Supplemental Methods

Animals and treatment

Mice were anesthetized in the fed state by an intraperitoneal injection of 300 µL of an anesthetic standard solution (4% xylazine, 16% ketamine, 80% NaCl 9 ‰). The depth of the anesthesia was checked by the absence of reflex of withdrawal following the pinching of the foot pads: the procedure was continued only when the animal was under deep narcosis. A needle was then inserted under the sternal plexus to reach the left ventricle. A volume of 1-2 mL was taken on average causing the death of the animal by exsanguination and collected in heparined tubes.

Serum Preparation

For patients, blood samples were immediately centrifuged for serum/plasma separation, and small aliquots were stored at – 80°C until biological analysis.

Routine laboratory analyses

In humans, glucose, total cholesterol (TC), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), triglycerides (TG), proteins and apolipoprotein AI (ApoAI) were determined on a Dimension Vista analyzer using dedicated reagents (Siemens, Saint-Denis, France).

In mice, the same laboratory analyses were used, except for ApoAI, which were measured with a sandwich enzyme immunoassay kit (Cloud-Clone Corp, TX, USA).

Isolation and characterization of HDL particles

The total HDL fraction (density=1.063-1.210g/mL) was isolated from 300 uL of serum by sequential ultracentrifugation at 4°C using a 50.4 rotor in an Optima L80-XP ultracentrifuge (Beckman Coulter). After ultracentrifugation at 50,000 rpm for 8h (density adjusted at 1.063 g/mL with KBr), the apolipoprotein B-containing fraction was withdrawn. Then, after a second ultracentrifugation at 50,000 rpm for 14h (density=1.210g/mL), the HDL fraction (300 µL) was collected.
HDL₂ and HDL₃ subfractions were isolated from 300 µL of plasma by sequential ultracentrifugation at 4°C using a 120.2 rotor in a TL-100 ultracentrifuge (Beckman Coulter) as previously described.²⁰

TC, TG, proteins and apoAI were measured in HDL fraction as described above for serum. Free cholesterol and phospholipids were measured in HDL on a Vista analyzer with Diasys reagents (Condom, France). Cholesteryl ester (CE) mass was calculated as (TC-free cholesterol)x1.67. TC was measured in HDL₂ and HDL₃ subfractions as described above for serum.

**Preparation of the labelling medium**

Labeling medium was prepared extemporaneously. A 20 mM methyl-beta-cyclodextrin solution was prepared in RPMI1640 glutamax, 10 mM HEPES with ACAT inhibitor (2 µg/ml). Cholesterol (100 mM) and Bopidy cholesterol (25 mM) solutions were prepared in chloroform. 500 nmoles of bodipy cholesterol and 2 µmoles of unlabeled cholesterol were dried under nitrogen in the dark in a round-bottom glass tube and resuspended with 10 ml of the methyl-beta-cyclodextrin solution. Solution was sonicated for 4 min. and filtered. A loading solution without fluorescent cholesterol was prepared in parallel.

**Materials and chemicals of lipidomic analysis**

Internal standards [dimyristoylphosphatidylcholine (DMPC), lysomyristoylphosphatidylcholine (LMPC), dimyristoylphosphatidylethanolamine (DMPE), d18:1/17:0 ceramide and 17:0 sphingosine 1-P(S1P)] and external standards [17:0/17:0 phosphatidylcholine (PC), d18:1/17:0 sphingomyelin (SM), 19:0 lysophosphatidylcholine (LPC), 18:0/20:4 phosphatidylinositol (PI), 17:0/17:0 phosphatidylethanolamine (PE), 18:0/18:1 PC-based plasmalogens (PC-p), 18:0/18:1 PE-based plasmalogens (PE-p), 20:0 ceramide (Cer), 18:0 Cer, 16:0 Cer and 18:1 S1P] (Avanti Polar Lipids, Alabaster, USA) were certified with a purity higher than 99% and were reconstituted if necessary according to the manufacturer's instructions. Chloroform, methanol, isopropanol, water (Fisher Chemical, Zurich, Switzerland) and ethyl acetate (VWR) were LC-MS grade quality. Acetic acid (Fisher Chemical), formic acid (Merck, Fontenay sous Bois, France), phosphate buffer saline (PBS, Lonza, Basel, Switzerland), ammonium acetate, ammonium formate and sodium chloride (Sigma Aldrich, St-Louis, USA) were also purchased for the lipid analysis.
Lipid extraction

Glycerophospholipids and sphingophospholipids were extracted from HDL according to a modified Folch’ procedure, as previously described. Sphingosine-1-Phosphate (S1P) and ceramides (Cer) were extracted from HDL as previously described. All reconstituted extracts were finally transferred to liquid chromatography auto-sampler screw-cap vials and stored at –20°C until analysis by tandem mass spectrometry.

Lipidomic (phospholipid and sphingolipid) analysis

Identification and quantitation of glycerophospholipids and sphingolipids in total lipid extracts from HDL fractions were performed by liquid chromatography tandem mass spectrometry, using a 6460 mass spectrometer (Agilent Technologies, Santa Clara, USA) equipped with an electrospray ionization source and MassHunter data system. For PC, SM, LPC, PE, LPE, pPE analysis, the liquid chromatography was performed on a Zorbax Eclipse plus C18 column (1.8 µ, 2.1 x 100 mm, Agilent) maintained at 55°C with a flow-rate at 0.25 mL/min using the following gradient conditions: 1 min at 60% mobile phase A, 60% to 5% A over 14 min, 1 min at 5% A, a return to 60% A over 1 min then 5 min at 60% A prior to the next injection. Mobile phases A and B consisted of water:methanol and isopropanol:methanol in the ratios 60:40 and 90:10 (v/v), respectively, both containing 10 mM ammonium acetate and 1 mM acetic acid. For Cer and S1Ps analysis, the liquid chromatography was performed on a Poroshell 120 EC-C8 column (2.7 µ, 2.1 x 100 mm, Agilent) maintained at 30°C with a flow-rate of 0.3 mL/min using the following gradient conditions: 1 min at 70% B, 70% to 100% B over 4 min, 5 min at 100% B, a return to 70% B over 1 min then 3 min at 70% B prior to the next injection. Mobile phases A and B consisted of 1 mM ammonium formate and 0.2% formic acid (v/v) in water and methanol, respectively.

Specific individual lipid species were identified using scheduled multiple-reaction monitoring (MRM) in the positive or negative ion mode. PC, SM, LPC species were identified by monitoring the ion production of the choline head group m/z=184 in the positive mode, whereas PE species were identified by monitoring the neutral loss of the phosphoethanolamine head group (m/z 141) in the positive mode.

Quantitative analyses were based on seven-point calibration curves generated from mixtures of external standards at concentrations surrounding those obtained with subjects’ samples. All ceramide species with a lateral chain containing at least 20 carbons were quantified using the d18:1/20:0 standard. The concentrations of phospholipid and sphingolipid species were calculated by relating the peak area of each species to the peak area of
the corresponding internal standard. Total lipids of each class were calculated by summing the individual lipid species.

**RNA extraction and RT-qPCR analysis**

Total RNA from tissues were isolated by Qiazol extraction and purification using Qiagen RNeasy minicolumns according to the manufacturer’s instructions (Qiagen, Courtaboeuf, France). For quantitative real-time PCR analysis, 1 μg of total RNA was reverse-transcribed using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA). SYBR Green reactions using the SYBR Green PCR Master mix (Applied Biosystems) were assembled along with 0.5 μM primers according to the manufacturer’s instructions and were performed using a Light Cycler system (Roche Diagnostics). Relative expression of mRNAs was determined after normalization to the reference genes, 36B4 and HPRT. The primers were designed with Primer3 Plus software and synthesized by Invitrogen (Life Technologies, Saint Aubin, France). Their sequences are available upon request.

**Preparation of extracts and immunoblot analysis**

Total liver extracts were solubilized in a lysis buffer containing 50 mM Tris–HCl (pH 8), 150 mM NaCl, 1% Triton-X-100, and a cOmplete™ Mini Protease Inhibitor Cocktail (Roche, Basel, Switzerland). After 15 min on ice, extracts were cleared by a 15,000 × g centrifugation at 4 °C for 15 min. Proteins (40 μg) were subjected to SDS–PAGE analysis on 7.5 % gels and transferred to nitrocellulose membranes. SRB1 protein was detected using a rabbit polyclonal antibody (#NB400-101, Novus Biologicals) and α-Tubulin was detected using a monoclonal antibody (#T5168, SIGMA-Aldrich, St. Quentin Fallavier, France). The immunoreactive bands were revealed using the ECL detection kit (Pierce ECL Western Blotting substrate, Rockford, IL USA) with the iBright Western blot imaging system (Thermo Fisher Scientific, Les Ulis, France). Quantification was performed using an imageJ program (Chemi Genius2 scan, GeneSnap; Syngene, Cambridge, UK).
In vivo kinetic of HDL

HDL fluorescent labelling (Bodipy) was performed essentially according to the technique described earlier. Briefly, human HDLs (2 mg of protein) were incubated for 4 hours at 37°C in bicarbonate buffer in the presence of 565 µg of Bodipy probe. Bicarbonate buffer and unbound label were then removed and lipoproteins were recovered in sterile PBS by filtration in centrifugal concentration tubes (cutoff, 30 kDa; Centricon, Merck-Millipore). For in vivo kinetics, HDLs (4 µg cholesterol per mouse) were injected intravenously and blood samples were drawn at indicated times. Fluorescence in plasma, reflecting the amount of labelled HDL, was measured with Tecan at indicated time (0, 5, 30, 60, 120, 360, 480 and 1440 min).

Supplemental Results

Does phospholipid and sphingolipid profile influence HDL-mediated cholesterol efflux?

The proportion of the different phospholipid and sphingolipid classes relative to total phospholipids and sphingolipids on the surface of HDL particles is summarized in supplemental table 1. As these proportions were not different in patients before and after GC treatment, we have not evaluated whether HDL-mediated cholesterol efflux was influenced by the proportion of the different phospholipid and sphingolipid classes.

To be exhaustive in our analysis, we assessed if there were differences in the phospholipid and sphingolipid distribution. The distribution of phospholipid and sphingolipid species is presented in supplemental figure 1. The distribution of several species in patients after GC treatment was significantly different from that in patients before GC treatment. We assessed if the distribution of the species which were modified, might be correlated with HDL-mediated cholesterol efflux (Supplemental table 2). HDL-mediated cholesterol efflux correlated positively with the proportion of SM18:1/24:1 (p=0.048) and Cer18:1/20:0 (p=0.015) and negatively with LPC18:2 (p=0.032), and Cer18:2/23:0 (p=0.028). CRP correlated positively with the proportion of SM18:1/24:1 (p=0.001) and Cer18:1/20:0 (p=0.006) and negatively with LPC18:2 (p=0.007), and Cer18:2/23:0 (p=0.004). In a multivariate analysis, HDL-mediated cholesterol efflux was neither associated with the 4 phospholipid and sphingolipid species which were modified after GC treatment nor with CRP.