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Article type : Commentary

'Interaction of chylomicron remnants and VLDLs during ultracentrifuge separation based on the Svedberg flotation rate.' – Authors' response

We thank Drs Stellaard and Lütjohann for their interest in our study and for their question regarding the nature of apoB48-containing lipoprotein particles found in the VLDL1 and VLDL2 density ranges (1). The correspondents are correct in pointing out that chylomicron remnants and apoB100-containing VLDL of liver origin co-exist in the VLDL1 (Sf60-400) and VLDL2 (Sf20-60) fractions, especially after a fat-rich meal. This is indeed what we reported in in Figure 1 (2) which gives examples of the apoB48 and apoB100 concentrations and tracer enrichments in these fractions during the postprandial phase. One of our objectives in exploring the kinetics of these two apoB species was precisely to address the point raised in the letter – that is, are apoB48 particles in VLDL generated solely from chylomicron lipolysis?

In developing an integrated model of chylomicron and VLDL metabolism (2), we did indeed start from the prevailing paradigm that the apoB48 appearing in the Sf 60-400 and Sf 20-60 fractions would be present only on chylomicron remnants (as represented by the flow from compartment 14 to compartment 15 in the apoB48 model sub-system shown in Figure 3). However, as set out in detail in Supplementary figure 2, this arrangement did not fit the observed tracer data in that the model-predicted rise in apoB48 tracer enrichment in VLDL1 and VLDL2 consistently fell below the observed data points. In fact, what we did find (to our surprise it must be said) was that, over the first 4 hours following the fat meal, tracer enrichment in apoB48 rose simultaneously in the chylomicron, VLDL1 and VLDL2 fractions (see panel G in Supplementary figure 2). When we introduced direct input of tracer (i.e. *de novo* synthesis) into compartments 16/17 (VLDL1 apoB48) and compartments 18/19 (VLDL2 apoB48) in the model (Figure 3) then a satisfactory fit was

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obtained to the observed data in all subjects (see panels C and D in Supplementary figure 2). Thus, the best explanation for our results was that the intestine, in response to the meal, released particles across the entire size and density range (chylomicrons-VLDL1-VLDL2) which was a new and important finding. Further, in an upcoming paper we are able to report in an expanded cohort of subjects with a wide spectrum of plasma triglyceride levels that approximately half of the apoB48 found in the VLDL1 and VLDL2 density ranges following a fat meal is derived from chylomicron lipolysis and half from direct secretion of intestinally derived VLDL-sized particles (Bjornson *et al* unpublished).

While the idea that the intestine secretes a wider range of particle sizes during fat absorption than previously thought is novel, as presented in the Discussion the concept that this organ is capable of direct secretion of apoB48-containing VLDL sized particles is not. The phenomenon was reported in a kinetic investigation that used a micro-meal design to establish a quasi-steady state (3), and cell culture and animal model studies show that the intestine has the ability to synthesise and secrete lipoproteins of this size (see references 50-52 in (2)).

1. Stellaard F and Lütjohann D. Interaction of chylomicron remnants and VLDLs during ultracentrifuge separation based on the Svedberg flotation rate. J Intern Med 2019;IN PRESS
2. Björnson E, Packard CJ, Adiels M et al. Investigation of human apoB48 metabolism using a new, integrated non-steady-state model of apoB48 and apoB100 kinetics. J Intern Med 2019;285:562-577.
3. Zheng C, Ikewaki K, Walsh BW and Sacks FM. Metabolism of apoB lipoproteins of intestinal and hepatic origin during constant feeding of small amounts of fat. *J Lipid Res*. 2006;47:1771-9.