Effects of Oxidation on Structural Stability and Functional Remodeling of Human Very Low-Density Lipoprotein

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SUPPLEMENTAL MATERIAL

Fig. S1. Circular dichroism spectra of intact and copper-oxidized VLDL. Total plasma VLDL were oxidized by Cu^{2+} at 37 °C to stages 1-6 as described in Figure 2 legend and in the Methods; 0 stands for intact VLDL. The samples were diluted to 0.1 mg/mL protein to record far-UV CD (A) or used at 0.5 mg/mL protein to record near-UV CD (B) at 25 °C. Insert: Far-UV CD difference spectrum, $[\Theta_0]$-$[\Theta_6]$, between the spectra of VLDL that were intact or oxidized to stage 6 suggests partial unfolding of the α-helical and, probably, β-sheet structure upon oxidation. Near-UV CD above 300 nm remained invariant upon oxidation (B), suggesting that VLDL did not rupture (37).

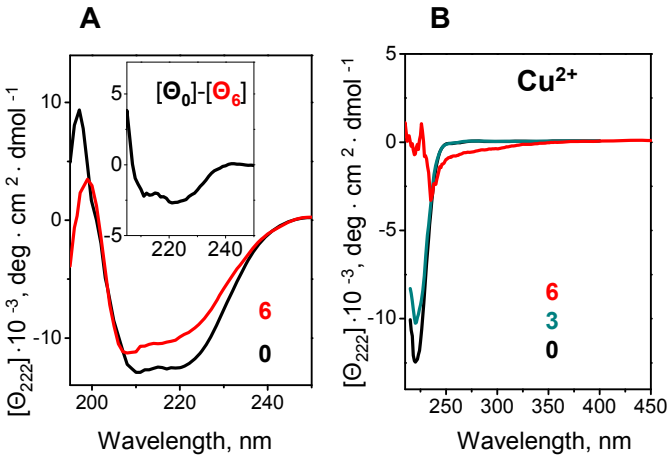
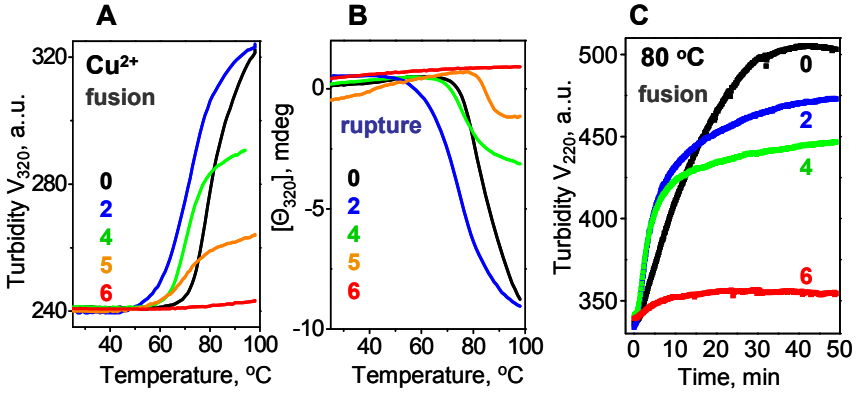


Fig. S2. Effects of oxidation on thermal stability of VLDL. VLDL that were intact (0) or oxidized by Cu^{2+} to stages 1-6 were diluted to 0.1 mg/mL protein in standard buffer and heated at a rate of 11 °C/h (A, B) or subjected to a temperature jump to 80 °C (C). Increase in the particle size due to fusion followed by rupture and coalescence into lipid droplets was monitored by turbidity V at 320 or 220 nm (A, C), which is proportional to the dynode voltage measured in CD experiments (47). Lipid repacking upon VLDL rupture and coalescence into lipid droplets was monitored by CD at 320 nm (B), $\Theta_{320}(T)$, which was measured simultaneously with $V_{320}(T)$ (A).



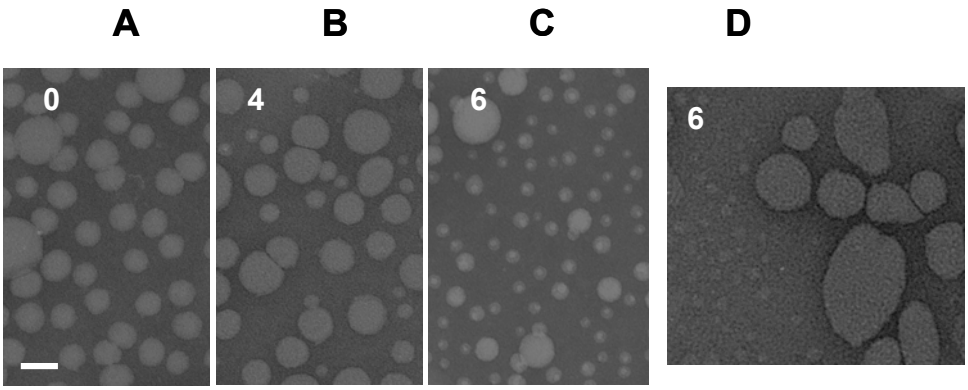


Fig. S3. Effects of oxidation on VLDL morphology at ambient temperatures. Total plasma VLDL that were intact (A) or oxidized by OCl^- to stages 4 (B) or 6 (C) or by Cu^{2+} to stage 6 (D) were visualized by negative staining electron microscopy at 22 ^oC. Panel D illustrates formation of large, sometimes non-spherical particles. In addition, small HDL-like particles are observed upon extensive oxidation by either agent (C, D). Bar size is 40 nm.

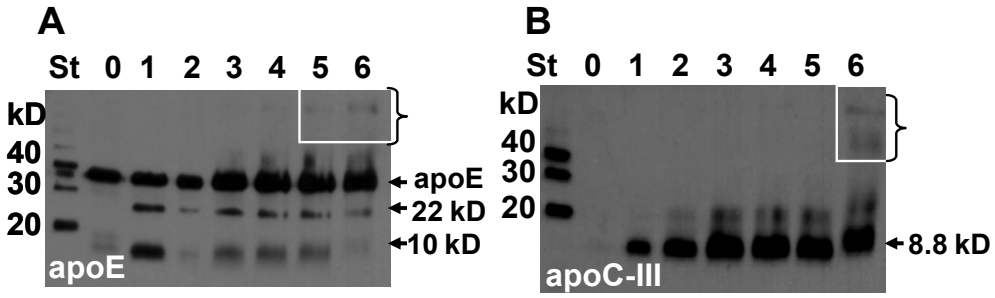


Fig. S4. Oxidative changes in the exchangeable apolipoproteins in VLDL assessed by SDS PAGE and immunoblotting. VLDL that were intact (0) or oxidized by Cu^{2+} to stages 1-6 (shown by numbers) were analyzed by 12% SDS PAGE followed by immunoblotting for apoE (A) or apoC-III (B). Arrows indicate full-size apoE (32 kD) and its N-terminal 22 kD (*) and C-terminal 10 kD (**) fragments (A), and full-size apoC-III (8,8 kD) (B). White boxes show cross-linked proteins.