commentary

Life is complicated: so is apoCIII[1]

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Apolipoprotein (apo)CIII, comprised of 79 amino acids and with a mass of 8.8 kDa, was first isolated and characterized 50 years ago by Brown et al. (1). Studies conducted during the following decade demonstrated that apoCIII was an inhibitor of both LPL (2) and the uptake of triglyceride-rich lipoproteins (TGRLs) and remnants by perfused livers (3, 4). Lipoprotein kinetic studies of two sisters with complete absence of apoCIII (5) demonstrated, in vivo, that absence of this protein resulted in a dramatic increase in lipolysis of VLDL-TG (6). Overexpression of apoCIII and targeted deletion of the *APOC3* gene in mice confirmed the human findings as well as the earlier rodent studies showing that apoCIII inhibited hepatic uptake of apoB-containing lipoproteins (7–9). Recent Mendelian randomization studies demonstrate a strong, direct relationship between loss of function of apoCIII and reduced risk of cardiovascular disease (10, 11). These data, together with the development of therapies to dramatically reduce apoCIII synthesis (12, 13), have reenergized the field and highlighted the need to define in detail the physiologic consequences of reducing apoCIII levels in humans (14–16).

In this issue of the *Journal of Lipid Research*, Ramms and colleagues extend their previous studies that dissected the hepatic pathways involved in apoCIII-mediated inhibition of TGRL uptake in mice (17) by focusing on the impact of apoE on apoCIII's effects on the latter pathway and on LPL-mediated hydrolysis of TG in apoB-containing lipoproteins. Our read of their "take-home" messages are that apoE determines which effects of apoCIII, inhibition of hepatic uptake of TGRL or inhibition of adipose LPL activity, will be dominant. The authors conclude that in the presence of apoE, apoCIII increases plasma TG by blocking hepatic uptake of TGRL, while in the absence of apoE, it is apoCIII inhibition of LPL that is the cause of increased plasma TG levels. In reaching these conclusions, the authors completed an impressive array of experiments with in vivo and in vitro approaches; we will summarize the key results and highlight the difficulty of addressing, even in innovative model systems, the dominant role of an apoprotein such as apoCIII, which has multiple effects on lipid and lipoprotein metabolism.

The authors used their *Ndst1*f/f*Alb-Cre*+ mouse, which lacks uptake of remnants by the heparan sulfate proteoglycan syndecan-1 pathway, to interrogate the relative roles of apoE and apoCIII on hepatic uptake of TGRL by the LDL and LDL receptor-related protein (LRP) pathways. These mice, being excellent models to study the roles of apoE and apoCIII in LDL and LRP1-mediated internalization of apoB-containing lipoproteins, were a logical choice based on the authors' recent publications (17, 18). However, studies in complex models such as this one, particularly when apoE is completely absent, may not allow extrapolation of the results to overall hepatic uptake of TGRL when the syndecan 1 pathway and apoE are present. Additionally, this model is expected to have normal LPL-mediated lipolysis of TG in apoB-lipoproteins and, therefore, effects of the apoC3 antisense oligonucleotide (ASO) on the other major pathway affected by apoCIII would have to be considered when interpreting effects of the ASO on hepatic uptake.

Because of these limitations, the experiments shown in the first two figures, designed to "determine the impact of apoE on apoC-III-mediated inhibition of LDLR/LRP1-mediated TRL clearance," must be interpreted with caution; TG levels, the key variable, will be determined by both TRL clearance and LPL-mediated lipolysis. One way to address the uncertain nature of these results would be to determine effects of the ASO on apoB100 and apoB48 levels using these nonexchangeable ligands for the receptors as measures of hepatic internalization of TGRL or their remnants. The authors show a single apoB48 Western blot result for each of the two experiments: it does not change in the chow-fed mouse but is reduced in the Western type diet-fed mouse. It would have helped readers to see more definitive apoB48 data. Additionally, it is not clear why apoB100 data is not presented. Mice expressing only apoB100 have significantly greater hypertriglyceridemia in response to overexpression of apoCIII than mice expressing only apoB48 (19).

The authors, cognizant of the problem of using TG as the outcome, turned to retinyl ester-labeled apoB48 lipoproteins lacking apoE and with or without apoCIII as their tracer for hepatic uptake; this reduced confounding by LPL-mediated lipolysis. The results of these experiments, shown in Fig. 3, indicate clearly that hepatic removal of apoB48 particles entering the circulation after oral

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administration was not affected by the level of apoCIII when apoE is absent. Maybe even more revealing was that, in the absence of apoE and syndecan 1, the rate of LPL-mediated lipolysis of TG on apoB48 particles, which we must assume was faster in the apoC3 ASO-treated mice, did not affect hepatic removal or remnant particles as reflected by retinol disappearance. A similar conclusion can be drawn from the identical plasma disappearance curves observed after intravenous injection of retinol-labeled apoB48-TGRLs. These in vivo studies were supported by a series of elegant studies, depicted in Fig. 4, with the same lipoproteins and primary hepatocytes from the double knockout mice. It must be noted, however, that conclusions drawn from these studies are limited to apoB48-lipoproteins that require apoE to interact with the LDL and LRP1 receptors. We make this point because it is very likely that increased LPL-mediated TG hydrolysis of apoB100-lipoproteins in the absence of apoCIII would increase the rate of their clearance by the liver, because lipolysis of apoB100-lipoproteins increases their affinity for the LDL receptor. This limitation of the mouse studies may explain the authors' finding that volanesorsen has similar efficacy in humans, who have much higher plasma concentrations of apoB100 TGRL, regardless of apoE phenotype, as shown in Fig. 9.

In the later part of the paper, the authors focused on the role of apoCIII in LPL-mediated lipolysis of TGRL and, based on a series of studies presented mainly in Fig. 7, concluded that apoCIII inhibits LPL-mediated lipolysis mainly in white adipose tissue. Most LPL is in white adipose tissue, so this would be expected. Regardless, the data are convincing, although these studies might have been confounded by the double knockout status of the mice. ApoE is also synthesized in adipose tissue and its absence is associated with smaller adipocytes and defects in accumulation of TG (20). Additionally, it has been reported that apoE can inhibit LPL activity (21). It is also interesting that the effects of apoC3 ASO treatment were only seen in fed mice; loss of function of apoCIII in humans results in both lower fasting and lower postprandial TGs. When individuals homozygous for *APOC3* loss of function were challenged with an oral fat load, there was a marked blunting of the rise in plasma TG levels (15).

There are several lessons to be learned from this paper. First, the small, exchangeable apolipoproteins, including apoCI and apoCII, in addition to apoE and apoCIII, have complicated and broad roles in the metabolism of lipids and lipoproteins. Second, innovative approaches to unraveling the hierarchy of roles played by each apoprotein, regardless of the uniqueness and specificity of the cell or mouse models used, may only provide insights that are unique and specific for those models; this is also true for studies in humans with mutations affecting these proteins, particularly homozygous mutations. Our two subjects with complete loss of apoCIII also lacked apoAI, apoAIV, and apoA5; we can only speculate as to how this affected our results (6). Our recent results, that 50% reductions in apoCIII in Old Order Amish were associated with marked increases in lipolysis of VLDL without increases in the rates of direct removal of VLDL from the circulation, may be unique to the subjects we studied, who have very low plasma levels of TG independent of the presence or absence of the apoCIII R19X loss-of-function mutation (16). It is very possible that individuals with a higher concentration of TG will have increased rates of clearance by both pathways when apoCIII is reduced. Finally, and particularly relevant to apoCIII, the question of whether reducing the circulating levels of this apoprotein will mirror the reduced rates of cardiovascular disease observed in the Mendelian randomization study will only be answered by a well-designed clinical trial.

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