Complexities of plasma apolipoprotein C-III metabolism[1]

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Apolipoprotein C-III (apoC-III) is a protein of 79 amino acids that is synthesized in the liver and to a lesser degree in the intestine (1). In the circulation, apoC-III is a constituent of both apoB- and apoA-I-containing lipoproteins. It is not evenly distributed between these lipoproteins however, with the majority of apoC-III found in the HDL fraction in normolipidemic individuals and on triglyceride-rich lipoproteins in patients with elevated levels of plasma triglyceride (2, 3). Furthermore, some VLDLs, intermediate density lipoproteins (IDLs) and LDLs contain many molecules of apoC-III, whereas others contain none (4). Semi-quantitative analysis suggests that less than half of apoA-I-containing lipoproteins in plasma (i.e., HDL) contain apoC-III (5).

ApoC-III plays a pivotal role in regulating the plasma metabolism of VLDL, IDL, and LDL, primarily by inhibiting receptor-mediated uptake of these lipoproteins by the liver (6–8). VLDL containing apoC-III are channeled down the lipolytic cascade to LDL, particularly to denser LDL that have a slower clearance rate from plasma (9). ApoC-III also enhances the hepatic assembly and secretion of VLDL (10), and overproduction of apoC-III and of apoB lipoproteins that contain apoC-III is a common feature of patients with hypertriglyceridemia (8, 9, 11). Thus, apoC-III is intricately involved in establishing hypertriglyceridemia and its associated dense LDL phenotype. Much less is known, however, about the function of apoC-III when it is a component of HDL. ApoC-III has been shown to inhibit hepatic lipase (12) and interact with receptors such as scavenger receptor class B type I (13) and ABCA1 (14), thus having the potential to affect the function or metabolism of HDL.

ApoC-III can also stimulate several processes involved in atherogenesis and vascular inflammation. ApoC-III stimulates blood-born monocytes and endothelial cells to produce cytokines such as tumor necrosis factor-α and adhesion molecules, and it activates insulin-resistance pathways in endothelial cells causing endothelial dysfunction (15, 16). Interaction of apoC-III with TLR-2 at the cell surface acts as an initiating event in this function. ApoC-III in LDL binds to vascular proteoglycans that may lead to LDL retention in the arterial wall (17). ApoC-III also stimulates adipocytes to produce cytokines and suppresses their production of adiponectin (18). These actions, converting adipocytes into a proinflammatory phenotype, may act indirectly to promote the development of atherosclerosis. More direct evidence for the atherogenicity associated with apoC-III comes from transgenic animal studies that have found that $ldlr^{-/-}$ mice overexpressing human apoC-III develop enhanced atherosclerotic lesions on a Western diet (19). Furthermore, apoC-III and the VLDL and LDL that contain it are strong independent predictors of cardiovascular events and of progression of coronary atherosclerosis (20, 21). In summary, evidence from several sources link apoC-III with atherosclerosis, providing a strong rationale to better understand factors affecting the plasma metabolism of apoC-III.

In the April issue of the *Journal of Lipid Research*, Ooi and colleagues (22) evaluated the metabolism of apoC-III in patients with chronic renal failure, a condition associated with a high incidence of cardiovascular disease. The principal finding was a low fractional catabolic rate (FCR) for apoC-III in VLDL of renal patients compared with controls. The FCR of VLDL apoC-III was strongly correlated with the FCR of VLDL apoB. These results provide additional evidence for an effect of apoC-III on clearance of triglyceride-rich lipoproteins and for the involvement of apoC-III in the dyslipidemia of patients with chronic renal impairment.

Ooi and colleagues, in this and previous research articles (22–24), have used their apoC-III kinetic data to make general conclusions about the plasma metabolism of apoC-III. They found that their tracer enrichment-time curves for apoC-III in VLDL and in HDL were similar. Consequently, the FCR for VLDL and HDL apoC-III were the same. From this observation, they concluded that apoC-III equilibrates rapidly and completely between these lipopro-

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teins (24). Because VLDL is cleared much more rapidly from plasma than HDL, these results and conclusions suggest that apoC-III transfers or exchanges between apoB- and apoA-I-containing lipoproteins (and perhaps also between different apoB-containing subspecies), but avoids being catabolized when these lipoproteins are cleared from the circulation. This scenario would suggest that apoC-III continually "jumps off" lipoproteins before they are taken up by cells. In addition, for a one-pool model of apoC-III kinetics to adequately explain plasma apoC-III metabolism, apoC-III must be cleared as free apoC-III or as a single lipoprotein type (i.e., "terminally catabolized triglyceride-rich lipoproteins"). Because free apoC-III is not found in human plasma (5, 25), and we find it hard to conceptualize how apoC-III escapes tissue uptake during the normal course of VLDL and HDL clearance, we have to question this oversimplified view of apoC-III metabolism.

We view the one-pool model for plasma apoC-III metabolism as only one of several possible interpretations of apoC-III tracer enrichment-time curves. Ooi and colleagues have not measured the movement of apoC-III between lipoproteins, so their interpretation that apoC-III rapidly and completely exchanges between lipoproteins is by inference only and not by direct measurement. One possibility is that enrichment curves could reflect an average of several distinctly different apoC-III pools within VLDL or HDL (which is incidentally the case for VLDL apoB and for HDL apoA-I when it is isolated and analyzed as one fraction). The pattern of apoC-III distribution within triglyceride-rich lipoproteins certainly does not suggest a random equilibration of apoC-III. ApoC-III-immunoaffinity chromatography separation of VLDL has demonstrated that VLDL containing apoC-III have as many as 100 apoC-III molecules per particle, yet a significant portion of VLDL do not contain apoC-III at all (4, 26).

Another problem with the one-pool concept for plasma apoC-III metabolism is that it fails to explain the asymmetrical presence of apoC-III among triglyceride-rich lipoproteins and HDL. ApoC-III is present across the entire spectrum of differently-sized plasma lipoproteins from large VLDL to small HDL. However, apoC-III is present on only 30–70% of VLDL, a smaller percentage of IDL, 5–15% of LDL, and a minority of HDL particles. The actual apoC-III distribution pattern, as demonstrated in **Fig. 1**, suggests that apoC-III transfer between various lipoproteins is a regulated process and requires other factors. If apoC-III is freely and rapidly exchangeable in a single pool, then exchange would be restricted to apoC-III-containing lipoprotein subtypes. This would require an unknown property of apoC-III-containing lipoproteins that permitted apoC-III to transfer freely among them and not to nonapoC-III containing lipoproteins.

Therefore, an unanswered question that needs to be resolved is the extent to which apoC-III exchanges between VLDL and HDL. Although some studies have demonstrated exchange and equilibration of apoC-III between VLDL and HDL (24, 27), others have found nonexchange-
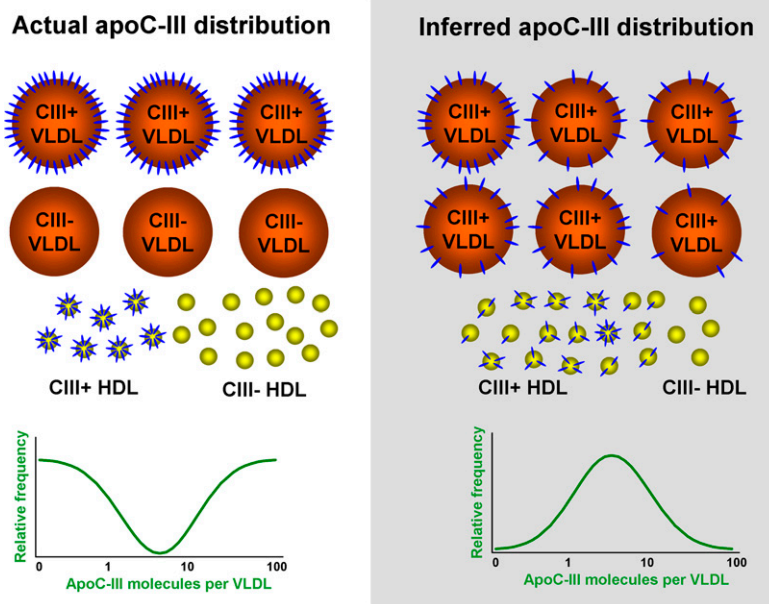
Fig. 1. Comparison between the actual lipoprotein distribution of apoC-III and the distribution inferred from the one-pool concept of plasma apoC-III metabolism. There are on average ~20–50 apoC-III molecules on each VLDL particle. About 50% of VLDL contain apoC-III (CIII+), and the other half of VLDL do not contain apoC-III at all (CIII−). The apoC-III distribution pattern within VLDL has an inverse bell shape (left panel). On the other hand, the one-pool concept of plasma apoC-III metabolism suggests that apoC-III exchanges freely and randomly within VLDL and HDL and also between VLDL and HDL. In this scenario, the vast majority of VLDL and HDL would contain some apoC-III molecules and would have a normal distribution pattern (right panel). There would be few VLDL or HDL containing large numbers of apoC-III molecules and one would be unlikely to find lipoproteins without apoC-III.

able pools of apoC-III that do not completely equilibrate between VLDL and HDL (11, 28). Based on in vitro experiments, Boyle et al. (29) found two kinetic pools of apoC-III that transferred between VLDL and HDL at different speeds. ApoC-III in the fast pool rapidly transferred from donor to recipient lipoproteins in a matter of minutes, whereas apoC-III in the slow pool followed a monoexponential rate of exchange with a $t_{1/2}$ of 3 h. The distribution of apoC-III between fast and slow pools was variable but apparently depended on the size of the donor particles. These results suggest that the two kinetically distinct pools may be related to conformational changes in individual apoC-III molecules on the lipoprotein surface. In a separate study, labeled apoC-III disappearance from plasma was found to follow tri-exponential kinetics, and differences in plasma and urine radioactivity curves suggested the presence of kinetically distinct pools of apoC-III (30). These findings for apoC-III are analogous to the properties of apoE, which is a fixed and exchangeable component within VLDL of various sizes (31).

Our tracer studies of apoB metabolism have shown that apoC-III-containing VLDL and IDL are converted to VLDL, IDL, and LDL without apoC-III (8, 9). More than half of apoC-III-containing VLDL and IDL follow this pathway, losing their triglyceride content together with apoC-III. They are thus transformed into slowly turning over lipoproteins that do not contain apoC-III. Partial loss of apoC-III also happens to triglyceride-rich lipoproteins with apoC-III as evidenced by their diminishing apoC-III content as they become smaller (26). It remains to be studied whether apoC-III molecules released from VLDL and IDL are relocated to other apoB lipoproteins or HDL or are cleared from circulation directly. Similarly, it is not entirely clear what processes regulate the transfer of apoC-III from HDL to VLDL. Although apoC-III can readily transfer from HDL to VLDL or chylomicron-sized lipid emulsions (24, 30), in our tracer studies, we have not found evidence in vivo for acquisition of apoC-III by circulating VLDL that do not yet have apoC-III. It is possible that if indeed apoC-III does move from HDL to VLDL in significant amounts, the preferred recipient VLDL are the large particles that already possess considerable amounts of apoC-III. One additional reason to question the simplicity of a one-pool model of plasma apoC-III metabolism is that apoC-III exists as three isoforms, i.e., apoC-III$_0$, apoC-III$_1$ and apoC-III$_2$, corresponding to 0-2 sialic acid molecules on the protein. The isoform with most sialic acid, apoC-III$_2$, tends to have a higher FCR compared with monosialylated apoC-III$_1$, and apoC-III$_0$, the less-predominant isoform, has a significantly slower FCR and lower production rates compared with the other two isoforms (32). These data suggest heterogeneity among apoC-III isoforms, regarding both secretion and clearance, which should be taken into consideration when dealing with disease conditions that directly affect apoC-III sialylation, including chronic renal failure (33).

Current modeling of apoC-III kinetic data has assumed that apoC-III is secreted on both VLDL and HDL, and that the distribution of secretion is proportional to their respective pool sizes. However, it is not clear if this assumption is correct. Recent experiments with hepatocytes expressing apoC-III show that the vast majority of apoC-III is found on HDL instead of VLDL in the media of cultured cells (10). It is unclear whether apoC-III is secreted together with HDL or if HDL acquires apoC-III after secretion. On the other hand, the same group reported that apoC-III seemed to play an important role in packaging lipids onto VLDL precursors (10, 34). Indeed, the majority of apoC-III in the microsomal lumen was found associated with IDL-sized lipid droplets, which later fused with VLDL precursors to form VLDL. Therefore, it is possible that apoC-III is secreted as an integral component of lipoproteins and is not a result of random acquisition by acceptor particles. Once again, apoC-III appears to have a specific and not simply a random role in regulating VLDL production by the liver.

In conclusion, it is our opinion that: *1*) hepatic apoC-III production (i.e., synthesis and secretion) plays an important role in determining the size or number of triglyceride-rich VLDL secreted by the liver; *2*) in the blood, different lipoproteins (whether apoB- or apoA-I-containing) have different numbers of apoC-III molecules, which may be determined by the structure or composition of the lipoproteins themselves; and *3*) irrespective of whether all apoC-III is exchangeable or not, it significantly affects the metabolism of the particle on which it resides, and in so doing, plays a central role in determining the concentration in the circulation of potentially atherogenic VLDL, IDL, and small dense LDL.

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