Supplementary Information:

Detailed Materials and Methods

Materials:

Apolipoprotein A-I (ApoA-I) was purified from human serum as previously described.[1] 22A peptide (PVLDLFRELLNELLEALKQKLK) was synthesized by GenScript Biotech (Piscataway, NJ). 5A (DWLKAFYDKVAEKLKEAF-P-DWAKAAYDKAAEKAKEAA) and 18A (Ac-DWLKAFYDKVAEKLKEAF-NH_2) were synthesized by Bachem Americas Inc. (Torrance, CA). 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and sphingomyelin (SM) were purchased from NOF America Corporation (White Plains, NY). Sphingomyelin [choline methyl-3H] was purchased from American Radiolabeled Chemicals (Saint Louis, MO). All other reagents were analytical grade and obtained from commercial suppliers.

Sphingomyelin Solubilization Assay:

The solubilization of sphingomyelin was measured based on our previously established protocol.[2] Sphingomyelin lipid vesicles were prepared by adding 10 mg of egg-sphingomyelin to 1mL of PBS and sonicating for 2 minutes at 3 watts using a Vibra-Cell probe sonicator (Sonics, Newtown, CT). 50 μL of the resulting suspension was added to each well of a 96-well plate followed by 50 μL of different apolipoprotein mimetic peptides. The final mass ratio of peptide:sphingomyelin was 1:1 in each well. The reduction in turbidity was determined via UV absorbance at 600 nm measured every 5 minutes over the course of 2 hours on a SpectraMax M3 plate reader (Molecular Devices, San Jose, CA).

Preparation and Characterization of Synthetic High-Density Lipoprotein Nanodiscs (sHDLs): sHDLs composed of the phospholipid, DMPC, and the apolipoprotein mimetic peptide, 22A, were synthesized by preparing a suspension of DMPC in PBS and adding an equal volume of 22A dissolved in PBS (final peptide:lipid mass ratio of 2:1). The resulting mixture was then vortexed and subjected to 3 rounds of heating at 50°C and cooling on ice for 5 minutes each until a clear solution was obtained. The sHDL solution was then passed through a 0.22μM syringe filter, and the particle size was measured via dynamic light scattering on a Zetasizer Nano ZSP (Malvern Panalytical, Westborough, MA). The purity of the sHDL was determined using size-exclusion chromatography on a Tosoh TSK gel G3000SWxl column (Tosoh Bioscience, King of Prussia, PA) with UV detection at 220 nm using PBS as the mobile phase with an isocratic flow rate of 1 mL/min on a Waters Breeze Dual Pump system (Waters, Milford, MA).

Negative Stain Transmission Electron Microscopy (TEM):
The morphology of sHDL nanodiscs were determined using TEM as previously described.[3] sHDL samples (4 μL of 10 μg/ml) were adsorbed for 1 min to a glow discharged 400-mesh copper grid covered with formvar/carbon-coated film (Electron Microscopy Sciences, Hatfield, PA). The grids were then negatively stained in 0.1% uranyl acetate. Images were obtained on an FEI Morgagni electron microscope run at 100 kV at a magnification of 22,000X (2.1 Å/pixel) and then recorded on a Gatan Orius charge-coupled device camera.

Radiolabeled Sphingomyelin Efflux Assay:

Quantification of the removal of sphingomyelin from ASMD skin fibroblasts was performed as previously described.[3] Cells (40,000/well) were cultured overnight in 24-well plates and then incubated with 1 µCi/mL sphingomyelin [choline methyl-3H]. After 24 hours, the cells were washed with PBS and treated with apolipoprotein mimetic peptides or sHDL nanodiscs at 10µM and 100µM based on previous efflux experiments for 24 hours.[3-5] Radioactivity in media and cells was counted using a PerkinElmer scintillation counter (PerkinElmer, Waltham, MA). Percent sphingomyelin effluxed from cells was calculated by dividing media counts by the total sum of media and cell counts and then multiplying by 100. Non-specific sphingomyelin efflux in untreated cells was subtracted from all treatment groups.

Cytotoxicity:

Cell viability was assessed using CellTiter 96 Aqueous One Solution (Promega, Madison, WI). Human ASMD skin fibroblasts were cultured in 96-well plates at 10,000 cells per well for 24 hours, washed with PBS, and treated with apolipoprotein mimetic peptides or sHDL nanodiscs at concentrations up to 500µM for 24 hours based on peptide plasma concentrations from unpublished pharmacokinetic experiments in mice. CellTiter 96 reagent was then added to each well (20 µL reagent per 100 µL of media), and the plate was incubated for 1 hour at 37 °C. Absorbance was read at 490 nm, and the average absorbance reading of untreated cells was set to 100%. The percent

viability was determined by dividing the average absorbance of treated over non-treated cells and multiplying by 100.

Mice:

Smpd1-/+[6] mice were maintained on the C57BL/6J background and intercrossed to generate *Smpd1-/-* mice and littermate wild type (WT) controls. Approximately equal numbers of males and females were used for all experiments. All procedures involving mice were approved by the University of Michigan Committee on Use and Care of Animals (PRO00008133) and conducted in accordance with institutional and federal guidelines.

Single-Dose Treatment with sHDL Nanodiscs:

Adult WT (n=4) or *Smpd1-/-* (n=3) mice were administered a single dose of sHDL at 100 mg/kg via intraperitoneal (IP) injection.

Mobilization of Sphingomyelin In Vivo:

Serum from each mouse was collected before treatment and 2, 6, and 24 hours after single-dose administration of sHDL. The amount of sphingomyelin in the serum was determined using an enzyme-based colorimetric plate assay (Cell Biolabs Inc., San Diego, CA) according to the manufacturer’s instructions.

Lipoprotein Profile:

Serum samples from WT or ASMD mice were pooled, and 50μL of serum was injected into a Waters HPLC system equipped with a Superose 6 Increase 10/300 GL column (GE Healthcare, Piscataway, NJ). The mobile phase consisted of 0.9% NaCl and 0.02% NaN_3 in water, and a flow rate of 0.6 mL/minute was used to separate lipoproteins with UV detection and 220 nm and 265 nm.

Repeated Dosing with sHDL Nanodiscs:

Smpd1-/- mice were randomly assigned to treatment or vehicle (saline) groups. Mice were administered 100 mg/kg 22A-DMPC sHDL or saline (both *Smpd1-/-* and WT) daily by I.P. administration for 4 weeks starting at 6 weeks of age.

Serum Analysis for Liver Function:

Whole blood was collected from mice at the end of treatment and allowed to clot for 5 min in BD microtainer® SST gold cap tubes (365967). Tubes were centrifuged for 5 min at 3000×*g* at 4ºC to remove the clot. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALKP) levels were analyzed by the University of Michigan In-Vivo Animal Core.

Balance Beam:

The balance beam consists of 5mm wide square beam suspended at 50cm. Mice were trained at 5 weeks of age to cross the beam and then tested every other week starting at 6 weeks, when mice were enrolled in treatment trial. For testing, mice were run 3

times across the beam, and the average time was taken. Maximum time was set at 20 sec and falls were scored as 20 sec.

Statistics:

Treatments were compared using ANOVA with a Tukey's test for multiple comparisons. Error bars in all figures are SEM. A p value < 0.05 was considered significant.

References:

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