A METHOD TO PURIFY APOB100 PARTICLES FROM APOB48 PARTICLES IN TRIGLYCERIDE-RICH LIPOPROTEINS (TRL)

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Background: The kinetics of VLDL and chylomicron lipoprotein particles as well as their respective triglyceride (TG) content are important to understand the mechanisms by which dietary or pharmacological interventions modify particle size, lipid profile and cardiovascular risk. Ultracentrifugation cannot separate remnant chylomicrons from large VLDL particles. Methods: We devised an immuno-affinity method to separate VLDL (apoB100) from chylomicron (apoB48) particles in triglyceride-rich lipoproteins (TRL) using an apoB100 antibody. The resin/sample mixture was incubated and centrifuged, resulting in a flow through (FT) containing chylomicrons, which was reapplied to fresh resin. This process was repeated for two consecutive passes. The columns were then washed with high salt and VLDLs were eluted with low pH. The separated apolipoproteins were analyzed by Silver Stain and by apoB specific ELISA. To further evaluate the separation process we examined the TG content of the separated VLDLs and chylomicrons by LC/MS or GC/MS analysis. Results: The Silver Stained gels showed a depletion of apoB100 in the sequential FTs with little or no apoB100 in FT 2 and the elution of apoB100 contained no apoB48 (apoB48 specific ELISA). LC and GC/MS analysis revealed a difference in the incorporation of 13C-acetate in palmitate between triglycerides in the VLDL versus chylomicron particles. Conclusions: These results demonstrate that we have developed and validated a method that allows us to isolate apoB48 particles from apoB100 particles in human TRL samples. Furthermore, these data suggest that the intestine converts sugar to fatty acids by de novo lipogenesis.