

SUPPLEMENTAL INFORMATION

Heparin binding triggers human VLDL remodeling by circulating lipoprotein lipase:

Relevance to VLDL functionality in health and disease

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This file contains supplemental figures S1-S5.

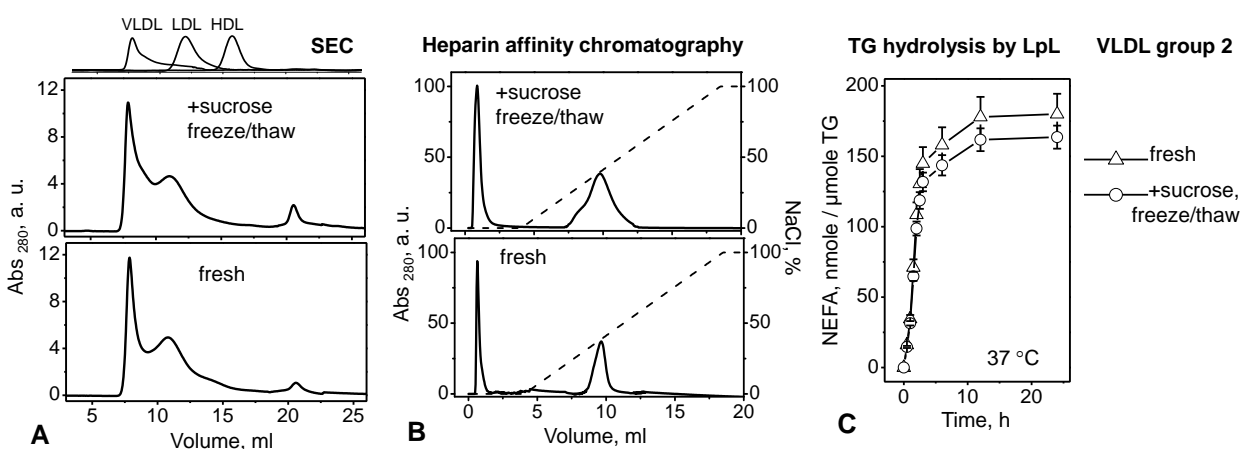


Figure S1. Effects of VLDL storage with sucrose followed by freeze/thaw on the particle size distribution, heparin affinity, and TG hydrolysis in VLDL. Single-donor group 2 VLDL samples from the same batch were used either fresh (no sucrose, no freeze/thaw) or after addition of 20% sucrose followed by freezing in 1ml aliquots, storage for 6-12 months, thawing and dialysis against the sucrose-free buffer (marked +sucrose freeze/thaw) prior to further studies. SEC (A), heparin affinity chromatography (B) and TG hydrolysis by the exogenous LpL (C) were performed as described in part 2 Methods. Values in panel C are shown as the mean \pm SD of three independent measurements.

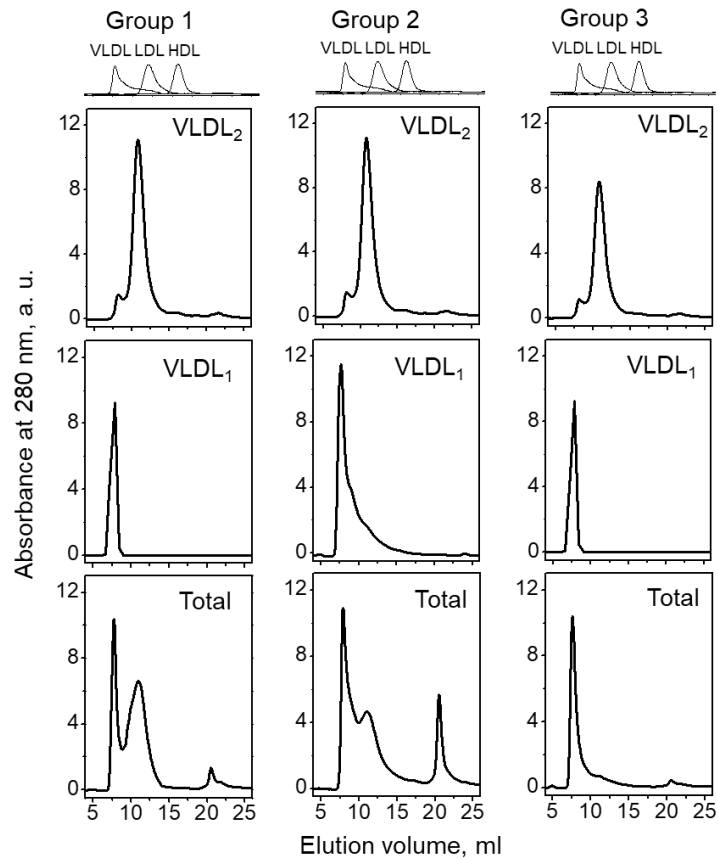


Figure S2. SEC data showing particle size distribution in total VLDL and its individual subclasses, VLDL₁ (large buoyant) and VLDL₂ (small dense). Single-donor total VLDL from groups 1-3 (as indicated) was isolated in the density range 0.94-1.006 g/ml; VLDL₁ and VLDL₂ subfractions were separated by density gradient ultracentrifugation following published protocols [Zao et al., 1995] with minor modifications, which included ultracentrifugation at 160,000 g for 2.5 hours at 4 °C in a SW40 Ti rotor. Fraction collection started from the top of the tube; the upper 1.5 mL represented VLDL₁ and the lower 5 mL represented VLDL₂. For SEC, VLDL samples containing 0.5 mg/ml total protein were injected into superose-6 10/300 GL column. Size markers for the major classes of plasma lipoproteins are shown at the top for comparison.

Reference:

S.P. Zhao, E.M. Bastiaanse, M.F. Hau, A.H. Smelt, J.A. Gevers Leuven, A. Van der Laarse, F.M. Van't Hof, Separation of VLDL subfractions by density gradient ultracentrifugation. *J. Lab. Clin. Med.* (1995) 125(5):641-649.

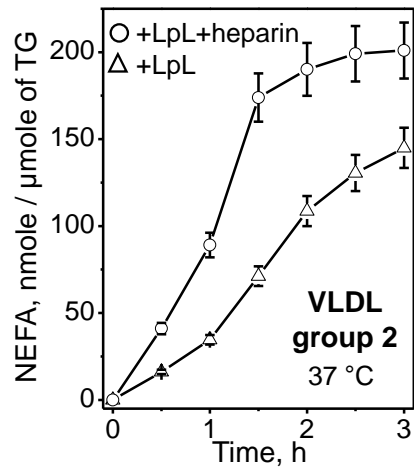


Figure S3. Heparin increases the catalytic activity of the exogenous LpL. Group 2 VLDL was incubated at 37 °C for 3 h either alone (control) or with exogenous LpL (25 U/ml) in the absence (+LpL, Δ) or in the presence of heparin (+LpL+heparin, \circ) using 1:1 wt/wt total protein to heparin ratio as described in Methods part 2.6. NEFA levels were measured at indicated time points; controls were subtracted from the data. Values are shown as the mean \pm SD of three independent measurements.

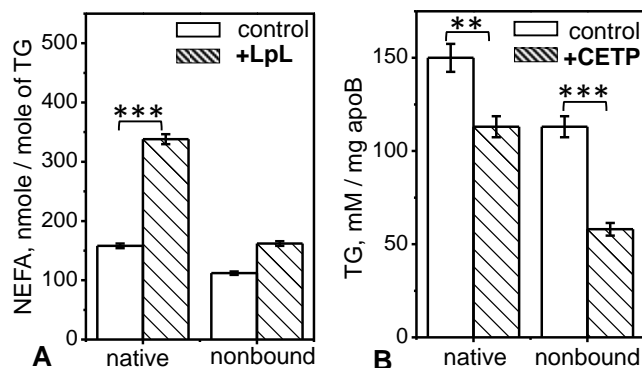


Figure S4. VLDL that does not bind heparin has impaired functionality. Fraction of nonbound VLDL was isolated by heparin affinity chromatography. Functional properties of nonbound VLDL as a substrate for the exogenous LpL (A) or CETP (B) were compared with those of native VLDL (before chromatography).

(A) TG hydrolysis by the exogenous LpL. VLDL were incubated at 37 °C with (+LpL) or without exogenous LpL (control), followed by NEFA measurements as described in Methods part 2.6.

(B) TG transfer from VLDL (donor) to HDL (acceptor) mediated by CETP. VLDL were incubated with HDL at 37 °C for 2 h with (+CETP) or without CETP (control), followed by VLDL re-solution and NEFA measurements as described in Methods part 2.7. Moderate ($p \leq 0.1$, **) and significant differences compared to controls ($p \leq 0.05$, ***) are indicated.

Figure S5. Characterization of VLDL from normolipidemic (NI) and type-2 diabetic patients before (Pc) and after treatment (Gc). Data for batches 1-4 are presented on two pages below; similar data for batch 5 are shown in Figure 7.

(A) Lipid and (B) protein analysis of intact VLDL. PL – phospholipids, TC – total cholesterol.

(C) Spontaneous TG lipolysis by the endogeneous LpL and (D) total lipolysis of VLDL.

(E) Oxidation index of freshly isolated VLDL measured by absorbance at 234 nm for conjugated dienes.

(F) SEC and (G) heparin affinity profiles of VLDL. Moderate ($p \leq 0.1$, **) and significant differences ($p \leq 0.05$, ***) between Pc and NI values are indicated.

