotting followed by densitometry to determine SR-BI protein level normalized to β-actin. There was a normal distribution of protein levels among clones expressing wild-type (10 clones) and G2S (22 clones) variant SR-BI. Two clones expressing the highest levels of SR-BI for each genotype were pooled in a 1:1 ratio based on cell counting.

Construction of pCDNA3.1-wild-type SR-BI and G2S variant. Oligonucleotides used for amplification of human *SCARB1* cDNA included 5'-GAT ACA ATT GAT GGG CTG CTC CGC CAA A-3' and 5'-GCG CTC TAG ACT ACA GTT TTG CTT CCT G-3'. PCR reactions using *Pfu* polymerase (Stratagene) were run according to manufacturer instructions. The reaction volume was 50 µl, and the template used was 1 ng of pSG5-SR-BI, which contains wild-type human *SCARB1* cDNA. Thermocycler conditions were as follows: initial melting at 95°C for 2 minutes, then 35 cycles of amplification using 95°C for 30 seconds, 58°C for 1 minute, and 68°C for 2 minutes. This was followed by a final extension step at 68°C for 5 minutes and dwell at 4°C. The reaction yielded a single PCR product of the expected size by gel electrophoresis. The product was cloned into pCR4Blunt-TOPO (Invitrogen) according to manufacturer instructions. The product was then digested using *Mfe*I and *Xba*I (New England Biolabs) according to manufacturer instructions and subcloned into the *Eco*RI and *Xba*I sites of pCDNA3.1 (Invitrogen) to create pCDNA3.1-wild-type SR-BI. The wild-type *SCARB1* cDNA sequence was verified by DNA sequencing. The G2S substitution mutation was constructed using the QuikChange mutagenesis kit (Stratagene) according to manufacturer protocols and verified by DNA sequencing. Mutagenic oligonucleotides included 5'-CTG GAA TTG ATG AGC TGC TCC GCC AAA-3' as the forward primer and 5'-TTT GGC GGA GCA GCT CAT CAA TTC CAG-3' as the reverse complement primer.

Transfection of cells. COS-7 cells were grown in 175 cm^{2} and maintained in DMEM with 10% heat-inactivated fetal bovine serum (FBS), 100 units/ml penicillin, 100 µg/ml streptomycin at 37˚C in 95% air / 5% CO_2. One day prior to the transfection of 150 ng pSG5-wild-type or G2S-SR-BI and 100 ng pSV-

βgal using FuGENE6 reagent Costar 6-well plates were seeded with $5*10^5$ cells per well. The total amount of transfected DNA was increased to 1 μg using pSG5 empty vector. Transfection efficiency was determined by measuring β-galactosidase expression. Twenty four hours after transfection, the cells were rinsed once with PBS and cells lysates collected to determine cellular SR-BI protein expression by western blotting and visualized bands were normalized to β-actin expression.

***In vitro* transcription and translation of human wild-type SR-BI and G2S variant.** The PROTEINscript II T7 kit was purchased from Ambion (#AM1281) and used according to manufacturer instructions. Approximately 225 ng of plasmid DNA (pCDNA3.1-wild-type SR-BI or G2S variant) served as template. Empty pCDNA3.1 vector was used as a negative control. Three independent experiments were performed. Transcription products were used for both gel electrophoresis to compare transcript quantities and subsequent *in vitro* translation using rabbit reticulocyte lysate according to manufacturer instructions. *In vitro* translated protein products were resolved on a 10% SDS-polyacrylamide gel which was then dried and exposed overnight at -80°C using a KODAK BioMax Transcreen (LE) intensifying screen.

SR-BI protein turnover experiments. RAW264.7 macrophages stably expressing wild-type SR-BI or the G2S variant were established as described. Approximately $1.5x10^5$ cells were seeded into triplicate wells of 12-well tissue culture plates. Media contained DMEM with 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 250 μg/mL geneticin (Invitrogen). On the day after seeding, media was changed to include 140 μg/mL cycloheximide (Sigma-Aldrich). Experiments were terminated at varying time points (0, 2, 4 and 7 hours) by rinsing cells twice in phosphate buffered saline, pH 7.4 and lyzing them in 100 μl of lysis buffer containing 50 mM Tris-HCl, pH 7.6, 5% SDS, protease inhibitor cocktail (1:100) (P8340, Sigma) and phenylmethylsulfonylfluoride (PMSF), followed by sonication. Protein concentrations were determined in triplicate samples using the BCA protein assay (Pierce) according to manufacturer instructions. Western blots were performed as described below. The experiments were performed three times and the results were pooled.

Western blotting. Total cell lysates from cells were prepared using 5% SDS, 50 mM Tris-Cl, pH 7.6 buffer in the presence of protease inhibitor cocktail and PMSF (1 mg/ml). Aliquots of the lysates (8 μg protein/lane) were subjected to SDS-PAGE in 4-10% gradient gels and then transferred onto Immobilon transfer membranes (Millipore) at 4°C. We had previously performed optimization assays to determine the linear range for the SR-BI protein concentration. Blots were blocked with 5% milk for 1 h, incubated with polyclonal anti-SR-BI (Novus) (1:1000) at 37°C for 1 h, rinsed three times with TBS-0.1% Tween plus 5% milk, reacted with anti-rabbit HR-peroxidase labeled IgG at room temperature for an additional hour, and then rinsed three more times with TBS-0.1% Tween. Bands were visualized using an Amersham ECL ™ chemiluminescence kit (GE Healthcare), quantitated by densitometric scanning and normalized to β-actin expression. .

Cholesteryl ester (CE) uptake and cell association assays. *CE uptake assays*: HDL isolated from the study subjects was first radiolabeled with [1,2-^{3}H]cholesteryl oleyl ether. In brief, 50 μCi of [1α,2α(n)-^{3}H]cholesteryl oleyl ether (Amersham Inc.) was dried under N_2 and then redissolved in 100 μl of dimethylsulfoxide (6). HDL (1 mg protein) was then added to the solution and allowed to incubate for 2 h at 40°C. At the end of the incubation period, the HDL preparations were dialyzed in 0.15M NaCl, 0.3 mM EDTA for 4 exchanges (4L each) at 4°C. We have previously shown that the biochemical characteristics of radiolabeled HDL (electrophoretic pattern and lipid content) were similar to control, unlabeled HDL. MDMs from the study subjects were incubated with HDL (50 μg protein/ml) radiolabeled with [^{3}H]cholesteryl oleyl ether for 24 h**.** At the end of the incubation period, the medium was collected and centrifuged at 1500 rpm for 10 minutes to pellet nonadherent cells. Cellular lipids were extracted with hexane:isopropanol (3:2,v/v) (7), the fractions were dried under N_2 and then subsequently redissolved in 1 ml of hexane. An aliquot of the medium and the cell lipid extract was counted for the presence of [^{3}H]cholesterol using liquid scintillation spectroscopy, and the CE uptake from HDL was calculated as (cellular [^{3}H]cholesteryl oleyl ether/[medium [^{3}H]cholesteryl oleyl ether + cellular [^{3}H]cholesteryl oleyl ether]) x 100%. We did not radiolabel the apoA-I moieties with ^{125}-I as we did not

have sufficient time between isolation of HDL and monocyte-macrophage culture to perform radioiodine labeling of HDL from each donor. *Degradation and cell association assays:* We examined degradation and cell association of HDL in the stably expressing wild-type and G2S RAW macrophages. ^{125}I-radiolabeled HDL was prepared using the method of McFarlane (8) as previously described (9). Unbound ^{125}I was removed by dialysis against four exchanges of 0.15 M NaCl containing 0.05% Na_2EDTA, pH 7.5. The ^{125}I radiolabeled HDL was then radiolabeled a second time with [^{3}H]cholesteryl oleyl ether as described above, and then subjected again to multiple dialysis exchanges of 0.15 M NaCl containing 0.05% Na_2EDTA, pH 7.5, before use in cell experiments. Stably expressing RAW macrophages were cultured in DMEM and 10% serum for approximately 24 hours and then the culture medium was aspirated and the cells rinsed four times with serum free medium. Cells were then incubated for 4 h with DMEM and 1% serum, after which the medium was aspirated and the cells were rinsed at least four times with serum free medium. Triplicate wells were incubated with [^{125}I,^{3}H]-labeled HDL (5 μg protein/ml) in the presence or absence of excess unlabeled HDL (250 μg protein/ml) for 16 h at 37^{o}C. Experiments were terminated by collecting the media from each well and then subjecting it to centrifugation to pellet non-adherent cells. For the degradation assay, an aliquot of the media was subjected to 10% trichloroacetic acid for protein precipitation, and then an aliquot of the supernatant was subjected to ^{125}I gamma counting. For the cell association assay, the cell monolayer was rinsed four times with phosphate buffered saline-bovine serum albumin (0.5%) and then 1.0 ml of 1 N NaOH was used to lyse the cells. The cell lysates were dried overnight at room temperature and then were redissolved in 1.0 ml of dH_20. An aliquot of the cell lysates were used for scintillation spectroscopy (^{3}H measurement) or gamma counting (^{125}I measurement) and for protein measurement by BCA. Specific cell association was determined as the difference between cell association in the absence of unlabeled HDL and cell association in the presence of excess unlabeled HDL.

***SCARB1* sequencing.** The entire coding DNA (exons 1-12), the immediately adjacent exon-intron junctions, 1 kB upstream putative promoter, 3'- and 5'-UTR regions were characterized by direct sequencing in both directions of PCR products. Fourteen pairs of primers were synthesized in order to amplify each of the exonic regions and intronic borders of the *SCARB1* gene. Sequence comparisons were determined using the Sequencher Program v.4.0 (Gene Code). The primer sequences and PCR conditions are available from the authors upon request.

References for supplementary Materials and Methods

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