SUPPLEMENTARY MATERIAL

Triglyceride increase in the core of high-density lipoproteins augments apolipoprotein dissociation from their surface: Potential implications for treatment of apolipoprotein deposition diseases

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%	Cholesterol	Triglycerides	Phospholipids	apoA-I	apoA-II	apoB	apoE	apoC-III
NL-HDL	15.3	4.5	28.4	41.4	8.6	0.0	0.4	1.3
DM-HDL	13.1	6.0	30.2	38.5	9.6	0.0	0.3	2.2

Table S1. Protein and lipid composition in HDL obtained from pooled plasma of normolipidemic (NL) or diabetes mellitus (DM) patients. The values are shown as % of total weight. Total cholesterol (esterified and unesterified) is listed. The variability in TG weight determination is ~5%.

%	PL	UC	CE	TG, TO	TG:CE	Protein
nHDL	41	4	18	1	0.06	46
TG-HDL	39	4	11	15	1.36	42
TO-HDL	42	4	15	12	0.8	44

Table S2. Biochemical composition of plasma HDL before and after the enrichment in either TG or TO. HDL were mixed either with VLDL (as a source of TG) or with PC/TO microemulsions and were incubated in the presence of CETP as described in Methods; TG or TO-enriched HDL particles were re-isolated by density gradient centrifugation to obtain TG-HDL and TO-HDL, respectively. CE - cholesteryl ester; UC - unesterified cholesterol; PL - phospholipids; Protein - total HDL protein. Particle composition, which was determined as described in Methods, is expressed as % of total weight; weight ratio of TG or TO to CE (TG/CE) is also shown. The values represent the mean of three independent experiments, with variability of ±2%.

	PL	UC	CE	TG	TG:CE	apoA-I
rHDL	35 ± 0.9	1.1 ± 0.1	27 ± 0.5	0		1
TG-rHDL	37 ± 1.1	1.5 ± 0.3	18 ± 0.5	8 ± 0.4	0.44	1

Table S3. Biochemical composition of reconstituted HDL used in this study before (rHDL) and after their enrichment in TG (TG-rHDL). Reconstituted HDL were enriched with TG by co-incubation with VLDL and CETP, followed by re-isolation of the HDL fraction as described in Methods. The tabulated values, which are mol fractions per mol of apoA-I, represent the mean ± STD of three independent measurements.

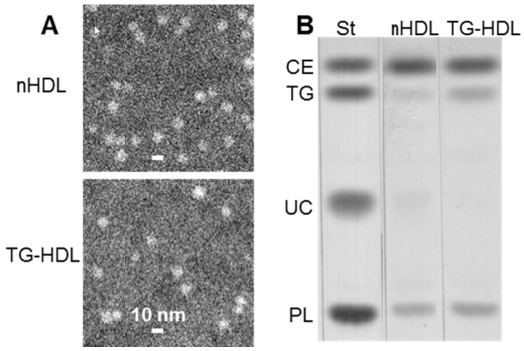

Figure S1. Isolated triglyceride-enriched HDL (TG-HDL) visualized by negative stain transmission electron microscopy (**A**) and analyzed by thin-layer chromatography for lipid composition (**B**). The bands corresponding to the major lipid constituents are indicated: CE – cholesterol esters; TG – triglycerides; UC – unesterified cholesterol; PL – phospholipids.

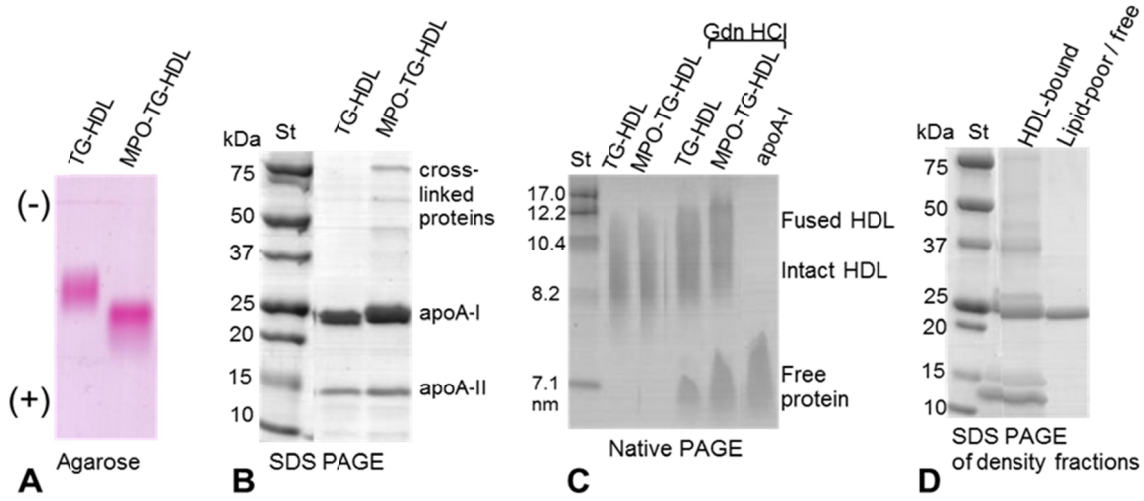

Figure S2. Oxidation of TG-HDL using MPO/H_2O_2/Cl^- promotes the release of monomeric apoA-I. TG-enriched HDL (TG-HDL) were oxidized with MPO/H_2O_2/Cl^- (to obtain MPO-TG-HDL) as described in Methods. (**A**) Agarose gel, which was performed using a TITAN gel lipoprotein electrophoresis system as described previously [54], shows a shift in the electrophoretic mobility of TG-HDL upon oxidation by MPO, indicating increased net negative charge on oxidized HDL. (**B**) SDS PAGE of MPO-TG-HDL shows oxidative cross-linking of the main HDL proteins. (**C**) TG-HDL and MPO-TG-HDL were incubated with 3M Gdn HCl as described in Methods to initiate protein release. Native PAGE shows that free protein is released from TG-HDL, and that a greater amount of free protein is released from MPO-TG-HDL. (**D**) To separate free and HDL-bound protein, MPO-TG-HDL that have been incubated with Gdn HCl were subjected to density gradient centrifugation as described in Methods. The top fraction contained HDL-bound proteins and the dense bottom fraction contained predominately lipid-poor/free proteins. SDS PAGE of these density fractions shows that the cross-linked proteins were retained in the HDL-bound fraction, while the free protein released from MPO-TG-HDL contained almost exclusively monomeric apoA-I. Mass spectrometry analysis of this released apoA-I indicated methionine oxidation (data not shown).

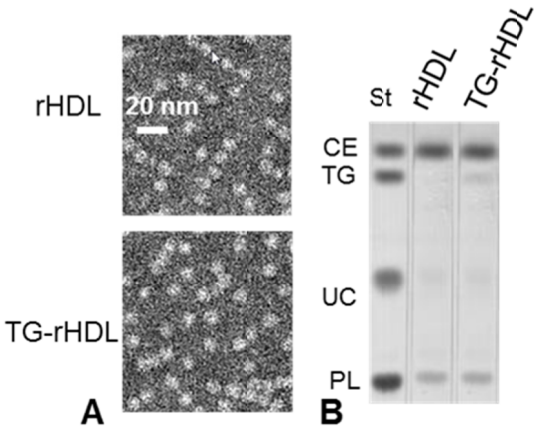

Figure S3. Characterization of spherical reconstituted HDL (rHDL). Discoidal HDL have been reconstituted from apoA-I, POPC and unesterified cholesterol (UC), and were subjected to LCAT reaction to generate cholesterol ester (CE) and thereby convert rHDL into spherical particles. These spherical rHDL were then enriched with TG via the CETP reaction to obtain TG-rHDL as described in Methods. The resulting particles were visualized by negative stain electron microscopy (**A**) and were analyzed by thin-layer chromatography to ascertain their lipid composition (**B**) as described in Methods (part 2.2). Major lipid bands are indicated.

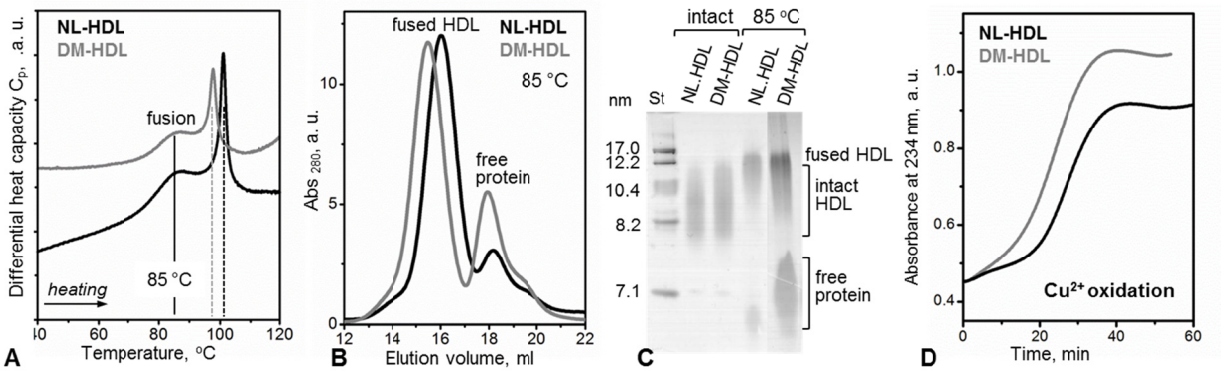

Figure S4. Thermal remodeling and oxidation of human plasma HDL from normolipidemic (NL) and diabetes mellitus (DM) subjects. (**A**) Heat capacity data, $C_p(T)$, of NL-HDL and DM-HDL recorded by differential scanning calorimetry (DSC) during heating at a rate of 90 °C/h. The samples have been heated to 85 °C in DSC experiments to trigger HDL fusion, followed by size-exclusion chromatography using Superose 6 10/300 GL column (**B**) and native PAGE analysis (4-20% gradient, Denville Blue protein stain) (**C**). DM-HDL, which have higher TG content (see Table S1 above), show more extensive thermal remodeling and protein release as compared to NL-HDL. (**D**) Time course of copper-induced lipoprotein oxidation performed as described in Methods shows accelerated oxidation in DM-HDL as compared to NL-HDL.