Supplemental Methods

Significance of the hydrophobic residues 225 to 230 of apoA-I for the biogenesis of HDL

HDL

Secretion of WT and mutant apoA-I forms. To assess the secretion of WT and mutant apoA-I forms, SW1783 human astrocytoma (HTB-13) cells grown to 80% confluence in Leibovitz's L-15 medium containing 2% heat-inactivated horse serum in 6-well plates were infected with adenoviruses expressing WT and mutant apoA-I forms at a multiplicity of infection of 10. Twenty-four hours post-infection, the cells were washed twice with PBS and incubated in serum-free medium for 2 h. Following an additional wash with PBS, fresh serum-free medium was added, and 24 h later was collected and analyzed by SDS-PAGE for apoA-I expression.

Plasma lipids and apoA-I levels, FPLC fractionation, and two-dimensional gel electrophoresis. The concentration of total cholesterol and triglycerides of plasma drawn four days post-infection was determined using the Total Cholesterol E, Free Cholesterol C and Phospholipids C reagents respectively (Wako Chemicals USA, Inc., Richmond, VA). Triglycerides were determined using the INFINITY triglycerides reagent (ThermoScientific, Waltham, MA), according to the manufacturer's instructions. Plasma apoA-I levels were determined by a turbidometric assay using AutoKit A-I (Wako Chemical USA, Inc., Richmond, VA) (1;2). For FPLC analysis of plasma, 17 μl plasma obtained from mice infected with adenovirus-expressing WT or mutant apoA-I forms were loaded onto a Sepharose 6 PC column (Amersham Biosciences, Piscataway, NJ) in a SMART micro FPLC system (Amersham Biosciences, Piscataway, NJ) and eluted with PBS. A total of 25 fractions of 50 μl volume each were collected for further analysis. The concentration of lipids in the FPLC fractions was determined as described above. The plasma HDL subpopulations were separated by two-dimensional electrophoresis. The proteins were

then transferred to a nitrocellulose membrane and apoA-I was detected by immunoblotting, using the goat polyclonal anti-human apoA-I antibody AB740 (Chemicon International, Billerica, MA) (3).

Fractionation of plasma by density gradient ultracentrifugation and electron microscopy (EM) analysis of the apoA-I containing fractions. For this analysis, 300 µl of plasma obtained from adenovirus-infected mice was diluted with saline to a total volume of 0.5 mL and fractionated by density gradient ultracentrifugation. Following ultracentrifugation, 0.5 mL fractions were collected and analyzed by SDS-PAGE as described (3). Fractions 6-7 obtained by the density ultracentrifugation, that float in the HDL region, were analyzed by electron microscopy using a Philips CM-120 electron microscope.

ApoA-I mRNA quantification. Total hepatic RNA was isolated by the Trizol® method (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RNA samples were adjusted to 0.1 µg/µl and cDNA was produced using the high capacity reverse transcriptase cDNA kit (Applied Biosystems, Foster City, CA). Apo A-I mRNA was quantified using Applied Biosystems Gene Array TaqMan® primers for apoA-I cDNA (Applied Biosystems, Foster City, CA, Cat# Hs00985000_g1), and 18s rRNA (Cat# 4319413E) and the TaqMan® Gene expression PCR Master Mix (Applied Biosystems, Foster City, CA, Cat# 4370048), using the Applied Biosystems 7300 Real-Time PCR System (4).

Preparation of apoA-I for physicochemical measurements. Before all analyses, lyophilized wild-type or mutant apoA-I forms were dissolved at a final concentration of 0.2mg/mL in 8M guanidine hydrochloride in DPBS. The protein samples were incubated for 1h at RT and then dialyzed extensively against DPBS pH 7.4. The samples were centrifuged at 12000g for 10 min to remove any precipitated protein. The supernatant solutions were quantitated by measuring their absorbance at 280nm. The proteins were kept at low concentrations (~0.1mg/mL) on ice to avoid aggregation. All analyses were performed on freshly refolded protein.

Circular dichroism measurements*:* A Jasco-715 spectropolarimeter connected to a Jasco PTC-348 WI Peltier temperature controller was used to record the far-UV CD spectra of the apoA-I samples from 190 to 260nm at 25˚C using a quartz cuvette with an optical path of 1nm. The protein samples were at 0.1mg/mL in DPBS (pH 7.4). The measurement parameters were as follows: bandwidth 1nm, response 8sec, step size 0.2nm and scan speed 50nm/min. Each spectrum was the average of 5 accumulations. The results were corrected by subtracting the buffer baseline. Helical content was calculated based on the molar ellipticity at 222nm as described by Greenfield et al. (5) using the equation: $\%\alpha\text{-helix}_{222nm}=([\Theta]_{222}+3000)/(36000+3000)\times100$. To record the thermal denaturation profile of the protein, we monitored the change in molar ellipticity at 222nm, while the temperature was raised from 20˚C to 80˚C, at a rate of 1˚C/min. The curve was fitted to a Boltzman sigmoidal model curve using the Graphpad Prism™ software.

Chemical denaturation. To record the chemical denaturation profile of ApoA-I, 0.1 mg/mL of freshly refolded protein was added to a 4 mL quartz fluorimeter cuvette and the intrinsic tryptophan protein fluorescence was measured after excitation at 295 nm. Small amounts of an 8.0 M guanidine hydrochloride (Applichem) solution were gradually added in the cuvette. The contents were continuously mixed using a magnetic stirrer. After each addition of guanidine hydrochloride, the sample was incubated in the dark for 2 min before measuring the fluorescence signal.

8-anilino-1-naphthalene-sulfonate (ANS) fluorescence. 1,8 ANS (1-anilinonaphthalene-8-sulfonic acid, Sigma-Aldrich) was dissolved in dimethylsulfoxide (DMSO) to a final concentration of 50 mM (ANS stock solution) and stored at -20°C. Freshly refolded apoA-I at 0.1mg/ml in DPBS pH 7.4 was placed into the wells of a 96-well microplate, and the fluorescence signal was measured by a TECAN Infinite M200 microplate reader. The excitation wavelength was set at 395 nm and the emission range from 420 to 600 nm. One microliter of ANS stock solution was added to each sample and mixed so that

the final ANS concentration was 310 μM. A control ANS spectrum in the absence of protein was also recorded to allow the calculation of ANS fluorescence enhancement in the presence of apoA-I.

Supplemental Table 1: Nucleotide sequence of primers used in PCR amplifications

Name	Sequence	Location of sequence
apoA-I [F225A/V227A/ V229A/L230A] F	5′- G CCC GTG CTG GAG AGC **GC**[a]C AAG G**C**C AGC **GC**C **GC**G AGC GCT CTC GAG GAG **-3′**	nt 764-812[b] (sense) (aminoacids +219 to +235)[c]
apoA-I [F225A/V227A/ V229A/L230A]R	5′- CTC CTC GAG AGC GCT C**GC** G**GC** GCT G**G**C CTT G**GC** GCT CTC CAG CAC GGG C **-3′**	nt 812-764 (antisense) (aminoacids +235 to +219)
LCAT F	5′- GA ***AGA TCT***[d] ACC ATG GGG CCG CCC GGC TCC CCA- 3′	-
LCAT R	5′- GCG ***GAT ATC***[d] CTA TTC AGG AGG CGG GGG CTC TGG - 3′	-

[a]Mutagenized residues are marked in boldface type and are underlined.

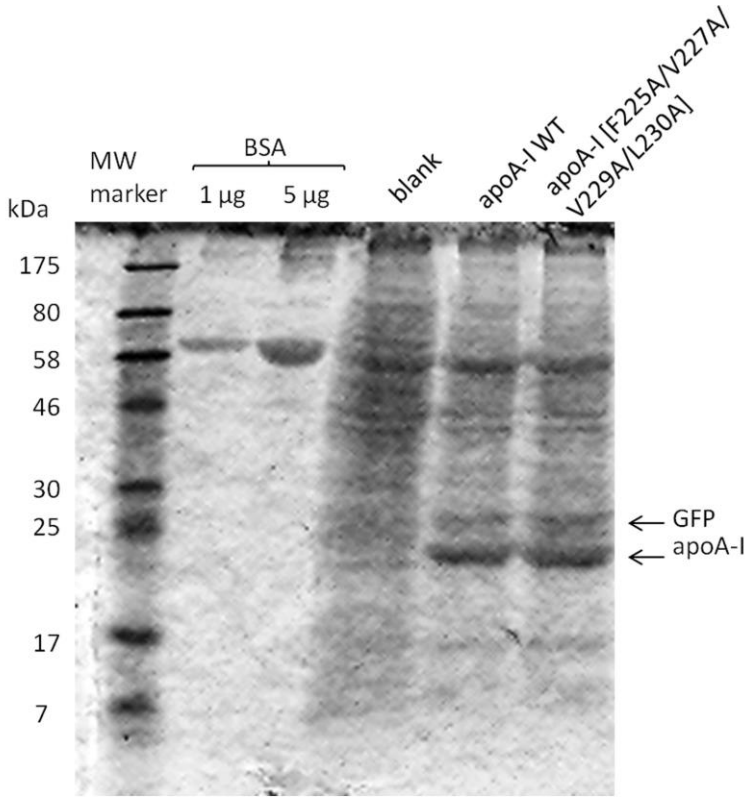
[b]Nucleotide number of the human apoA-I cDNA sequence, oligonucleotide position relative to the translation initiation ATG condon.

[c]Aminoacid position (+) refers to the mature plasma apoA-I sequence.

[d]The Bgl-II and EcoRV restriction sites.

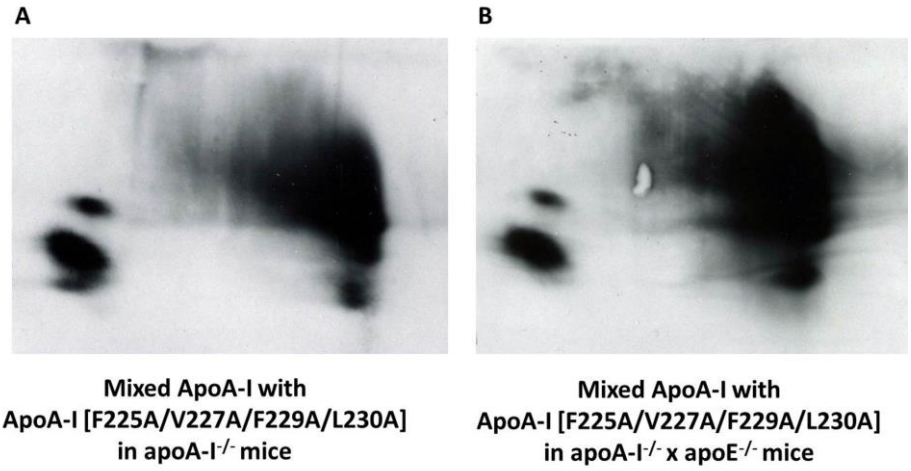
Supplemental Figures:

Supplemental Figure 1:



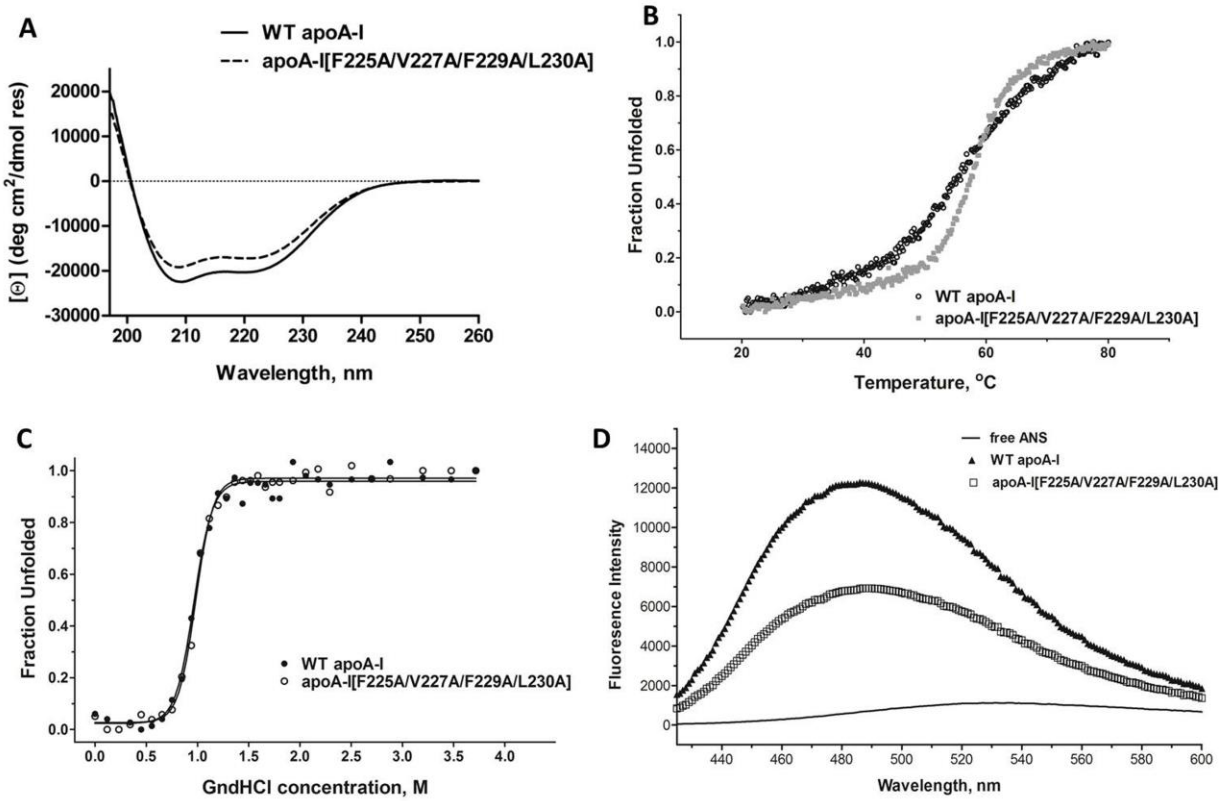
Supplemental Figure 1: ApoA-I secretion following infection of HTB-13 cells with apoA-I expressing adenoviruses. SDS-PAGE analysis of 100 microliter of culture medium of HTB-13 cells infected with adenovirus expressing the WT and the apoA-I[F225A/V227A/F229A/L230A] mutant form at a MOI (multiplicity of infection) of 20 viruses per cell as described in experimental procedures. The position of apoA-I and the green fluorescent protein (GFP) that is also expressed independently by the same virus are shown.

Supplemental Figure 2:



Supplemental Figure 2 (A, B)*:* Two dimensional gel electrophoresis of a mixture of samples obtained from plasma of apoA-I$^{-/-}$ (A) or apoA-I$^{-/-}$ x apoE$^{-/-}$ (B) mice expressing the either the WT apoA-I or the apoA-I[F225A/V227A/F229A/L230A] mutant.

Supplemental Figure 3:



Supplemental Figure 3 (A-D)*:* Far-UV CD spectra of WT apoA-I and apoA-I [F225A/V227A/F229A/L230A] obtained at 25˚C *(A)*. Thermal denaturation profiles of WT apoA-I and apoA-I [F225A/V227A/F229A/L230A] determined by changes in molar ellipticity at 222nm. Samples were denatured by increasing the temperature up to 80˚C. Experimental points depicted as dots *(B)*. Chemical denaturation profile of WT apoA-I and apoA-I [F225A/V227A/F229A/L230A] mutant. The intrinsic fluorescence signal of tryptophan of apoA-I was monitored while titrating with GndHCl. Solid line represents non-linear regression to a simple Boltzmann model. The experimental points are depicted as dots *(C)*. ANS fluorescence spectra obtained in the presence of 100 micrograms/mL WT apoA-I or the apoA-I [F225A/V227A/F229A/L230A] mutant, or in the presence of buffer alone *(D)*.

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