Effects of phospholipase A_2 and its products on structural stability of human low-density lipoprotein: Relevance to formation of LDL-derived lipid droplets

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SUPPLEMENTARY MATERIAL

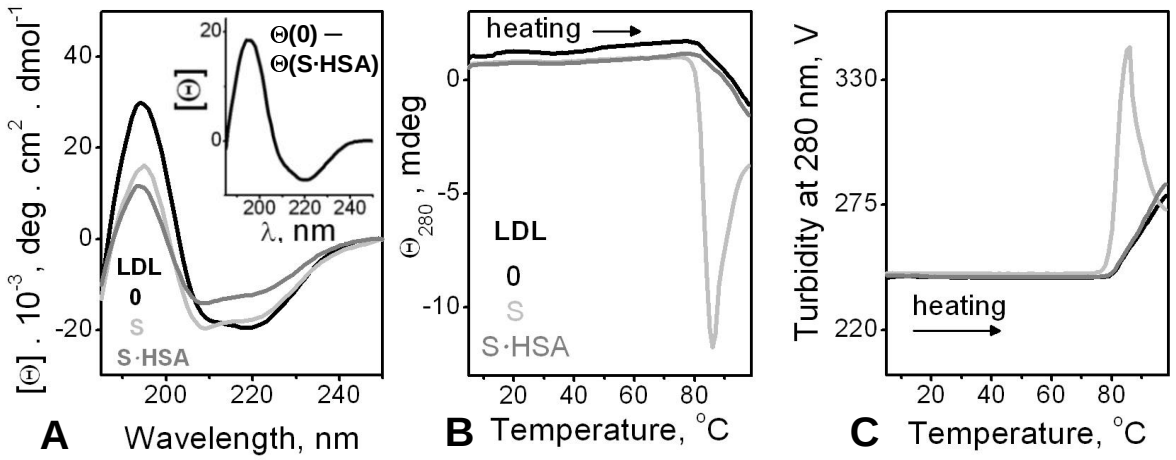

Figure S1. Effects of spontaneous PC hydrolysis on the structure and stability of human LDL assessed in circular dichroism experiments. LDL were native (0) or hydrolyzed spontaneously upon overnight incubation at 37 ^{o}C in the absence (S) or in the presence (S-FFA) of 20 mg/ml FFA-free albumin. (**A**) Far-UV CD spectra of LDL indicate loss of the protein secondary structure upon hydrolysis and albumin treatment. **Insert** shows the difference spectrum between intact and spontaneously hydrolysed and albumin-treated LDL, [Θ](0)-[Θ](S·HSA). The spectral shape is characteristic of the β-sheet, indicating that the secondary structure lost upon hydrolysis and albumin treatment is predominantly β-sheet in apoB. (**B, C**) Melting data of LDL that were intact or spontaneously hydrolyzed with or without albumin treatment. LDL solutions (2 mg/mL protein in buffer A) were heated at a rate of 11 ^{o}C/h; the data were recorded at 280 nm by CD to monitor repacking of apolar lipids (**B**) and by turbidity (dynode voltage, V) to monitor increase in the particle size upon rupture and coalescence into lipid droplets (**C**).

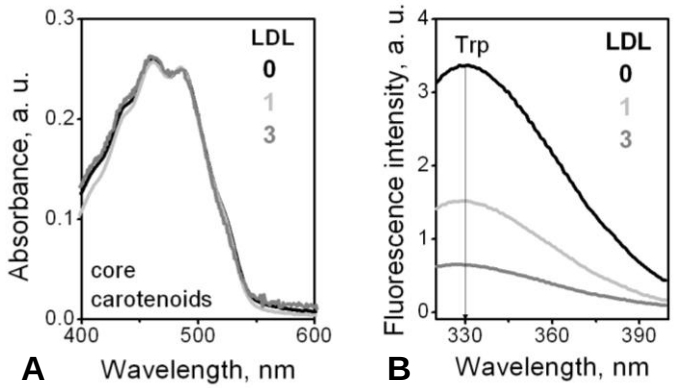

Figure S2. Changes in LDL core and surface upon hydrolysis by PLA_2 monitored by absorption and fluorescence spectroscopy. LDL were intact (0) or have been hydrolyzed by using 0.05 or 5 μg/ml PLA_2 to stages 1 or 3, respectively. The spectra were recorded at room temperature. (**A**) Visible absorption spectra show a characteristic triple peak with maxima at 435, 459 and 484 nm indicating intact carotenoids in the core of LDL. (**B**) Intrinsic Trp fluorescence of LDL (0.1 mg/mL protein, 280 nm excitation wavelength) shows that hydrolysis leads to a progressive reduction in the emission intensity, suggesting altered packing of the Trp and/or their chemical modifications. The wavelength of maximal fluorescence remains invariant at 333 nm, indicating largely buried Trp.

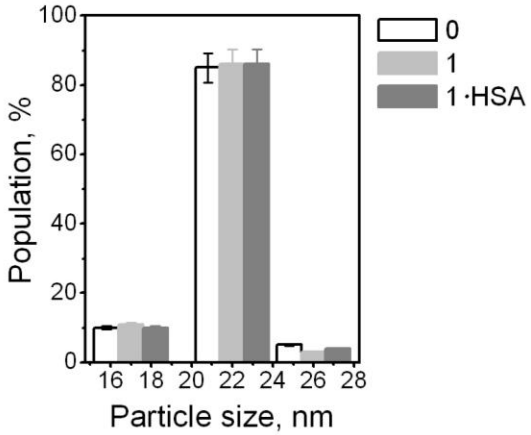

Figure S3. Particle size distribution in LDL hydrolyzed to stage 1. Particle size was determined based on the electron micrographs shown in Figures 4 A-C. Intact LDL (0), LDL hydrolyzed to stage 1 by using 0.05 μg/ml PLA_2 (1), and LDL hydrolyzed to stage 1 and treated with human serum albumin (HSA) to remove FFA (1·HSA) are shown. Error bars represent deviations among different measurements. The results show that the particle size distribution before heating does not significantly change upon PC hydrolysis by PLA_2 and FFA removal by albumin.

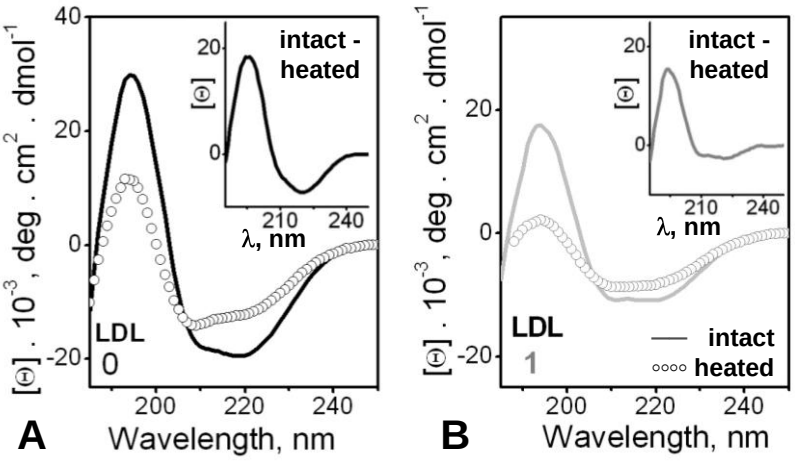

Figure S4. Effects of LDL heating on the secondary structure of apoB. Far-UV CD spectra of intact LDL (**A**) and of LDL that have been hydrolyzed by PLA_2 to stage 1 (**B**) are shown. The spectra in each panel were recorded from the same LDL sample (2 mg/mL protein in buffer A) at room temperature before (solid lines) and after heating to 100 ^{o}C (open circles). Inserts show difference spectra between the CD recorded before and after heating.

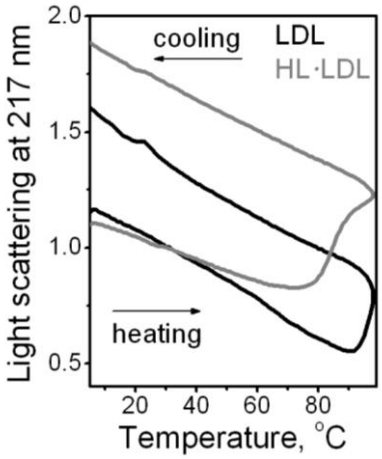

Figure S5. Effect of LDL lipolysis by hepatic lipase (HL) on the lipoprotein stability. HL from human pancreas was from Sigma. LDL (2 mg/mL protein) were incubated with 0.01 μg of HL at 37 ^{o}C for 12 h in 10 mM Tris buffer, pH 7.5. The reaction was quenched by adding 250 mM EDTA. Hydrolyzed LDL were immediately separated by gel filtration using Superose 6 10/300 GL column by elution at a flow rate of 0.5 mL/min in buffer A. Under these conditions, no protein modifications were detected by SDS PAGE (not shown). TLC analysis of HL-hydrolyzed LDL showed nearly 10 % increase in lyso-PC and FFA and decrease in PC. LDL solutions (0.1 mg/mL protein) were heated and cooled at a rate of 11 ^{o}C /h, and lipoprotein rupture and coalescence into lipid droplets was monitored by right-angle light scattering at 217 nm. The results show a large low-temperature shift by >10^{o}C in the rupture transition of LDL upon hydrolysis (HL·LDL). Thus, similar to PLA_2 hydrolysis, HL hydrolysis destabilizes LDL against rupture and promotes lipid droplet formation. Similar destabilizing effect was observed in HL-treated HDL (data not shown). HL hydrolysis of VLDL under similar conditions led to lipoprotein rupture even before heating, resulting in increased turbidity observed visually and by EM (data not shown). This prevented further biophysical studies of the HL-hydrolyzed VLDL.

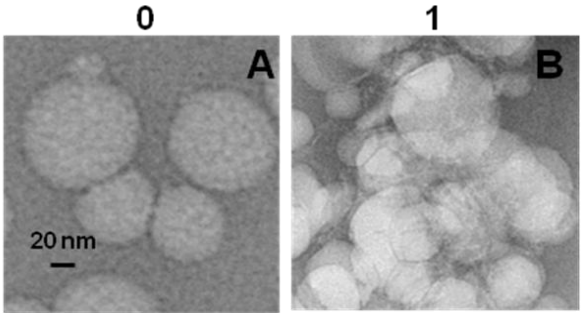

Figure S6. Negative stain electron microscopy of unheated VLDL that were native (A) or hydrolyzed by PLA_2 to stage 1 (B). Hydrolyzed VLDL were destabilized to such an extent that they underwent rupture and coalescence into lipid droplets without heating (B). This prevented stability studies of such hydrolyzed VLDL.