Supplemental Data

Helical Domains That Mediate Lipid Solubilization and ABCA1-Specific Cholesterol Efflux in Apolipoproteins C-I and A-II

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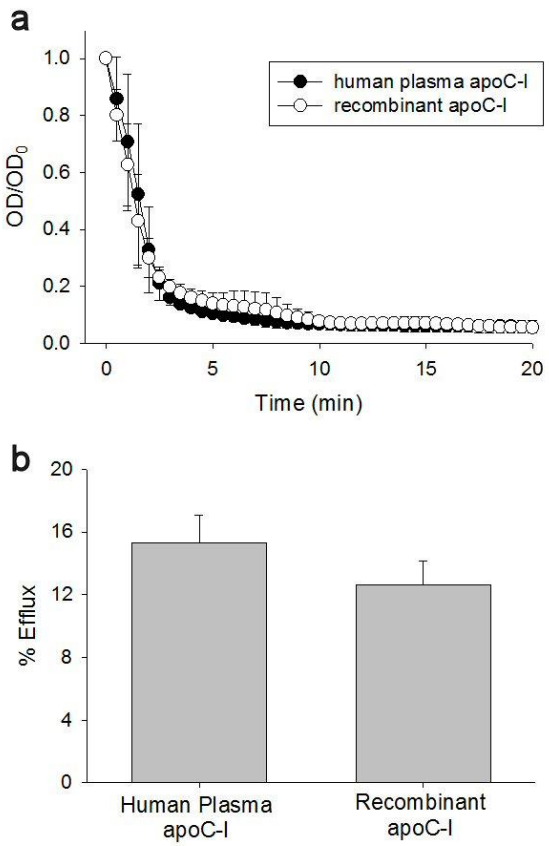

Supplement Figure 1. Comparison of human plasma and recombinant apoC-I in clearance of DMPC liposomes and promotion of ABCA1-mediated cholesterol efflux. a) ApoC-I was added to DMPC liposomes at a lipid:protein mass ratio of 2.5:1 at 24.5 °C. For each sample, the light scattering at time 0 min was normalized to 1.0 and each subsequent reading was expressed as optical density (OD) divided by the initial optical density (OD_0). b) RAW mouse macrophages were exchange labeled with tritiated cholesterol and then treated with or without 8-Br-cAMP to upregulate ABCA1 expression as described in *Methods*. 5 µg/ml of each apolipoprotein was then incubated with the cells for 8 h with or without 8-Br-cAMP and the radioactivity appearing in the media were assessed by scintillation counting. The data are expressed as the % of label present in the cells immediately before the incubation period (T=0 h). Only cAMP dependent efflux is shown. The experiment was run in triplicate and expressed as averages ± 1 sample standard deviation. While the recombinant and plasma forms of apoC-I were not statistically different, we did see a consistent trend across experiments for the plasma form to be more efficient than the recombinant form. This may be due to residual lipopolysaccaride (LPS) present in the recombinant preparations which is known to affect ABCA1 expression (1).

Supplement Table 1. Physical characteristics of peptides used in this study.

Peptide	Amino acids	MW (Da) [a]	Charge [b]	Hydrophobic moment/residue[c]	Hydrophobic Index/residue[d]
ApoC-I	57	6630.6	1	ND[e]	ND[e]
ApoC-$I_{helix\ 1}$	27	3173.6	0	2.2	1.7
ApoC-$I_{helix\ 2}$	24	2970.5	2	1.6	2.3
ApoC-$I_{helix\ 2+1}$	56	6584.6	1	ND[e]	ND[e]
ApoC-$I_{reverse}$	57	6630.6	1	ND[e]	ND[e]
ApoA-II	77	8707.9	-2	ND[e]	ND[e]
ApoA-$II_{helix\ 1}$	24	2837.2	-1	2.1	1.9
ApoA-$II_{helix\ 2}$	16	1923.2	1	1.8	1.6
ApoA-$II_{helix\ 2\text{-}long}$	19	2267.6	-1	1.7	1.8
ApoA-$II_{helix\ 3}$	23	2609.1	0	1.9	1.8
ApoA-$II_{helix\ 1+2}$	22	2591.0	-1	1.6	2.0
ApoA-$II_{helix\ 2+3}$	36	4149.8	1	ND[e]	ND[e]

[a] In the case of synthesized peptides, the MW includes the sequence of each peptide (see **Fig. 3** in the main text) as well as an N-terminal acetyl group and a C-terminal amide.

[b] Estimated charge at pH 7.0 based on sequence analysis.

[c] Hydrophobic moment per residue as determined by the WHEEL program (2).

[d] Hydrophobic index per residue as deterimend by the WHEEL program (2).

[e]..In many multihelical constructs or proteins, there was a turn sequence that appeared to change the orientation of the individual helical faces when drawn as a single helical wheel. Therefore, parameters such as hydrophobic moment and hydrophobic index were not applicable.

References

1. Baranova, I., T. Vishnyakova, A. Bocharov, Z. Chen, A. T. Remaley, J. Stonik, T. L. Eggerman, and A. P. Patterson. 2002. Lipopolysaccharide down regulates both scavenger receptor B1 and ATP binding cassette transporter A1 in RAW cells. *Infect. Immun.* **70:** 2995-3003.

2. Jones, M. K., G. M. Anantharamaiah, and J. P. Segrest. 1992. Computer programs to identify and classify amphipathic alpha helical domains. *J. Lipid Res.* **33:** 287-296.